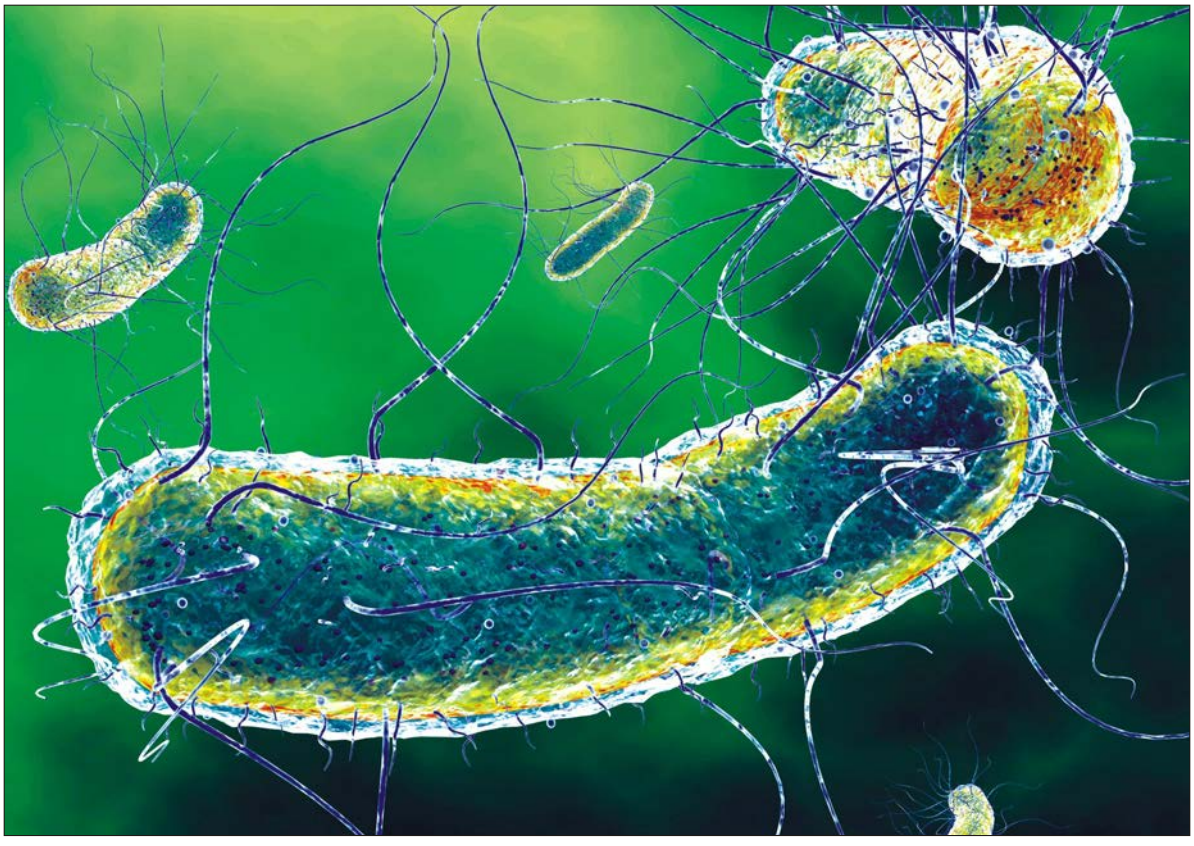

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Two Pathotypes of *Xanthomonas oryzae* pv. *oryzae* Virulence Identified in West Africa

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Abstract: Pathotyping analysis of 50 Xanthomonas oryzae pv. oryzae (Xoo) isolates from seven West African countries against 18 rice cultivars was carried out to identify and characterize Xoo virulence. The study revealed two major pathotypes (Pta and Ptb) of Xoo virulence. Pta has 29 virulence (Vr) Xoo isolates while Ptb has 21 mildly virulence (MVr) Xoo isolates. Pta has three subgroup pathotypes (Pta1, Pta2 and Pta3) and Ptb has two subgroup pathotypes (Ptb1 and Ptb2). At country level the study revealed the presence of Pta1, Ptb1 and Ptb2 in Niger, Pta3, Ptb1 and Ptb2 in Benin and Nigeria, Pta1, Pta3 and Ptb1 in Burkina Faso, Pta1, Pta3, Ptb1 and Ptb2 in Mali, Pta1, Pta2, Pta3, Ptb1 and Ptb2 in Guinea and Pta1, Pta2, Ptb1 and Ptb2 in the Gambia. The existence of five subgroups was likely due to mutations and interactions among isolates that originally constituted Pta and Ptb pathotypes. The study revealed information on Xoo virulent population structure in West Africa as well as possible Xoo pathogen migration between these countries and this provide useful information for selection and deployment of cultivars with durable resistance to BLB disease in West Africa.

Key words: Bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae*, lesion length virulence, pathotype, rice cultivars, West Africa

INTRODUCTION

Rice is perhaps the most widely cultivated food crop world over, but its production is constrained by diseases of fungal, bacterial and viral origin. Bacterial Leaf Blight (BLB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the oldest known diseases and was first noticed by the farmers of Japan in 1884. Subsequently, its incidence has been reported from different parts of Asia, northern Australia, Africa and USA (Mew *et al*., 1993; Awoderu *et al*., 1991; Sigee, 1993; Sere *et al*., 2005).

In Asia, BLB became a major disease after the introduction and widespread cultivation of high yielding but susceptible rice cultivars. Consequently, comprehensive studies on pathogen virulence diversity were undertaken, which provided useful information on the Xoo virulence population structure and resistance genes used in Asian breeding programs. However, little information is available on Xoo virulence population structure in Africa. This makes it very important to study the status of this bacterial disease in West African countries. Recent studies on BLB disease survey and samplings at different rice ecologies in Niger, Burkina Faso, Nigeria, Benin and Mali, revealed that BLB frequently occurred in farmers fields across these countries with incidence ranged from 70-85% and yield loss

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ranged from 50-90% (Sere *et al*., 2005). This indicates a wide spread of BLB in farmers fields across West African countries. Some selected Xoo isolates have shown high level of pathogenicity and virulence on the cultivated rice varieties (Sere *et al*., 2005). Research studies have also revealed that BLB is an important rice disease in irrigated rice ecosystems in West Africa.

Bacterial leaf blight is characterized by a high degree of race-cultivar specificity. There are over 30 reported races of isolates from several countries (Adhikari *et al*., 1999; Mew *et al*., 1993). A set of races identified in the Philippines using five differential rice cultivars (Mew *et al*., 1993) has been used widely for identifying and classifying resistance to BLB in other cultivars (Lee *et al*., 2003). It has been noted, however, that screening for resistance to pathogen populations specific to particular geographical locations and tailoring regional breeding programmes accordingly are important (Mew *et al*., 1993). Xoo also has a high degree of genetic diversity among different isolates, based on Restriction Fragment Length Polymorphism (RFLP) and pathotype analyses of more than 300 strains from different parts of Asia, using a repetitive Insertion Sequence (IS) element as the RFLP probe (Adhikari *et al*., 1999). In the study, isolates formed five clusters, each with more than one pathotype. Some correlation of clusters with geographical distribution and specific pathotypes was observed, indicating that tailoring breeding programmes for specific regions isindeed a tenable approach to control, although there was also evidence of movement of strains among regions. However, the present study aimed at conducting pathotyping analysis of 50 *Xanthomonas oryzae* pv. *oryzae* (Xoo) isolates from seven West African countries against 18 rice cultivars in order to identify and characterize Xoo virulence. The characterization of Xoo virulent population structure in West Africa will provide wide useful information for selection and deployment of cultivars with durable resistance.

MATERIALS AND METHODS

Bacterial Isolates

Xanthomonas oryzae pv. *oryzae* (Xoo) isolates (Table 1) used in this study were obtained from Plant Pathology Unit, Africa Rice Center (WARDA), Cotonou, Benin Republic, where their identity had been confirmed by oxidative biochemical test.

Near-Isogenic Lines (NILs)

Fourteen near isogenic lines (NILs) (Table 2) used for Xoo pathotyping study were obtained from International Rice Research Institute (IRRI). These are rice NILs with known resistance gene to Bacterial Leaf Blight (BLB). Other varieties such as IR64 and PNA647F4-56 were included as susceptible check (SCK) to BLB while Gigante and TOG5681 (highly resistant to Rice yellow mottle virus) were also included to study their current BLB resistant status.

Experimental Design

Split plot design with 3 replications was used. Fifty Xoo isolates (Table 1) were used to screen 18 varieties (Table 2) inside the screenhouse at Africa Rice Center (WARDA), Cotonou, Benin Republic. The experiment was carried out between February to May 2007. Rice grains were first pregerminated in steriled petri dishes under sterile condition. One plastic pot per variety per isolate in three replications was used.

Fertilizer Application

At transplanting 1.0 g of NPK per pot was applied and at 21 days after transplanting 0.2 g of Urea per pot was applied.

Table 1: List of *Xanthomonas oryzae* pv. *oryzae* isolates used for the study

Isolates code	Host plant	Country
XN-1	D52-37	Niger
XN-2	D52-37	Niger
XN-3	IR15296829	Niger
XN-4	IR15296829	Niger
XN-5	WITA 8	Niger
XN-6	WITA 8	Niger
XB-7	Local	Benin
XB-8	Local	Benin
XB-9	Local	Benin
XB-10	Local	Benin
XB-11	Local	Benin
XNG-12	WITA9	Nigeria
XNG-13	WITA9	Nigeria
XNG-14	WITA 4	Nigeria
XNG-15	WITA 4	Nigeria
XNG-16	WITA 8	Nigeria
XBF-17	TS2	Burkina Faso
XBF-18	TS2	Burkina Faso
XBF-19	FKR14	Burkina Faso
XBF-20	FKR19	Burkina Faso
XBF-21	FKR14	Burkina Faso
XBF-22	Chinese	Burkina Faso
XM-23	Adventices	Mali
XM-24	Kogoni	Mali
XM-25	Kogoni	Mali
XM-26	Kogoni	Mali
XM-27	Kogoni	Mali
XM-28	Kogoni	Mali
XM-29	Jamajigi	Mali
XM-30	Nionoka	Mali
XG-31	Weed	Guinea
XG-32	Weed	Guinea
XG-33	Weed	Guinea
XG-34	Local	Guinea
XG-35	Local	Guinea
XG-36	Local	Guinea
XG-37	Local	Guinea
XG-38	Local	Guinea
XG-39	Local	Guinea
XG-40	Local	Guinea
XTG-41	Local	The Gambia
XTG-42	Local	The Gambia
XTG-43	Local	The Gambia
XTG-44	Local	The Gambia
XTG-45	Local	The Gambia
XTG-46	Local	The Gambia
XTG-47	Local	The Gambia
XTG-48	Local	The Gambia
XTG-49	Weed	The Gambia
XTG-50	Weed	The Gambia

Isolates Inoculation

Fifty Xoo isolates of 20 μL each from stock culture were grown in Glucose Yeast Extract (GYE) liquid medium at 28°C to promote accelerated growth at incubation time of 36 h to obtain most active and effective bacterial cells (Sere *et al*., 2005). Inoculum was prepared by suspending the bacterial cells in sterile distilled water and adjusted to a concentration of 10^9 cfu mL^{-1} (OD_{650} = 0.5) prior to inoculation. Inoculation was by clipping method (Sere *et al*., 2005). The whole leaves of each plant in each plastic pot were clip inoculated 21 days after sowing.

Measurement of Parameters

Temperature and relative humidity within the screen house were measured by thermohygrometer throughout the experiment period. At 14 days after inoculation, lesion length and total leaf length from the cut leaf tip were measured in centimeter (Sere *et al*., 2005). From the collected lesion length and total leaf length data, percentage lesion length was estimated. BLB disease reaction was categorized according to percentage lesion length.

Data Analysis

Using the percentage lesion length data, Analysis of Variance (ANOVA), genotype by environment (GxE) interaction and additive main effect and multiplicative interaction (AMMI) analyses were conducted using IRRISTAT software to identify different Xoo pathotypes and virulence groups (Ebdon and Gauch, 2002; Bruckner and Slanger, 1986; Aleong and Howard, 1985; Xiaoping and Ognjen, 2005).

RESULTS AND DISCUSSION

Considerable diversity was observed in the reactions of 50 Xoo isolates to 18 rice cultivars in terms of percentage lesion length due to Bacterial Leaf Blight (BLB) disease. Analysis of Variance (ANOVA) for percentage lesion length due to BLB disease caused by inoculated Xoo isolates from seven West African countries revealed significant strong interaction, between Xoo isolates and rice cultivars and between isolates country of origin and rice cultivars (Table 3). Means percentage lesion length were significantly different

Table 2: List of near isogenic lines (NILs) and other varieties used for *Xoo* pathotyping

NILs	Resistance genes
IRBB1	*Xa-1*
IRBB2	*Xa-2*
IRBB3	*Xa-3*
IRBB4	*Xa-4*
IRBB5	*xa-5*
IRBB7	*Xa-7*
IRBB8	*Xa-8*
IRBB10	*Xa-10*
IRBB11	*Xa-11*
IRBB13	*Xa-13*
IRBB14	*Xa-14*
IRBB21	*Xa-21*
IRBB53	-
IRBB59	*xa-5, xa-13, Xa-21*
IR24	SCK
PNA647F4-56	SCK
Gigante	-
TOG5681	-

SCK = Susceptible check

Table 3: Analysis of variance for percentage lesion length due to BLB diseases caused by inoculated *Xoo* isolates from different West African countries

Source	DF	SS	Mean square	F-value
Rep	2	47049.1	47049.095	72.61**
Variety (V)	17	125897.8	7405.753	11.43**
Isolate (I)	49	175206.2	4074.562	6.29**
Country (C)	6	74842.97	12473.828	19.25**
V*I	833	1418353	1940.292	2.99**
V*C	102	225909	2214.794	3.42**
Error	907	582524.5	647.969	
Total	1916	2649783		

** = Significant at 1% level

both for BLB disease caused by Xoo isolates of the same and different country of origin (Table 4, 5). Percentage lesion length relative to Xoo isolate country of origin was between 8.2-23.6% (Niger), 6.3-26.6% (Benin Republic), 9.3-28.3% (Nigeria), 12.2-53.6% (Burkina Faso),14.4-31.4% (Mali), 17.6-46.2% (Guinea) and 10.8-57.9% (The Gambia) (Table 4, 5). Xoo

Table 4: Analysis of means comparison for percentage lesion length due to BLB disease caused by inoculated *Xoo* isolates

		Variety (V)								
Isolate (I)	Origin	V1	V2	V3	V4	V5	V6	V7	V8	V9
XN-1	C1	37.26bc	82.15ab	60.81a-d	55.65ab	99.50a	3.63cd	50.84ab	5.93cd	3.95b
XN-2		1.36c	2.92c	2.00d	2.60b	2.63c	150.00a	2.46b	2.31d	3.95b
XN-3		2.49c	3.19c	2.45d	1.37b	99.50a	3.98cd	92.85a	2.37d	99.50a
XN-4		90.00ab	49.09abc	1.24d	2.75b	15.46bc	10.72cd	1.10b	2.48d	2.96b
XN-5		2.68c	1.51c	3.95d	1.63b	51.43abc	2.16d	99.50a	3.88cd	10.17b
XN-6		2.07c	5.80c	37.46bcd	2.50b	4.78bc	4.94cd	0.89b	0.67d	0.50b
XB-7	C2	1.92c	8.66c	16.68cd	3.09b	1.31c	1.24d	5.71b	1.20d	2.46b
XB-8		1.75c	1.88c	1.61d	5.95b	2.85c	2.88d	2.49b	2.66cd	2.84b
XB-9		2.62c	27.00bc	6.38d	15.21b	4.34bc	8.52cd	12.43b	6.52cd	20.94b
XB-10		51.43abc	2.50c	22.59cd	4.60b	17.39bc	2.90d	2.22b	75.00ab	2.67b
XB-11		1.78c	2.13c	3.95d	3.02b	52.29abc	52.00bcd	3.28b	99.50a	2.68b
XNG-12	C3	2.61c	2.24c	2.36d	5.73b	99.50a	1.95d	1.64b	11.60bcd	1.97b
XNG-13		1.24c	75.00ab	3.92d	8.22b	15.07bc	3.97cd	16.46b	28.51bcd	2.84b
XNG-14		1.83c	4.09c	8.60d	9.15b	99.50a	3.11d	6.67b	2.77cd	99.50a
XNG-15		38.55bc	2.88c	3.27d	3.57b	25.56bc	2.90d	43.85ab	52.00a-d	52.00ab
XNG-16		9.88c	3.20c	3.00d	3.25b	99.50a	2.96d	1.69b	0.77d	7.90b
XBF-17	C4	5.00c	6.31c	2.61d	5.32b	2.69c	2.00d	2.14b	3.04cd	52.00ab
XBF-18		5.00c	2.96c	2.96d	53.19ab	3.00c	14.30cd	55.49ab	3.02cd	99.50a
XBF-19		2.71c	2.84c	3.11d	5.23b	3.92c	97.28ab	2.72b	10.07cd	52.00ab
XBF-20		5.59c	3.80c	75.81abc	2.13b	99.50a	20.92cd	50.96ab	67.35abc	52.00ab
XBF-21		99.50a	94.00a	56.42a-d	99.50a	24.74bc	4.35cd	12.48b	99.50a	4.22b
XBF-22		2.82c	13.95c	2.02d	5.62b	61.00abc	99.50ab	3.73b	3.95cd	19.74b
XM-23	C5	8.60c	11.72c	8.65d	10.25b	38.06bc	1.46d	99.50a	50.68a-d	52.00ab
XM-24		13.46c	6.47c	1.33d	9.54b	2.35c	4.30cd	2.91b	20.56bcd	2.91b
XM-25		65.09abc	2.84c	3.82d	99.50a	12.66bc	2.61d	1.45b	52.40a-d	5.90b
XM-26		8.85c	5.50c	51.67a-d	0.77b	2.99c	4.33cd	1.81b	3.50cd	2.64b
XM-27		2.66c	6.00c	8.51d	3.65b	3.52c	10.34cd	5.02b	52.00a-d	1.69b
XM-28		2.80c	80.00ab	2.61d	3.73b	3.95c	3.10d	7.07b	65.18a-d	4.09b
XM-29		11.81c	3.95c	3.04d	6.27b	2.84c	2.72d	3.62b	3.43cd	2.77b
XM-30		9.88c	2.74c	1.59d	1.37b	1.39c	2.81d	4.50b	2.77cd	4.50b
XG-31	C6	1.34c	41.25abc	1.78d	5.47b	54.55abc	53.68bcd	4.94b	19.21bcd	99.50a
XG-32		99.50a	94.00a	86.11ab	6.92b	99.50a	4.89cd	4.50b	6.93cd	52.23ab
XG-33		99.50a	99.50a	3.99d	5.21b	68.75ab	3.50cd	52.00ab	52.00a-d	64.93ab
XG-34		18.07c	3.84c	1.88d	16.29b	12.88bc	2.72d	4.50b	8.06cd	2.25b
XG-35		2.97c	5.86c	99.50a	99.50a	12.91bc	13.73cd	5.17b	37.64bcd	3.07b
XG-36		1.70c	80.00ab	99.50a	8.23b	99.50a	4.00cd	5.34b	2.67cd	52.00ab
XG-37		4.44c	6.50c	5.45d	32.69b	3.95c	13.38cd	5.67b	2.67cd	12.00b
XG-38		2.91c	90.00a	2.81d	3.27b	99.50a	53.34bcd	54.82ab	3.00cd	52.00ab
XG-39		3.25c	99.50a	3.95d	13.10b	13.56bc	3.95cd	104.17a	3.95cd	3.95b
XG-40		52.50abc	90.00a	1.42d	7.27b	8.59bc	3.07d	99.50a	4.63cd	4.04b
XTG-41	C7	3.54c	7.36c	54.38a-d	53.34ab	99.50a	99.50ab	99.50a	2.96cd	105.00a
XTG-42		1.77c	99.50a	3.19d	52.00ab	99.50a	3.95cd	62.00ab	2.36d	52.00ab
XTG-43		1.63c	2.69c	1.83d	3.82b	99.50a	12.72cd	99.50a	51.79a-d	24.92b
XTG-44		2.50c	9.00c	4.44d	14.67b	99.50a	6.58cd	1.49b	24.95bcd	5.42b
XTG-45		13.97c	2.66c	1.40d	56.25ab	5.20bc	2.95d	17.78b	1.49d	3.50b
XTG-46		2.91c	53.43abc	63.54a-d	4.07b	5.36bc	99.50ab	8.00b	3.45cd	99.50a
XTG-47		99.50a	94.00a	99.50a	62.50ab	52.00abc	52.00bcd	97.68a	16.58bcd	99.50a
XTG-48		99.50a	90.00a	3.25d	2.37b	8.15bc	68.18bc	99.50a	62.73a-d	99.50a
XTG-49		3.26c	4.89c	3.02d	16.22b	4.24bc	53.69bcd	4.48b	11.87bcd	4.50b
XTG-50		1.81c	1.27c	2.05d	52.73ab	3.23c	3.95cd	2.82b	4.55cd	5.78b
V-Mean		20.19	29.85	18.95	19	38.06	21.74	28.61	21.18	30.37

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.Country: C1 = Niger; C2 = Benin Republic; C3 = Nigeria; C4 = Burkina Faso; C5 = Mali; C6 = Guinea; C7 = The Gambia. Variety: V1 = IRBB1; V2 = IRBB2; V3 = IRBB3; V4 = IRBB4; V5 = IRBB5: V6 = IRBB7; V7 = IRBB8; V8 = IRBB10; V9 = IRBB11

isolates from The Gambia produced the highest percentage lesion length of 57.9%, followed by those from Burkina Faso (53.6%), Guinea (46.2%), Mali (31.4%), Nigeria (28.3%), Benin Republic (26.6%) and Niger (23.6%) (Tables 4, 5). According to mean percentage lesion length by isolates country of origin, Burkina Faso produced the highest mean percentage

Table 5: Analysis of means comparison for percentage lesion length due to BLB disease caused by inoculated *Xoo* isolates

		Variety (V)									
Isolate (I)	Origin	V10	V11	V12	V13	V14	V15	V16	V17	V18	I-Mean
XN-1	C1	4.00b	2.00c	5.76d	2.00c	1.24b	3.60c	1.08b	4.00b	2.00b	23.63
XN-2		1.17b	2.96bc	34.54cd	2.02c	4.12b	1.50c	100.00a	2.35b	62.09ab	21.16
XN-3		3.09b	52.00abc	3.95d	3.25c	3.62b	2.66c	3.57b	1.55b	2.00b	21.3
XN-4		3.95b	2.64bc	3.95d	0.67c	2.46b	7.53c	1.09b	2.37b	53.00ab	14.08
XN-5		8.12b	8.87bc	3.18d	5.03c	52.00ab	2.65c	2.43b	2.20b	58.34ab	17.76
XN-6		3.95b	1.01c	2.90d	2.53c	0.68b	2.48c	41.36ab	3.95b	29.15b	8.2
XB-7	C2	2.39b	0.72c	59.23bcd	0.50c	2.81b	0.41c	1.31b	2.50b	2.00b	6.34
XB-8		52.00ab	3.95bc	4.22d	3.95c	17.61b	6.30c	21.10b	52.00ab	99.50a	15.86
XB-9		5.85b	12.65bc	0.40d	3.95c	6.88b	2.72c	3.06b	99.50a	99.50a	18.8
XB-10		2.77b	3.09bc	3.55d	3.95c	10.56b	2.72c	99.50a	4.36b	63.34ab	20.84
XB-11		3.57b	20.35bc	99.50ab	8.25c	3.95b	15.46bc	97.68a	5.93b	2.76b	26.56
XNG-12	C3	52.00ab	1.41c	2.14d	2.80c	51.24ab	5.80c	4.73b	51.98ab	55.00ab	19.81
XNG-13		7.00b	1.38c	63.73bcd	5.47c	1.02b	20.34bc	2.72b	99.50a	55.56ab	22.88
XNG-14		3.65b	3.64bc	44.89bcd	3.09c	3.39b	99.50a	11.70b	52.18ab	52.00ab	28.29
XNG-15		3.95b	2.96bc	80.77abc	4.68c	4.38b	13.79bc	7.21b	5.44b	55.17ab	22.38
XNG-16		8.30b	1.06c	4.00d	3.47c	2.39b	6.41c	2.36b	3.98b	2.84b	9.27
XBF-17	C4	5.28b	1.30c	4.37d	12.00bc	3.59b	50.66abc	5.16b	4.92b	52.00ab	12.24
XBF-18		15.54b	11.01bc	99.50ab	4.50c	2.93b	94.50a	99.50a	52.15ab	52.00ab	37.28
XBF-19		3.95b	2.98bc	4.18d	70.18ab	1.67b	2.90c	99.50a	59.95ab	99.50a	29.15
XBF-20		8.00b	58.34abc	99.50ab	99.50a	52.00ab	62.50abc	99.50a	56.06ab	52.00ab	53.64
XBF-21		4.83b	19.17bc	99.50ab	3.95c	1.91b	3.54c	99.50a	6.94b	52.00ab	43.67
XBF-22		99.50a	1.44c	99.50ab	2.61c	1.87b	13.49bc	12.87b	99.50a	52.00ab	33.06
XM-23	C5	4.73b	50.43abc	3.21d	55.82abc	50.47ab	2.73c	12.63b	5.36b	99.50a	31.43
XM-24		1.67b	2.96bc	21.32cd	2.77c	99.50a	4.22c	2.36b	2.53b	60.00ab	14.51
XM-25		7.09b	3.09bc	54.72bcd	2.91c	11.97b	2.44c	7.20b	99.50a	52.00ab	27.06
XM-26		2.46b	3.02bc	1.76d	52.00abc	4.86b	3.41c	5.75b	52.00ab	66.24ab	15.2
XM-27		2.65b	3.79bc	4.73d	50.82abc	8.32b	2.81c	3.50b	99.50a	25.26b	16.37
XM-28		51.37ab	2.77bc	2.86d	2.72c	3.17b	2.61c	12.59b	4.38b	4.38b	14.41
XM-29		16.43b	50.50abc	3.95d	3.82c	8.82b	99.50a	3.31b	3.95b	52.00ab	15.71
XM-30		3.11b	3.95bc	4.33d	3.95c	99.50a	99.50a	99.50a	17.82b	18.83b	21.22
XG-31	C6	2.35b	52.00abc	99.50ab	5.00c	99.50a	5.55c	5.67b	52.00ab	52.00ab	36.4
XG-32		99.50a	2.84bc	51.00bcd	3.82c	99.50a	9.69c	6.12b	52.00ab	52.00ab	46.17
XG-33		99.50a	99.50a	99.50ab	3.11c	1.02b	52.00abc	11.50b	3.95b	2.91b	45.69
XG-34		8.16b	1.38c	99.50ab	5.40c	3.54b	52.91abc	17.46b	6.32b	52.00ab	17.62
XG-35		12.00b	52.00abc	6.55d	52.00abc	57.86ab	90.24a	99.50a	54.70ab	52.00ab	42.06
XG-36		8.43b	8.16bc	52.00bcd	3.95c	6.45b	73.50ab	13.54b	2.72b	52.00ab	31.87
XG-37		17.00b	4.09bc	3.25d	3.43c	52.00ab	4.15c	99.50a	99.50a	52.00ab	23.42
XG-38		5.00b	2.21bc	5.62d	12.21bc	3.85b	7.16c	20.89b	6.55b	5.92b	23.95
XG-39		3.95b	3.66bc	6.65d	4.31c	3.95b	3.54c	2.59b	99.50a	99.50a	26.5
XG-40		2.78b	2.91bc	9.34d	5.00c	62.10ab	99.50a	3.19b	15.94b	50.00ab	28.99
XTG-41	C7	2.22b	8.66bc	32.52cd	2.80c	11.66b	2.69c	3.43b	63.02ab	53.01ab	39.17
XTG-42		99.50a	59.23abc	3.68d	7.47c	99.50a	13.96bc	8.87b	10.09b	6.48b	38.06
XTG-43		59.30ab	2.84bc	7.00d	5.00c	1.94b	2.91c	15.79b	7.00b	52.00ab	25.12
XTG-44		52.82ab	3.85bc	4.28d	3.89c	2.76b	2.46c	4.91b	52.00ab	52.00ab	19.3
XTG-45		51.50ab	5.98bc	3.67d	4.94c	2.65b	2.13c	2.55b	10.89b	99.50a	16.05
XTG-46		5.34b	11.91bc	4.78d	57.78abc	2.50b	4.50c	58.08ab	4.31b	52.00ab	30.05
XTG-47		2.70b	54.55abc	99.50ab	4.94c	99.50a	2.87c	96.66a	4.78b	2.77b	57.86
XTG-48		4.83b	2.30bc	1.20d	3.50c	5.92b	2.11c	7.98b	5.75b	99.41a	37.01
XTG-49		3.82b	1.39c	8.51d	3.59c	1.74b	51.54abc	3.95b	9.14b	4.50b	10.8
XTG-50		4.50b	66.85ab	130.72a	17.37bc	4.86b	3.63c	7.72b	2.67b	52.00ab	20.47
V-Mean		18.75	15.63	32.98	12.65	22.83	22.51	29.07	30.42	47.52	25.57

In a column, means followed by a common letter are not significantly different at the 5 % level by Duncan's Multiple Range Test. Country: C1 = Niger; C2 = Benin Republic; C3 = Nigeria; C4 = Burkina Faso; C5 = Mali; C6 = Guinea; C7 = The Gambia. Variety: V10 = IRBB13; V11 = IRBB14; V12 = IRBB21; V13 = IRBB53; V14 = IRBB59; V15 = IR24: V16 = PNA647F4-56; V17 = Gigante; V18 = TOG5681

lesion length of 34.4%, followed by Guinea (31.9%), The Gambia (28.3%), Nigeria (20.5%), Mali (19.5%), Niger (17.7%) and Benin Republic (17.1%) (Table 5; Fig. 1). Xoo isolates that produced percentage lesion length greater than 15% were considered virulence (Table 6). Xoo isolates from Burkina Faso have the highest percentage virulence of 45.4%, followed by Guinea (42.2%), The Gambia (38.3%), Nigeria (30.0%), Benin Republic (27.8%), Mali (26.4%) and Niger (23.1%) (Table 7). At the level of individual isolate, all the 50 Xoo isolates were virulence with the exception of 5 isolates (XN-6, XB-7, XNG-16, XBF-17 and XTG-49) which were less virulence (Fig. 2).

According to additive main effects and multiplicate interaction (AMMI) analysis, all the Xoo isolates were responsible mainly for unfavourable interactive conditions leading to significant increase in percentage lesion length in all the rice cultivars (Fig. 3, 4 and 5). Xoo virulence and interactive conditions were similar among isolates from Guinea and Burkina Faso, Mali and Nigeria, Benin and Niger with the exception of the Gambia which was distinct (Fig. 4, 5). Based on cluster dendrogram classification of Xoo isolates virulence, two major Xoo pathotypes (*Pta* and *Ptb*) were revealed (Fig. 6). *Pta* pathotype was made up of 29 virulence (*Vr*) Xoo isolates while *Ptb* pathotype constituted 21 mildly virulence (*MVr*) Xoo isolates (Fig. 6). *Pta* pathotype was further divided into three subgroup pathotypes (*Pta*1,

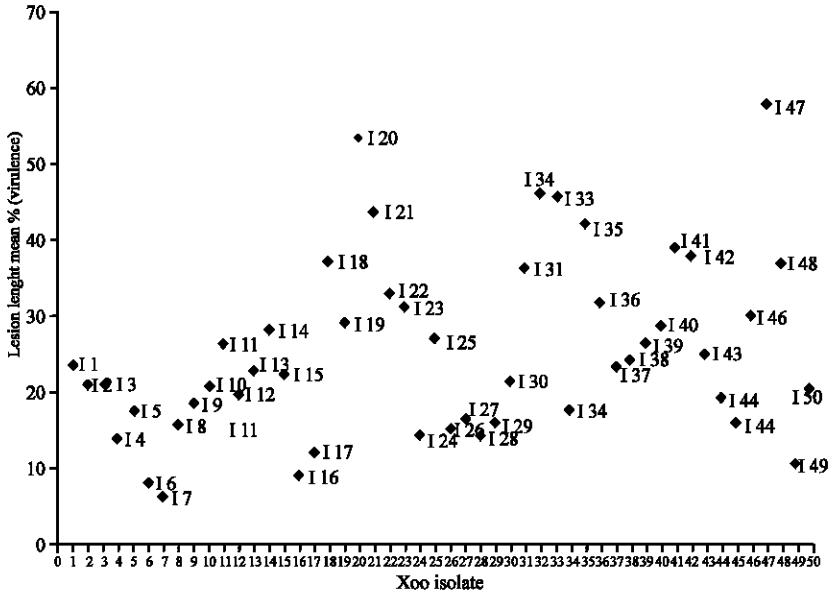

Fig. 1: Virulence status of 50 Xoo isolates against 18 rice varieties. Isolate: I1 = XN-1; I2 = XN-2; I3 = XN-3; I4 = XN-4; I5 = XN-5; I6 = XN-6; I7 = XB-7; I8 = XB-8; I9 = XB-9; I10 = XB-10; I11 = XB-11; I12 = XNG-12; I13 = XNG-13; I14 = XNG-14; I15 = XNG-15; I16 = XNG-16; I17 = XBF-17; I18 = XBF-18; I19 = XBF-19; I20 = XBF-20; I21 = XBF-21; I22 = XBF-22; I23 = XM-23; I24 = XM-24; I25 = XM-25; I26 = XM-26; I27 = XM-27; I28 = XM-28; I29 = XM-29; I30 = XM-30; I31 = XG-31; I32 = XG-32; I33 = XG-33; I34 = XG-34; I35 = XG-35; I36 = XG-36; I37 = XG-37; I38 = XG-38; I39 = XG-39; I40 = XG-40; I41 = XTG-41; I42 = XTG-42; I43 = XTG-43; I44 = XTG-44; I45 = XTG-45; I46 = XTG-46; I47 = XTG-47; I48 = XTG-48; I49 = XTG-49; I50 = XTG-50

Table 6: Analysis of means comparison for percentage lesion length due to BLB disease relative to inoculated *Xoo* isolates country of origin

Variety		Country (C)							
(V)	Resistance gene	C1 (N=6)	C2 (N=5)	C3 (N=5)	C4 (N=6)	C5 (N=8)	C6 (N=10)	C7 (N=10)	V-Mean
V1	*Xa-1*	22.65abc	11.90cd	10.82de	20.11cde	15.39bc	28.62b-e	23.04a-d	18.93
V2	*Xa-2*	24.11abc	8.34d	17.52b-e	20.65cde	14.90bc	61.04a	36.48a-d	26.15
V3	*Xa-3*	17.98abc	10.14cd	4.23de	23.82cde	10.15bc	30.64b-e	23.66a-d	17.23
V4	*Xa-4*	11.08bc	6.37d	5.98de	28.50cde	16.88bc	19.80b-e	31.80a-d	17.2
V5	*Xa-5*	45.55a	15.63cd	67.83a	32.47cde	8.47bc	47.37ab	47.62ab	37.85
V6	*Xa-7*	29.24abc	13.51cd	2.98e	39.72bcd	6.21c	15.63cde	40.30abc	21.08
V7	*Xa-8*	41.27a	5.23d	14.06cde	21.25cde	15.74bc	34.06a-e	49.28ab	25.84
V8	*Xa-10*	2.94c	36.98abc	19.13b-e	31.15cde	31.31abc	14.08de	17.82cd	21.91
V9	*Xa-11*	20.17abc	6.32d	32.84bcd	46.58abc	9.56bc	34.60a-e	49.96a	28.57
V10	*Xa-13*	4.05c	13.32cd	14.98cde	22.85cde	11.19bc	25.87b-e	28.65a-d	17.27
V11	*Xa-14*	11.58bc	8.15d	2.09e	15.71de	15.06bc	22.88b-e	15.57cd	13
V12	*Xa-21*	9.05bc	23.69bcd	39.11bc	67.76a	12.11bc	43.29abc	16.22cd	30.17
V13	?	2.58c	4.12d	3.90e	32.12cde	21.85abc	9.82e	11.13d	12.22
V14	*xa-5,xa-13,Xa-21*	10.68bc	8.36d	12.49cde	10.66e	35.83ab	38.94a-d	23.31a-d	20.04
V15	SCK	3.43c	4.52d	29.17b-e	30.67cde	24.66abc	32.75b-e	8.88d	19.15
V16	SCK	24.92abc	44.49ab	5.75de	69.34a	18.35bc	27.99b-e	20.99bcd	30.26
V17	?	2.74c	32.86a-d	42.61b	46.59abc	35.63abc	39.32a-d	16.97cd	30.96
V18	?	34.43ab	53.42a	44.11ab	59.92ab	47.28a	47.04ab	47.36ab	47.65
C-Mean		17.69	17.07	20.53	34.44	19.47	31.87	28.28	24.19

In a column, means followed by a common letter are not significantly different at the 5 % level by Duncan's Multiple Range Test. SCK = Susceptible check. N = Number of Xoo isolates. Country: C1 = Niger; C2 = Benin Republic; C3 = Nigeria; C4 = Burkina Faso; C5 = Mali; C6 = Guinea; C7 = The Gambia. Variety: V1 = IRBB1; V2 = IRBB2; V3 = IRBB3; V4 = IRBB4; V5 = IRBB5: V6 = IRBB7; V7 = IRBB8; V8 = IRBB10; V9 = IRBB11; V10 = IRBB13; V11 = IRBB14; V12 = IRBB21; V13 = IRBB53; V14 = IRBB59; V15 = IR24: V16 = PNA647F4-56; V17 = Gigante; V18 = TOG5681

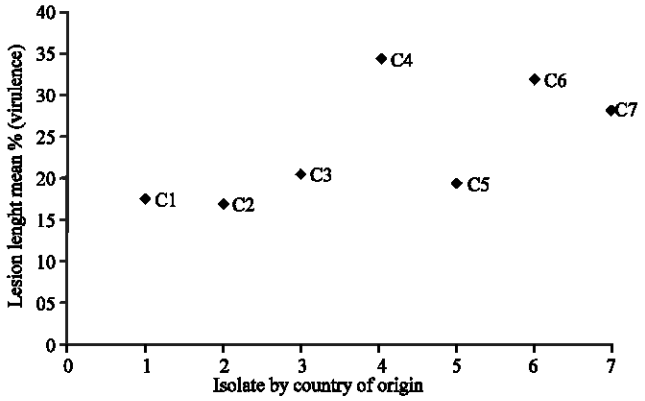

Fig. 2: Virulence status of Xoo isolates relative to country of origin. Country: C1 = Niger; C2 = Benin Republic; C3 = Nigeria; C4 = Burkina Faso; C5 = Mali; C6 = Guinea; C7 = The Gambia

*Pta*2 and *Pta*3) and *Ptb* pathotype into two subgroup pathotypes (*Ptb*1 and *Ptb*2). *Pta*1 was made up of 10 isolates (XTG-47, XBF-20, XBF-21, XG-35, XG-33, XG-32, XBF-18, XM-23, XBF-22 and XG-31), *Pta*2 was 8 isolates (XG-36, XTG-46, XTG-41, XTG-42, XTG-48, XN-1, XN-2 and XN-3), *Pta*3 was 11 isolates (XB-10, XNG-13, XB-11, XNG-15, XNG-14, XBF-19, XM-25, XM-30, XG-40, XG-39 and XG-38), *Ptb*1 was 10 isolates (XG-37, XTG-43, XTG-50,

Table 7: Xoo virulence and variety resistance status

			Variety (V)																
Isolate	Origin	Vr (%)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18
XN-1	C1	23.1	S	S	S	S	S	R	S	R	R	R	R	R	R	R	R	R	R	R
XN-2			R	R	R	R	R	S	R	R	R	R	R	S	R	R	R	S	R	S
XN-3			R	R	R	R	S	R	S	R	S	R	S	R	R	R	R	R	R	R
XN-4			S	S	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S
XN-5			R	R	R	R	S	R	S	R	R	R	R	R	R	S	R	R	R	S
XN-6			R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S
XB-7	C2	27.8	R	R	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
XB-8			R	R	R	R	R	R	R	R	R	S	R	R	R	S	R	S	S	S
XB-9			R	S	R	S	R	R	R	R	S	R	R	R	R	R	R	R	S	S
XB-10			S	R	S	R	S	R	R	S	R	R	R	R	R	R	R	S	R	S
XB-11			R	R	R	R	S	S	R	S	R	R	S	S	R	R	S	S	R	R
XNG-12	C3	30.0	R	R	R	R	S	R	R	R	R	S	R	R	R	S	R	R	S	S
XNG-13			R	S	R	R	S	R	S	S	R	R	R	S	R	R	S	R	S	S
XNG-14			R	R	R	R	S	R	R	R	S	R	R	S	R	R	S	R	S	S
XNG-15			S	R	R	R	S	R	S	S	S	R	R	S	R	R	R	R	R	S
XNG-16			R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
XBF-17	C4	45.4	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	R	R	S
XBF-18			R	R	R	S	R	R	S	R	S	S	R	S	R	R	S	S	S	S
XBF-19			R	R	R	R	S	R	R	R	S	R	R	R	S	R	R	R	S	S
XBF-20			R	R	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	S
XBF-21			S	S	S	S	S	R	R	S	R	R	S	S	R	R	R	S	R	S
XBF-22			R	R	R	R	S	S	R	R	S	S	R	S	R	R	R	R	S	S
XM-23	C5	26.4	R	R	R	R	S	R	S	S	S	R	S	R	S	S	R	R	R	S
XM-24			R	R	R	R	R	R	R	S	R	R	R	S	R	S	R	R	R	S
XM-25			S	R	R	S	R	R	R	S	R	R	R	S	R	R	R	R	S	S
XM-26			R	R	S	R	R	R	R	R	R	R	R	R	S	R	R	R	S	S
XM-27			R	R	R	R	R	R	R	S	R	R	R	R	S	R	R	R	S	S
XM-28			R	S	R	R	R	R	R	S	R	S	R	R	R	R	R	R	R	R
XM-29			R	R	R	R	R	R	R	R	R	S	S	R	R	R	S	R	R	S
XM-30			R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
XG-31	C6	42.2	R	S	R	R	S	S	R	S	S	R	S	S	R	S	R	R	S	S
XG-32			S	S	S	R	S	R	R	R	S	S	R	S	R	S	R	R	S	S
XG-33			S	S	R	R	S	R	S	S	S	S	S	S	R	R	S	R	R	R
XG-34			S	R	R	S	R	R	R	R	R	R	R	S	R	R	S	S	R	S
XG-35			R	R	S	S	R	R	R	S	R	R	S	R	S	S	S	S	S	S
XG-36			R	S	S	R	S	R	R	R	S	R	R	S	R	R	S	R	R	S
XG-37			R	R	R	S	R	R	R	R	R	S	R	R	R	S	R	S	S	S
XG-38			R	S	R	R	S	S	S	R	S	R	R	R	R	R	R	S	R	R
XG-39			R	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	S	S
XG-40			S	S	R	R	R	R	S	R	R	R	R	R	R	S	S	R	S	S
XTG-41	C7	38.3	R	R	S	S	S	S	S	R	S	R	R	S	R	R	R	R	S	S
XTG-42			R	S	R	S	S	R	S	R	S	S	S	R	R	S	R	R	R	R
XTG-43			R	R	R	R	S	R	S	S	S	S	R	R	R	R	R	S	R	S
XTG-44			R	R	R	S	S	R	R	S	R	S	R	R	R	R	R	R	S	S
XTG-45			R	R	R	S	R	R	S	R	R	S	R	R	R	R	R	R	R	S
XTG-46			R	S	S	R	R	S	R	R	S	R	R	R	S	R	R	S	R	S
XTG-47			S	S	S	S	S	S	S	S	S	R	S	S	R	S	R	S	R	R
XTG-48			S	S	R	R	R	S	S	S	S	R	R	R	R	R	R	R	R	S
XTG-49			R	R	R	S	R	S	R	R	R	R	R	R	R	R	S	R	R	R
XTG-50			R	R	R	S	R	R	R	R	R	R	S	S	S	R	R	R	R	S

Country: C1 = Niger; C2 = Benin Republic; C3 = Nigeria; C4 = Burkina Faso; C5 = Mali; C6 = Guinea; C7 = The Gambia. Variety: V1 = IRBB1; V2 = IRBB2; V3 = IRBB3; V4 = IRBB4; V5 = IRBB5: V6 = IRBB7; V7 = IRBB8; V8 = IRBB10; V9 = IRBB11; V10 = IRBB13; V11 = IRBB14; V12 = IRBB21; V13 = IRBB53; V14 = IRBB59; V15 = IR24: V16 = PNA647F4-56; V17 = Gigante; V18 = TOG5681.R = Resistance (% lesion length less than 15 %); S = Susceptible (% lesion length greater than 15%); Vr = Virulence

XN-5, XN-4, XB-8, XB-9, XNG-12, XBF-17 and XM-24) and *Ptb*2 was 11 isolates (XM-26, XM-27, XM-28, XM-29, XG-34, XTG-44, XTG-45, XTG-49, XN-6, XB-7 and XNG-16) (Fig. 6).

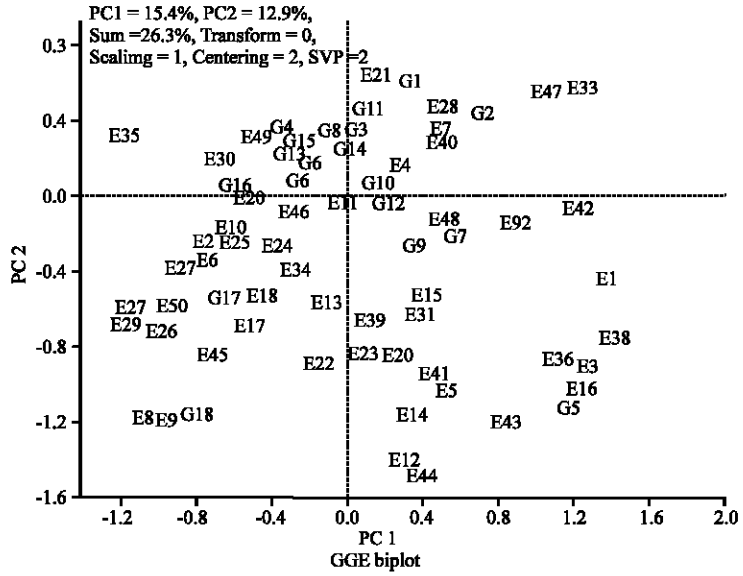

Fig. 3: Genotype (cultivar) by environment (isolate) interaction effects on percentage lesion length using additive main effects and multiplicate interaction (AMMI) analysis. Genotype (G): G1 = IRBB1; G2 = IRBB2; G3 = IRBB3; G4 = IRBB4; G5 = IRBB5: G6 = IRBB7; G7 = IRBB8; G8 = IRBB10; G9 = IRBB11; G10 = IRBB13; G11 = IRBB14; G12 = IRBB21; G13 = IRBB53; G14 = IRBB59; G15 = IR24: G16 = PNA647F4-56; G17 = Gigante; G18 = TOG5681. Environment (E): E1 = XN-1; E2 = XN-2; E3 = XN-3; E4 = XN-4; E5 = XN-5; E6 = XN-6; E7 = XB-7; E8 = XB-8; E9 = XB-9; E10 = XB-10; E11 = XB-11; E12 = XNG-12; E13 = XNG-13; E14 = XNG-14; E15 = XNG-15; E16 = XNG-16; E17 = XBF-17; E18 = XBF-18; E19 = XBF-19; E20 = XBF-20; E21 = XBF-21; E22 = XBF-22; E23 = XM-23; E24 = XM-24; E25 = XM-25; E26 = XM-26; E27 = XM-27; E28 = XM-28; E29 = XM-29; E30 = XM-30; E31 = XG-31; E32 = XG-32; E33 = XG-33; E34 = XG-34; E35 = XG-35; E36 = XG-36; E37 = XG-37; E38 = XG-38; E39 = XG-39; E40 = XG-40; E41 = XTG-41; E42 = XTG-42; E43 = XTG-43; E44 = XTG-44; E45 = XTG-45; E46 = XTG-46; E47 = XTG-47; E48 = XTG-48; E49 = XTG-49; E50 = XTG-50

The occurrence and distribution of Xoo pathotypes varied among isolates country of origin (Table 8). *Pta*1, a virulence (*Vr*) pathotype, was known to exist in four countries (Burkina Faso, Mali, Guinea and The Gambia) with 20% occurrence, *Pta*2 (*Vr*) has 16% occurrence in three countries (Niger, Guinea and The Gambia) and Pta3 (*Vr*) has 22% occurrence in five countries (Benin Republic, Nigeria, Burkina Faso, Mali and Guinea) (Table 8). *Ptb*1, a mildly virulence (*MVr*) pathotype, was known to exist in all the seven countries (Niger, Benin Republic, Nigeria, Burkina Faso, Mali, Guinea and The Gambia) with 20% occurrence and *Ptb*2 (*MVr*) has 16% occurrence in six countries (Niger, Benin Republic, Nigeria, Mali, Guinea and the Gambia) (Table 8). Thus, in Niger the study revealed the presence of *Pta*1, *Ptb*1 and *Ptb*2 Xoo pathotypes, in Benin and Nigeria (*Pta*3, *Ptb*1 and *Ptb*2), in Burkina Faso (*Pta*1, *Pta*3 and *Ptb*1), in Mali (*Pta*1, *Pta*3, *Ptb*1 and *Ptb*2), in Guinea (*Pta*1, *Pta*2, *Pta*3, *Ptb*1 and *Ptb*2) and in The Gambia (*Pta*1, *Pta*2, *Ptb*1 and *Ptb*2) (Table 8).

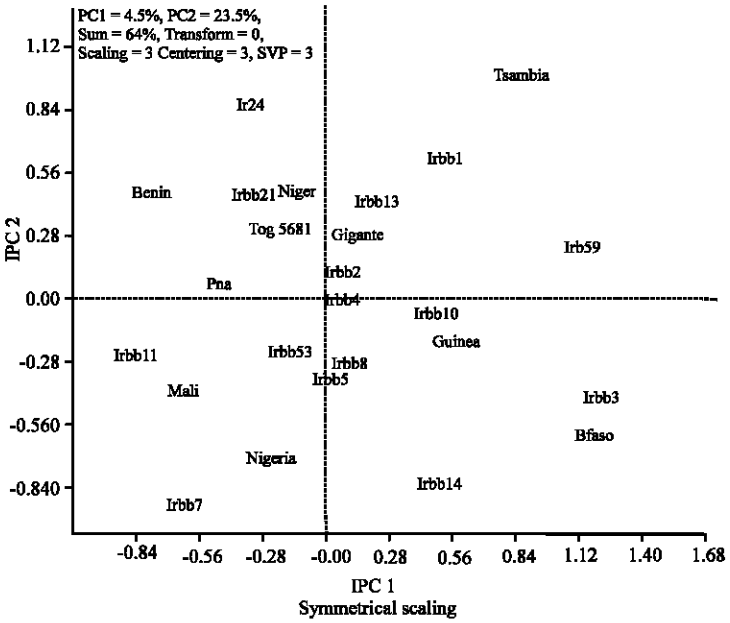

Fig. 4: Genotype (cultivar) by environment (isolate relative to country of origin) interaction effects on percentage lesion length using additive main effects and multiplicate interaction (AMMI) analysis

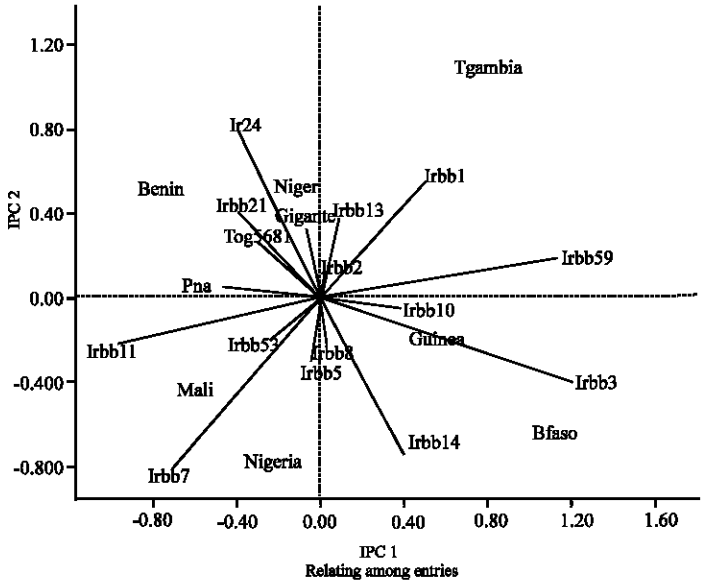

Fig. 5: Relationship among rice variety and Xoo isolate country of origin as revealed by genotype (cultivar) by environment interaction effects on percentage lesion length using additive main effects and multiplicate interaction (AMMI) analysis

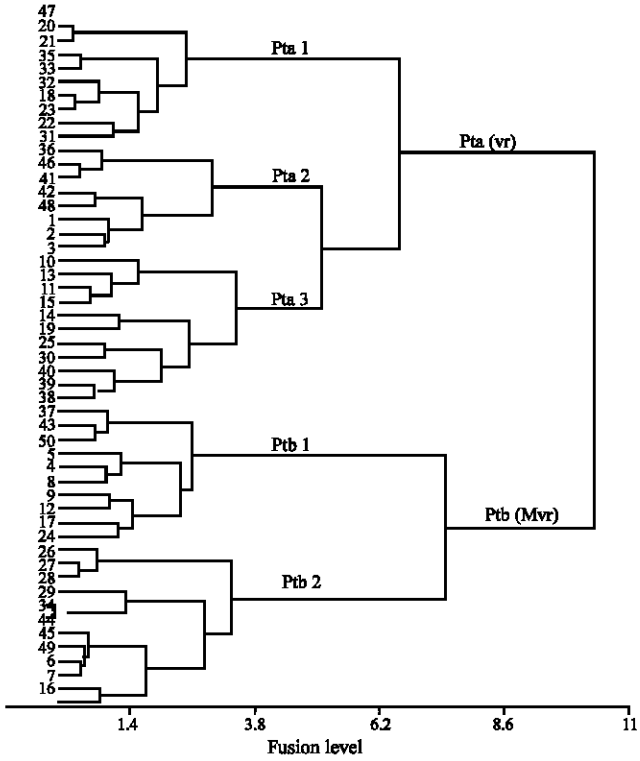

Fig. 6: Analysis of Xoo isolates virulence as revealed by pathotyping using Additive Main effects and Multiplicate Interaction (AMMI) analysis

Table 8: *Xoo* isolate group, virulence and distribution relative to country of origin

			Isolate origin and distribution							
Main group	Subgroup	Virulence	Niger	Benin	Nigeria	Burkina Faso	Mali	Guinea	The Gambia	Occurrence (%)
Pta	*Pta1*	*Vr*	-	-	-	4	1	4	1	20
	Pta2	*Vr*	3	-	-	-	-	1	4	16
	Pta3	*Vr*	-	2	3	1	2	3	-	22
Ptb	*Ptb1*	*MVr*	2	2	1	1	1	1	2	20
	Ptb2	*MVr*	1	1	1	-	4	1	3	22

Pta = Pathotype a; *Ptb* = Pathotype b; *Vr* = Virulence; *Mvr* = Mildly virulence

Information on the existing population structure of the pathogen in a region can be useful in the identification and characterization of useful resistant germplasm (Choi *et al*., 1998). For example, Nelson *et al*. (1994) used knowledge of the *Xoo* population structure in the Philippines to select representative strains for screening rice germ plasm collections for resistance. The Additive Main effect and Multiplicative Interaction (AMMI) analysis was shown to be effective in understanding complex Genotype by Environment (GE) interactions typical of National Turfgrass Evaluation Program (NTEP) variety trials (Ebdon and Gauch, 2002). Interactions in such complex data sets are difficult to understand with ordinary Analysis of Variance (ANOVA). Genotype by environment interaction can be defined as the differential response of varying genotypes under changes in the environment. AMMI analysis used in this study has revealed diversity and extent of 50 *Xoo*

isolates interaction among 18 rice cultivars that lead to the classification of Xoo isolates virulence into two major pathotypes (*Pta* and *Ptb*) that were responsible for unfavourable interactive conditions that lead to significant increase in percentage lesion length in all the rice cultivars. The existence of *Pta*1, *Pta*2 and *Pta*3 and *Ptb*1and *Ptb*2 subgroups pathotypes were likely due to mutations and interactions among isolates and strains that originally constituted *Pta* and *Ptb* pathotypes (Innes *et al*., 2001).

The movement of Xoo pathogens has important implications for the control of BLB disease. If migration is far reaching, the development and deployment of resistant germplasm would require knowledge about the structure and dynamics of distant, as well as local populations of the Xoo pathogen. Thus, it is important to understand pathogen migration and how it influences population genetic structure and potential for disease. Several examples of pathogen migration have been documented, including intercontinental movement of *Phytophthora infestans* (Fry *et al*., 1992) and *Puccinia graminis* (Burdon *et al*., 1982) and movement of Erysiphe graminis from continental Europe to England (Brown *et al*., 1991). In most cases, the arrival of immigrant genotypes has been associated with increased BLB disease problems. In the present study, *Pta* pathotype a virulence (*Vr*) type and *Ptb* pathotype a mildly virulence (*MVr*) type were known be to present in Mali, Nigeria, Benin, Burkina Faso, Niger, Guinea and the Gambia and Xoo virulence and interactive conditions were similar among isolates in these countries suggesting possible Xoo pathogen migration between these countries (Adhikari *et al*., 1995). However, such migration could be from germplasm exchange of contaminated seed among regional countries (Cheng *et al*., 1994). Since previous studies (Sere *et al*., 2005) were only conducted with Xoo isolates from Mali, the studies could not reveal Xoo pathogen migration and germplasm exchange of contaminated seeds across regional countries as revealed by the present study. The two major pathotypes (*Pta* and *Ptb*) of Xoo virulence obtained in this study has revealed information on Xoo virulence population structure in West Africa and this would provide wide useful information for selection and deployment of cultivars with durable resistance to BLB disease in West Africa.

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