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Pathogenic Diversity of *Xanthomonas oryzae* pv. *oryzae* Isolates in Togo

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ABSTRACT

The present study aimed at determining *Xanthomonas oryzae* pv. *oryzae* pathogenic diversity. Therefore, the disease survey was conducted in three ecozones of Togo-Forest savanna transition, Forest and dry savanna zones and the diseased samples were collected, the bacteria isolated and characterized by testing their virulence on 21 rice genotypes. The results revealed the occurrence of the bacterial leaf blight in the three ecozones. High bacterial leaf blight incidence of 50-65% was observed in Forest savanna transition and Forest zones, while it was up to 70% in the dry savanna zone. The highest incidence (70%) was recorded in dry savanna zone and the lowest (25%) was frequently observed in Forest zone. Pathotyping analysis of 13 bacterial isolates from samples collected from the infected fields against 21 rice genotypes to isolate and characterize bacteria virulence was carried out. Thus, AMMI cluster analysis revealed the existence of 3 Pathotypes (Pt) among these isolates: PtA highly virulent has 1 isolate, PtB virulent was made up of 3 isolates and PtC moderately virulent was made up of 9 isolates. At ecozone the analysis revealed the presence of PtB and PtC in the Forest savanna transition zone, PtA and PtC in Forest zone and PtB and PtC in the dry savanna zone. The present results provided knowledge on the bacteria virulence and its structure across ecozones of Togo and provided useful information for selection of genotypes for durable resistance to the disease.

Key words: Rice, bacterial leaf blight, isolates, virulence, pathotypes

INTRODUCTION

Rice is one of the most important staple crops worldwide. Unfortunately, its production is constrained by several diseases. Bacterial leaf Blight (BB), caused by the vascular pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo) (Swings *et al*., 1990), is one of the most serious diseases of rice. The disease is widespread throughout Asia and also was reported from rice-growing areas in Australia, united states and several countries in Latin America and Africa (Mew *et al*., 1993; Sere *et al*., 2005). Bacterial leaf blight may cause damage at seedling stage resulting in complete wilting or death of affected tillers. The infection at the maximum tillering stage results in blighting of leaves. Yield losses of up to 80% due to BB have been reported (Ou, 1985). Recently, BB disease was reported in several farmers' fields across ecozones in Niger, Burkina Faso, Nigeria, Benin and Mali, with high incidence of 70-80 and yield losses ranging from 50-90% (Sere *et al*., 2005).

In Togo, BB was reported to occur in several rice-growing areas in the North part of Togo, with high incidence and severity in most of the fields visited (Dewa *et al*., 2011). The authors observed a wide spread of BB in farmers fields in West African rice growing countries including Togo. Investigation on the pathological characterization of some Xoo isolates from these countries have been undertaken by testing the virulence of the bacteria and revealed a high level of virulence on cultivated rice varieties (Sere *et al*., 2005; Dewa *et al*., 2011). Bacterial leaf blight is characterized by a high degree of race-cultivar specificity. Pathotyping analysis of 50 Xoo isolates from seven West African countries against 18 rice cultivars revealed two major pathotypes of Xoo virulence (Onasanya *et al*., 2009). Although, preliminary studies on BB survey in the North of Togo and the virulence test of Xoo isolates, no investigation on the diversity of the pathogen was carried out. However, investigation on the Xoo virulent population structure using isolates from different ecozones is important for selection of varieties for durable resistance to BB. Therefore, the present study aimed at collecting bacterial leaf blight samples from infected farmers' fields in different ecozones of Togo and characterizing Xoo isolates virulence for identification of pathotypes.

MATERIALS AND METHODS

Survey of bacterial leaf blight and collection of samples: BB disease survey was conducted from 26 June to 5 July 2010 across three of the four main ecozones of Togo. A total of eight localities were covered by the survey: Lome Kovie and Davie in the Forest savanna transition zone (Maritime Region), Kpele Atime, Kpele Tutu and Sodo in the Forest zone (Plateau Region) and Koumbeloti and Tantiegou in the dry savanna zone (Savanna Region). In each locality, fields were visited and the number of infected plants were assessed in the four corners (1 m^2 per corner) and in the centre of the field in order to establish the incidence of the disease at field level. In the infected fields, diseased leaf samples were collected from rice and/or weed plants showing typical BB symptoms, conditioned in envelopes and conserved in cool plastic boxes for transport. Once in laboratory, the samples were kept into a refrigerator at 4°C for further process.

Bacteria isolation: Bacterial isolation was carried out using Nutrient Agar (NA) medium and purified on Glucose Yeast Calcium Agar (GYCA) medium for further investigations. In laboratory, leaf samples were gently washed under running water, rinsed with sterile distilled water and superficially sterilized in 1% hypochlorite solution and rinsed again with sterilized distilled water. Then, leaves were immerged into sterile distilled water for 30 min to reactivate bacteria cells in leaf tissues. Very small pieces in size of each leaf sample were cut and incubated in some drops of sterile distilled water in tubes and incubated for about 3 h. Thereafter loopfuls of the suspension were streaked on NA medium in petri dishes, which were incubated at 28°C for 48-72 h for bacterial growth. Then, typical Xoo-like colonies identified according to Agarwal *et al*. (1994) were checked and purified on fresh GYCA medium. To confirm the identity of the isolates obtained, biochemical tests were performed: Gram test was carried out using KOH_3 and the growth test on a selective medium was conducted using the nutrient agar+copper nitrate ($Cu(NO_3)_2$) 0,001% (Sere *et al*., 2005).

Pathotyping of Xoo isolates

Bacterial isolates: For the pathotyping test 13 Xoo isolates from different ecozones were used. These consisted of one or two isolates per locality depending on the occurrence of BB disease on rice plants only or on both rice and weeds plants.

Plant materials: Thirteen Near Isogenic Lines (NILs) with known resistance gene to bacteria leaf blight obtained from the International Rice Research Institute (IRRI), six improved genotypes from Africa Rice Center, Cotonou, Benin and two improved genotypes from Institut Togolais de Recherche Agronomique (ITRA), Togo, widely grown in Togo were used for *X. oryzae* pv. *oryzae* pathotyping test (Table 1).

Experimental design: The experiment was carried out using the split plot design with 3 replications. Thirteen isolates (Table 2) from different ecozones were used to screen 21 genotypes (Table 1) in the screen house at Africa Rice Center in Cotonou, Benin. Rice grains were first pre-germinated in sterilized petri dishes under sterile conditions for 5 days and were transplanted in plastic pots. One plastic pot per genotype per Xoo isolate in 3 replications was used.

Table 1: Varieties and near isogenic lines used for pathotyping of Xoo isolates

NILs	Resistance genes	Improved genotypes	Origin
IRBB1	Xa-1	TGR203 (WITA4)	ITRA Togo
IRBB2	Xa-2	IR841	ITRA Togo[a]
IRBB3	Xa-3	NERICA4	AfricaRice[a]
IRBB4	Xa-4	NERICA8	AfricaRice[a]
IRBB5	Xa-5	NERICA14	AfricaRice[a]
IRBB7	Xa-7	NERICA19	AfricaRice[a]
IRBB8	Xa-8	Gigante	AfricaRice
IRBB10	Xa-10	TOG 5681	AfricaRice
IRBB11	Xa-11		
IRBB13	Xa-13		
IRBB14	Xa-14		
IRBB21	Xa-21		
IR24	Xa-18		

[a]Cultivated in Togo, NILs: Near isogenic lines

Table 2: Xoo isolates, their host plants and their reaction to biochemical tests

			KOH_3 test	$Cu(NO_3)_2$ test
Isolates	Host plant	Localities	----------	----------
I1: KV4-2	IR841[a]	Kovie (FST)	Gram-	+
I2: KV14-2	*Sorghum arundinaceum*[c]	Kovie	Gram-	+
I3: IL23-1	IR841[1]	Lome (FST)	Gram-	+
I4: DV39-1	NERICA 8[1]	Davie (FST)	Gram-	+
I5: DV58-2	*Digitaria horizontalis*[c]	Davie	Gram-	+
I6: KA63-2	*Panicum maximum*[c]	Kpele Atime (F)	Gram-	+
I7: KT83-2	IR841[1]	Kpele Tutu (F)	Gram-	+
I8: KT84-2	*Leersia hexandra*[c]	Kpele Tutu	Gram-	+
I9: SD94-1	Rice[b]	Sodo (F)	Gram-	+
I10: KM101-1	IR841[1]	Koumbeloti DS	Gram-	+
I11: KM129-2	*D. horizontalis*[c]	Koumbeloti	Gram-	+
I12: TN135-2	Rice[2]	Tantiegou (DS)	Gram-	+
I13: TN160-2	*Echinochloa colona*[c]	Tantiegou	Gram-	+

[a]Rice genotypes, [b]Not identified rice cultivars, [c]Weed species, FST: Forest savanna transition zone, F: Forest zone, DS: Dry savanna zone

Fertilizer application: For fertilization, 1.0 g of NPK 15-15-15 per pot was applied 8 days after transplanting and 0.2 g of Urea 46% per pot was applied 15 days after transplanting.

Bacterial suspension and inoculation: Inoculum was prepared using a 48 h old culture of Xoo isolates produced on GYCA was harvested from agar plates and suspended in sterile distilled water and adjusted to a concentration of 10^9 CFU mL^{-1} (OD650 = 0.5) prior to use. Inoculation was by clipping method (Sere *et al*., 2005). The whole leaves of each plant in the plastic pot were clip inoculated 21 days after transplanting.

Data assessment: Evaluation consisted on the measurement of the lesion length due to bacterial leaf blight disease induced by inoculation with each of the isolates and also the measurement of the total leaf length 14 days after inoculation (Sere *et al*., 2005). From these data, the percentage of lesion length was determined for each inoculated leaf.

Data analysis: Using the percentage lesion length, Analysis of Variance (ANOVA) and Additive Main Effect and Multiplicative Interaction (AMMI) analysis were performed using IRRISTAT software to Xoo isolates virulence and identify eventual pathotypes (Aleong and Howard, 1985; Bruckner and Slanger, 1986; Ebdon and Gauch, 2002; Zhu and Kuljaca, 2005). AMMI analysis was shown to be effective in understanding complex Genotype by Environment interactions trials that are difficult to understand using ordinary ANOVA (Ebdon and Gauch, 2002).

RESULTS

Survey and bacteria isolates: The survey revealed the occurrence of BB in the three ecozones, Forest savanna transition zone, Forest zone and dry savanna zone with variable incidence ranging from 25-70%. BB incidence of 25% was recorded in Lome (Forest savanna transition zone), Kpele Atime and Kpele Tutu (Forest zone), while it was 50% in Davie (Forest savanna transition zone), 60% in Sodo and Tantiegou (dry savanna zone), 65% in Kovie (Forest savanna transition zone) and 70% in Koumbeloti (dry savanna zone). BB was observed on rice plants but also on some weed species in some localities.

A total of 134 isolates have been obtained as *X. oryzae* pv. *oryzae* (Xoo) after biochemical tests with the pathogens isolated. The identified Xoo bacteria were obtained not only from rice plants but also from some weed plants. A total of eight weed species of three families were found to be host plants of Xoo: *Commelina benghalensis* (Commelinaceae) at Davie, *Digitaria horizontalis* (Poaceae) at Davie and Koumbeloti, *Echinochloa colona* (Poaceae) at Davie and Tantiegou, *Leersia hexandra* (Poaceae) at Kovie, Kpele Atime and Kpele Tutu, *L. oryzoides* at Kovie, Koumbeloti and Tantiegou, *Panicum maximum* (Poaceae) at Kovie and Kpele Atime, *Sorghum arundinaceum* (Poaceae) at Kovie and *Zizania latifolia* (Poaceae) at Tantiegou.

Virulence of Xoo isolates and pathotyping: The results on the percentage of lesion length due to bacterial leaf blight revealed considerable variability in the reactions of the 13 Xoo isolates to 21 genotypes tested. Analysis of Variance (ANOVA) for the percentage lesion length due to inoculation with Xoo isolates from different ecozones revealed significant interaction ($p<0.05$) between Xoo isolates and rice genotypes (Table 3). Differential reaction was observed for Xoo isolates of the same ecozone or locality of origin (Table 4). Significant different lesion length was

Table 3: Analysis of variance for percentage lesion length induced by Xoo isolates

Source	df	SS	MS	Fisher-value
Repetition	2	3.59	1.79	2.85 ns
Treatment	272	484.28	1.78	2.82**
Variety (V)	20	126.74	6.34	10.05**
Isolate (I)	12	170.62	14.22	22.56**
V×I	240	186.92	0.78	1.24*
Error	544	342.87	0.63	
Total	818	830.75		

*,**Significant at 5 and 1% level, ns: Non significant

Table 4: Means of percentage BB disease lesion length due to inoculation with Xoo isolates from different ecozones

Geno-	Isolates													
types	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	G-Mean
G1	5.02^{ab}	2.47^{b}	2.41^{b}	5.05^{ab}	2.68^{b}	2.31^{b}	7.95^{ab}	9.56^{ab}	3.62^{b}	10.27^{ab}	2.85^{b}	14.47^{a}	11.50^{ab}	6.17
G2	12.35^{a}	1.31^{c}	1.60^{c}	8.74^{ab}	2.29^{bc}	2.49^{bc}	8.22^{abc}	12.53^{a}	5.63^{abc}	1.66^{bc}	2.31^{bc}	4.27^{abc}	6.15^{abc}	5.35
G3	4.99^{bc}	1.72^{c}	2.00^{c}	2.92^{c}	1.47^{c}	1.38^{c}	1.90^{c}	17.94^{a}	3.68^{bc}	1.31^{c}	1.63^{c}	4.36^{bc}	13.11^{ab}	4.49
G4	3.62^{abc}	1.46^{bc}	4.91^{abc}	3.05^{abc}	1.58^{bc}	5.05^{abc}	3.62^{abc}	8.08^{ab}	4.04^{abc}	1.09^{c}	2.56^{bc}	5.57^{abc}	12.49^{a}	4.39
G5	3.95^{abc}	1.39^{c}	2.51^{bc}	2.18^{bc}	0.68^{c}	0.90^{c}	1.56^{bc}	1.48^{c}	5.19^{abc}	1.54^{bc}	1.54^{bc}	8.13^{ab}	12.75^{a}	3.37
G6	3.63^{cde}	12.37^{a-d}	1.51^{e}	18.93^{a}	1.41^{e}	1.47^{e}	7.52^{b-e}	13.27^{ab}	12.31^{abc}	2.33^{de}	1.27^{e}	4.28^{cde}	17.80^{a}	7.54
G7	6.19^{ab}	1.11^{b}	2.49^{b}	3.93^{ab}	1.49^{b}	1.99^{b}	1.59^{b}	11.02^{a}	2.26^{b}	1.18^{b}	1.20^{b}	10.10^{a}	12.63^{a}	4.40
G8	17.62^{bc}	1.78^{d}	3.11^{d}	29.15^{b}	5.67^{d}	2.10^{d}	4.20^{d}	49.38^{a}	8.63^{cd}	3.16^{d}	2.43^{d}	24.96^{b}	24.47^{b}	13.59
G9	1.40^{b}	0.93^{b}	0.83^{b}	1.85^{ab}	1.62^{b}	0.71^{b}	0.97^{b}	7.84^{a}	0.98^{b}	1.43^{b}	0.90^{b}	2.16^{ab}	3.47^{ab}	1.93
G10	1.34^{a}	0.91^{a}	1.08^{a}	1.41^{a}	0.88^{a}	0.97^{a}	2.83^{a}	2.23^{a}	0.84^{a}	0.55^{a}	0.69^{a}	6.12^{a}	2.88^{a}	1.75
G11	4.18^{a}	0.93^{a}	1.68^{a}	4.70^{a}	1.67^{a}	2.13^{a}	1.20^{a}	3.57^{a}	1.57^{a}	0.83^{a}	1.85^{a}	5.42^{a}	2.21^{a}	2.46
G12	2.45^{b}	1.59^{b}	1.85^{b}	1.31^{b}	2.02^{b}	1.02^{b}	0.98^{b}	5.58^{ab}	1.12^{b}	1.09^{b}	1.22^{b}	10.18^{a}	5.17^{ab}	2.74
G13	1.73^{b}	1.17^{b}	1.83^{b}	3.38^{b}	2.07^{b}	2.29^{b}	1.21^{b}	1.88^{b}	6.22^{ab}	1.11^{b}	1.10^{b}	13.54^{a}	21.19^{a}	4.52
G14	2.11^{a}	3.08^{a}	1.98^{a}	2.65^{a}	1.29^{a}	2.57^{a}	1.51^{a}	2.27^{a}	1.63^{a}	0.62^{a}	1.10^{a}	2.67^{a}	5.68^{a}	2.24
G15	1.68^{b}	1.41^{b}	2.16^{b}	5.59^{ab}	1.04^{b}	1.03^{b}	2.55^{b}	11.48^{a}	1.59^{b}	3.20^{b}	0.92^{b}	3.67^{ab}	6.15^{ab}	3.27
G16	2.53^{a}	0.85^{a}	1.06^{a}	3.52^{a}	1.51^{a}	0.91^{a}	1.03^{a}	1.43^{a}	3.23^{a}	0.70^{a}	0.86^{a}	2.31^{a}	1.56^{a}	1.65
G17	2.20^{a}	1.13^{a}	3.15^{a}	7.81^{a}	2.21^{a}	0.78^{a}	1.57^{a}	1.48^{a}	2.05^{a}	0.75^{a}	1.20^{a}	6.45^{a}	5.25^{a}	2.77
G18	2.35^{a}	1.72^{a}	1.25^{a}	3.44^{a}	1.10^{a}	1.82^{a}	2.09^{a}	7.09^{a}	0.98^{a}	1.11^{a}	0.95^{a}	3.64^{a}	4.13^{a}	2.44
G19	2.08^{a}	0.88^{a}	1.43^{a}	3.66^{a}	4.20^{a}	0.99^{a}	1.40^{a}	2.88^{a}	2.69^{a}	1.36^{a}	1.09^{a}	3.14^{a}	1.98^{a}	2.14
G20	1.83^{a}	1.08^{a}	0.92^{a}	1.62^{a}	3.35^{a}	3.57^{a}	1.77^{a}	6.11^{a}	6.46^{a}	2.96^{a}	1.20^{a}	4.74^{a}	2.13^{a}	2.90
G21	1.91^{ab}	0.64^{b}	2.65^{ab}	2.54^{ab}	3.98^{ab}	3.94^{ab}	4.42^{ab}	3.71^{ab}	1.86^{ab}	0.85^{ab}	1.03^{ab}	7.55^{a}	3.85^{ab}	3.00
I-Mean	4.05^{b}	1.90^{c}	2.02^{c}	5.59^{b}	2.11^{c}	1.92^{c}	2.86^{c}	8.61^{a}	3.65^{b}	1.86^{c}	1.42^{c}	7.03^{a}	8.41^{a}	3.96

Isolates; I1: KV4-2, I2: KV14-2, I3: IL23-1, I4: DV39-1, I5: DV58-2, I6: KA63-2, I8: KT84-2, I9: SD94-1, I10: KM101-1, I11: KM129-2, I12: TN135-2, I13: TN160-2, Rice genotypes; G1: TGR203 (WITA4), G2: IR841, G3: NERICA4, G4: NERICA8, G5: NERICA14, G6: NERICA19, G7: Gigante, G8: TOG 5681, G9: IRBB1, G10: IRBB2, G11: IRBB3, G12: IRBB4, G13: IRBB5, G14: IRBB7, G15: IRBB8, G16: IRBB10, G17: IRBB11, G18: IRBB13, G19: IRBB14, G20: IRBB21, G21: IR24, Values within row with different letters are significantly different at $p<0.01$

observed between I1 (KV4-2) and I2 (KV14-2) from Kovie, with 12.35 and 1.31% of lesion length, respectively, I4 (DV39-1) and I5 (DV58-2) from Davie, with 29.15 and 5.67%, respectively in the Forest savanna transition zone, between I7 (KT83-2) and I8 (KT84-2) from Kpele Tutu, with 4.20 and 49.38%, respectively in the Forest zone, between I12 (TN135-2) and I13 (TN160-2) from Tantiegou, with 4.28 and 17.80%, respectively in the dry savanna zone. Also, Differential reactions were observed for Xoo isolates of different ecozones or localities.

Significant differences in percentage of lesion length ($p<0.01$) were observed between isolate I8 (KT84-2) (49.38%) from the Forest zone and I2 (KV14-2) (1.78%) from Forest savanna transition zone, between I11 (KM129-2) (2.43%) from the dry savanna zone and I4 (DV39-1) (29.15%) from Forest savanna transition zone and between I8 (KT84-2) (17.94%) from the Forest zone and I10 (KM101-1) (1.31%) from the dry savanna zone (Table 4).

Isolate I8 (KT84-2) recorded the highest percentage of lesion length of 49.39% on genotype G8 (TOG5681) and induced more than 12% of lesion length on three other genotypes. However, this isolate induced less than 3% of lesion on 7 of the 21 genotypes tested. Isolates I12 (TN135-2) and I13 (TN160-2) induced more than 12% of lesion length on at least 3 genotypes and reached the percentage of about 25%. However, they caused less than 3% of lesion length on at least three genotypes. Also, isolate I4 (DV39-1) exhibited high virulence on the genotypes G6 (NERICA19) and G8 (TOG5681) with the lesion length of 18.93 and 29.15%, respectively but induced less than 3% of lesion length on eight genotypes (Table 4).

The results showed that the highest means percentage of disease lesion length due to inoculation was recorded by the isolates I8 (KT84-2) with 8.61%, while the lowest lesion length was recorded by isolate I11 (KM129-2) with 1.42%. Analysis of variance for the means percentage of disease lesion length due to inoculation revealed significant difference between isolates ($p<0.01$) and classified them into 3 groups: The first group was made up of isolates I8 (KT84-2), I13 (TN160-2) and I12 (TN135-2) which recorded the highest means lesion length of 8.61, 8.41 and 7.03%, respectively; the second group included isolates I4 (DV39-1), I1 (KV4-2) and I9 (SD94-1) with means lesion length of 5.59, 4.05 and 3.65%, respectively and the third group was made up of isolates I11 (KM129-2), I10 (KM101-1), I2 (KV14-2), I6 (KA63-2), I3 (IL23-1), I5 (DV58-2) and I7 (KT83-2) with means lesion length ranging from 1.42-2.86% (Table 4).

Additive Main effects and Multiplicative Interaction (AMMI) analysis of percentage lesion length revealed differential reactions of isolates on genotypes tested and the cluster allowed identifying 3 groups of pathotypes among the 13 isolates from different ecozones: Pathotype A (PtA) made up of one isolate I8 (KT84-2) highly virulent, Pathotype B (PtB) made up of 3 virulent isolates including I4 (DV39-1), I12 (TN135-2) and I13 (TN160-2) and Pathotype C (PtC) made up of 9 moderately virulent isolates I1 = KV4-2; I2 = KV14-2; I3 = IL23-1; I5 = DV58-2; I6 = KA63-2; I7 = KT83-2; I9 = SD94-1; I10 = KM101-1; I11 = KM129-2 (Fig. 1, Table 5). At ecozone level cluster analysis revealed the presence of PtB and PtC in the Forest savanna transition zone, PtA and PtC in the Forest zone and PtB and PtC in the dry savanna zone. This indicates variability of the pathogen within and across ecozones.

Table 5: Xoo identified pathotypes, their virulence and distribution across ecozones

		Isolate origin and distribution between ecozones			
Pathotypes	Virulence	FST	FZ	DS	Occurrence(%)
PtA	++++	-	1	-	8
PtB	+++	1	-	2	23
PtC	++	4	3	2	69

PtA: Pathotype A, PtB: Pathotype B, PtC: Pathotype C, ++++: Highly virulent, +++: Virulent, ++: Moderately virulent, FST: Forest savanna transition zone, FZ: Forest zone, DS: dry savanna zone

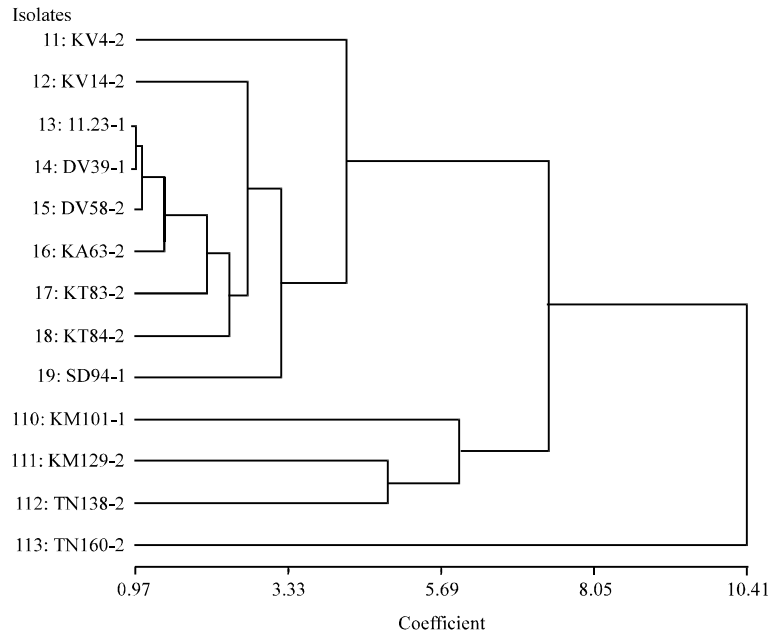

Fig. 1: Pathotyping dendrogram of *X. oryzae* pv. *oryzae* isolates using AMMI analysis, coefficient: fusion level

DISCUSSION

Bacterial leaf blight incidence was generally higher in the dry savanna zone than in the other ecozones. Previous survey on rice diseases in the North part of Togo reported higher BB incidence of >75% in dry savanna zone than in Wet savanna zone with incidence <50% (Dewa *et al.,* 2011). Recent research conducted in five West African countries including Niger, Burkina Faso, Nigeria, Benin and Mali revealed frequent occurrence of BB in farmers' fields across these countries with incidence ranging from 70-85% (Sere *et al.*, 2005). In the present study, high incidence of 70% was found in some ecozones. This indicates a wide spread of bacterial leaf blight in farmers fields across West African countries including Togo. The present survey coupled with the earlier investigation (Dewa *et al.*, 2011) revealed that BB is widely spread in rice growing areas of Togo.

The variability of the BB incidence observed across ecozones could be related to the environmental conditions. Earlier observations on cassava bacterial blight caused by *X. axonopodis pv. manihotis* reported that the disease incidence was higher in dry savanna zone than in Forest savanna transition, Wet savanna and Forest zones (Banito *et al.*, 2007). Also, cassava bacterial blight was found in various ecozones across four West African countries, with generally higher incidences in the savanna zones than in the transition Forest zones (Wydra and Verdier, 2002). The generally lower incidence of bacterial disease in the Forest zone compared to the savanna zones may be due to the climatic conditions, since great differences in night versus day temperatures in the savanna zones were reported to promote the disease (Lozano, 1986). This could explain the higher BB incidence found in dry savanna zone than in Transition and Forest zones.

Bacterial leaf blight was found to occur on some weed species such as Poaceae, Cyperaceae and Commelinaceae in six of the eight prefectures visited. The occurring on weeds such as *L. oryzoides* (L.) Sw., *Z. latifolia* (Griesb) Turcz. ex Stapf, *Leptochloa chinensis* (L.) Nees and *Cyperus rotundus* L. has been reported (Webster and Gunnell, 1992).

Host plant resistance is the best mean to control bacterial diseases of plant. Therefore, knowledge on the pathogen population structure is important to identify and select representative strains for screening genotypes for durable resistance to the disease (Nelson *et al*., 1994; Choi *et al*., 1998). In the present study, the virulence of *X. oryzae* pv. *oryzae* isolates and the genotype by isolate interaction were evaluated by inoculating 21 genotypes with 13 isolates from different ecozones of Togo. Virulence test of 11 Xoo isolates revealed most of the isolates virulent and one isolate from the dry savanna zone was highly virulent, while 3 isolates were moderately virulent (Dewa *et al*., 2011).

AMMI analysis revealed diversity among isolates tested and identified 3 pathotypes. Diversity in bacterial population and its structure and identification of pathotypes have widely been documented (Restrepo *et al*., 2004; Wydra *et al*., 2004; Jeung *et al*., 2006). Pathotyping analysis of 50 Xoo isolates from seven West African countries against 18 rice cultivars was carried out and Xoo virulence was identified and characterized and two main groups of pathotypes were found and clustered under five subgroups of pathotypes (Onasanya *et al*., 2009). Virulence analysis undertaken by Liu *et al*. (2007) revealed nine Xoo races from China rice grown regions. Also, Banito *et al*. (2010) revealed the existence of pathotypes among highly virulent *X. axonopodis* pv. *manihotis* strains from different African origins.

The spatial movement of *X. oryzae* pv. *oryzae* pathogens is an important factor to be considered in controlling bacterial leaf blight disease. In the present study, virulent PtB was found in the Forest savanna transition zone and in the dry savanna zone, while the PtC moderately virulent was found in the Forest savanna transition zone, the Forest zone and the dry savanna zone, indicating a certain movement of Xoo pathogens across ecozones. This is likely due to possible contaminated germplasm exchange between ecozones. Several investigations on pathogen migration have been carried out; for example the European continental movement of the *Erysiphe graminis* (Brown *et al*., 1991) has been reported. Recently, the presence of two pathotypes PtA (virulent) and PtB (middle virulent) was reported in 7 West African countries including Mali, Nigeria, Benin, Burkina Faso, Niger, Guinea and Gambia and suggested the possible movement of the pathogen across these countries (Onasanya *et al*., 2009). The present results revealed important genotype by pathotype interaction and knowledge on population structure of Xoo virulence across ecozones of Togo and provided useful information for selection of genotypes with durable resistance to bacterial leaf blight. However, experiments under field conditions in different environments are also needed.

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