Journal of Applied Biosciences 53: 3742 – 3748

ISSN 1997–5902

Screening of rice varieties for resistance to bacterial leaf blight

[1]Agnassim BANITO, [1]Essohouna Ago KADAI and [2]Yacouba SERE

[1]Ecole Supérieure d'Agronomie, Université de Lomé, BP 2348, Lomé, Togo

[2]Plant Pathologist – MICCORDEA Project Coordinator AfricaRice, Micocheni B/Kawe, Avocado Street P.O.Box 33581 Dar-es-Salaam, Tanzania

Corresponding Author: Agnassim Banito, PhD; Email: bagnassim@hotmail.com

Original submitted in on 5th April 2012. Published online at www.m.elewa.org on May 29th 2012.

ABSTRACT

Objectives: In order to find control measures against bacterial leaf blight (BLB), one of the most destructive diseases of rice, the present study aimed to evaluate rice varieties cultivated in Togo and some near isogenic lines from IRRI for resistance to BLB in screen house.

Methodology and Results: The lesions after inoculation with 13 *X. oryzae* pv. *oryzae* strains from Togo were evaluated on 21 rice varieties and near isogenic lines under screen house. The results revealed differential reactions of these genotypes in disease expression. AMMI analysis identified three groups of genotypes: resistant group made up of 12 lines IRBB1, IRBB2, IRBB3, IRBB4, IRBB7, IRBB8, IRBB10, IRBB11, IRBB13, IRBB14, IRBB21 and IR24, medium resistant group made up of three cultivars grown in Togo, NERICA4, NERICA8 and NERICA14, and one cultivar from AfricaRice, Giganté, and susceptible group including five genotypes TGR203 and IR841 from ITRA, NERICA19 and TOG5681 from AfricaRice, and the line IRBB5.

Conclusions and application of findings: The results provided useful information indicating that none of rice varieties grown in Togo was resistant to bacterial leaf blight, thus revealed a potential risk of epidemics in the growing areas since these varieties were only medium resistant (NERICA4, NERICA8 and NERICA14) to susceptible (TGR203, IR841 and NERICA19). Besides, the present results are prerequisite for further screening under field conditions across ecozones of Togo (forest zone, forest savanna transition zone, wet savanna zone and dry savanna zone) and years to find out stable resistant varieties for durable production of rice.

Keywords: Bacterial leaf blight of rice, strains, inoculation, host plant resistance.

INTRODUCTION

Bacterial leaf blight of rice (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Swings *et al*., 1990), is one of the most widespread and destructive diseases of rice in several countries in tropical rice-growing areas of Asia, Australia, United States, Latin America and Africa (Mew, 1987, 1989; Mew *et al*., 1993; Sere *et al*., 2005). BLB was observed to occur in fields with high incidence of 70 to 80% in several West African countries (Sere *et al*., 2005). Yield losses due to BLB ranging from 50 to 90% have been reported (Ou, 1985; Sere *et al*., 2005). Recent studies in West African countries such as Burkina Faso, Niger and Mali revealed the occurrence of BLB causing significant crop damages (Basso *et al*., 2011). Recent survey reported the occurrence of

bacterial leaf blight in most of rice-growing ecozones of Togo with high incidence and severity, and the virulence of the pathogen was determined (Kadai, 2010; Déwa *et al*., 2011). Studies of the resistance of rice genotypes have been undertaken (Sere *et al.,* 2005; Ouedraogo *et al*., 2007). However, investigation on the resistance of rice varieties to bacterial leaf blight has never been initiated in Togo. Therefore, the present study aimed at testing rice varieties widely grown in Togo their resistance to bacterial leaf blight.

MATERIALS AND METHODS

Plant materials: Six improved genotypes from Africa Rice Center (WARDA), and two improved genotypes from Institut Togolais de Recherche Agronomique (ITRA, Togo) widely grown in Togo were tested (Table 1). Thirteen near isogenic lines (NILs) with known resistance gene to bacteria leaf blight obtained from the International Rice Research Institute (IRRI) were used as positive control.

Bacterial strains: The experiment was conducted using 13 X. oryzae pv. oryzae strains from different ecozones of Togo.

Experimental design: The trial was carried out using the split plot design with 3 replications. Thirteen strains 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 pregerminated in sterilized Petri dishes under sterile conditions for 5 days and were transplanted in plastic pots. One plastic pot per genotype per *Xoo* strain in 3 replications was used.

Table 1: Rice genotypes tested and *Xoo* strains used for inoculation

Rice genotype			*Xoo* strains			
Origin	**Name**	**Code**	**Zones**	**Localities**	**Name**	**Code**
IRRI	IRBB1	V9	FST	Kovié	KV4-2	I1
IRRI	IRBB2	V10	FST	Kovié	KV14-2	I2
IRRI	IRBB3	V11	FST	Lomé	IL23-1	I3
IRRI	IRBB4	V12	FST	Davié	DV39-1	I4
IRRI	IRBB5	V13	FST	Davié	DV58-2	I5
IRRI	IRBB7	V14	Forest	Kpélé Atimé	KA63-2	I6
IRRI	IRBB8	V15	Forest	Kpélé Tutu	KT83-2	I7
IRRI	IRBB10	V16	Forest	Kpélé Tutu	KT84-2	I8
IRRI	IRBB11	V17	Forest	Sodo	SD94-1	I9
IRRI	IRBB13	V18	DS	Koumbéloti	KM101-1	I10
IRRI	IRBB14	V19	DS	Koumbéloti	KM129-2	I11
IRRI	IRBB21	V20	DS	Tantiégou	TN135-2	I12
IRRI	IR24	V21	DS	Tantiégou	TN160-2	I13
ITRA Togo	TGR203	V1				
ITRA Togo[a]	IR841	V2				
AfricaRice[a]	NERICA4	V3				
AfricaRice[a]	NERICA8	V4				
AfricaRice[a]	NERICA14	V5				
AfricaRice[a]	NERICA19	V6				
AfricaRice	Giganté	V7				
AfricaRice	TOG5681	V8				

[a] = cultivated in Togo; NILs = near isogenic lines; FST = Forest savanna transition zone; Forest = 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-hour old culture of *Xoo* strains 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 (Kauffman *et al*., 1973; Sere *et al*., 2005). The whole leaves of each plant in the plastic pot were clip inoculated 21 days after transplanting.

Data assessment and management: Evaluation consisted on the measurement of the lesion length due to bacterial leaf blight disease induced by inoculation with each of the strains, 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. Plant reactions to the disease were categorized according to lesion length. The reactions of differential genotypes were presented as the means of lesion length due to each of the *Xoo* strains used. For each strain, the reaction of genotypes were classified referring to the methods used by Sanchez *et al*. (2000), Lee *et al*. (2003) and Onasanya *et al*. (2009): resistant (R) - lesion length < 12.5% and susceptible (S) - lesion length > 112.5%.

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 genotypes disease expression (Aleong & Howard, 1985; Bruckner & Slanger, 1986; Ebdon & Gauch, 2002; Xiaoping & Ognjen, 2005). AMMI analysis was shown to be effective in understanding complex Genotype by Environment interactions trials that are difficult to understand using ordinary ANOVA as recommended by Ebdon & Gauch (2002). This analysis allowed classifying varieties and NILs regarding their expression of the disease after inoculation.

RESULTS

The data of the percentage of lesion length due to bacterial leaf blight revealed significant variability ($p<0.05$) in the reactions of 21 cultivars to the inoculation with 13 *Xoo* strains from different ecozones (Table 2). The lesion length caused by inoculation varied from 0.68% V5 (NERICA14) to about 49% V8 (TOG5681), with a total of seventeen values ≥ 17% of lesion length. Significant cultivars x strains interactions ($p<0.05$) with Xoo strains from the same locality or ecozone of origin were observed (Table 2). Cultivars V2 (IR841) was susceptible to KV4-2 and resistant to KV14-2 from Kovié, V6 (NERICA19) and V8 (TOG5681) were susceptible to DV39-1 and resistant to DV58-2 from Davié in the Forest savanna transition zone, V3 (NERICA4), V7 (Giganté) and V8 (TOG5681) were susceptible to KT84-2; and resistant to KT83-2 from Kpélé Tutu in the Forest zone, while V1 (TGR203) was susceptible to KM101-1 and resistant to KM129-2 from Koumbéloti and V6 (NERICA19) was susceptible to TN160-2 and resistant to TN135-2 from Tantiégou in the Dry savanna zone. Differential reactions were observed for rice genotypes to *Xoo* strains from the different ecozones or localites of origin. Most of the cultivated rice cultivars were susceptible to strains KT84-2 and TN135-2, while all of them were susceptible to strain TN160-2 from the Dry savanna zone, but were resistant to strains IL23-1, DV58-2, KA63-2, KT83-2, KM101-1 and KM129-2. Also the near isogenic lines revealed resistant reaction to all strains except for V13 (IRBB5) to strains TN135-2 and TN160-2 with lesion length of 13.54% and 21.19%, respectively.

Table 2: Analysis of variance for percentage lesion length after inoculation

SV	DF	SS	MS	F
REP (R)	2	3.59	1.79	2.85 ns
TREATMENT	272	484.28	1.78	2.82 **
VARIETE (V)	20	126.74	6.34	10.05 **
ISOLAT (I)	12	170.62	14.22	22.56 **
VxI	240	186.92	0.78	1.24 *
ERREUR	544	342.87	0.63	
TOTAL	818	830.75		

** = significant at de 1% level; * = significant at 5% level; ns = none significant

Additive Main effects and Multiplicative Interaction (AMMI) analysis of percentage lesion identify three groups of genotypes according to the level of their reaction to inoculation with *Xoo* strains (Figure 1):

- The *susceptible* genotypes (S), made up of five genotypes V1 (TGR203) and V2 (IR841) from ITRA, V6 (NERICA19) and V8 (TOG5681) from AfricaRice, and the near isogenic line V13 (IRBB5);
- The *medium resistant* genotypes (MR), made up of three genotypes cultivated in Togo, V3 (NERICA4), V4 (NERICA8) and V5 (NERICA14), and one genotype from AfricaRice, V7 (Giganté);
- The *resistant* genotypes (R) made up of only the near isogenic lines with known resistant genes: V9 (IRBB1), V10 (IRBB2), V11 (IRBB3), V12 (IRBB4), V14 (IRBB7), V15 (IRBB8), V16 (IRBB10), V17 (IRBB11), V18 (IRBB13), V19 (IRBB14), V20 (IRBB21) and V21 (IR24).

AMMI analysis revealed that none of the varieties grown in Togo was resistant to bacterial leaf blight however three of these were medium resistant. The cultivars IR841 and NERICA19 which are among the widely grown varieties in the country were susceptible. All the near isogenic lines with known resistant genes were resistant against bacterial leaf blight due to inoculation except IRBB5 which was susceptible.

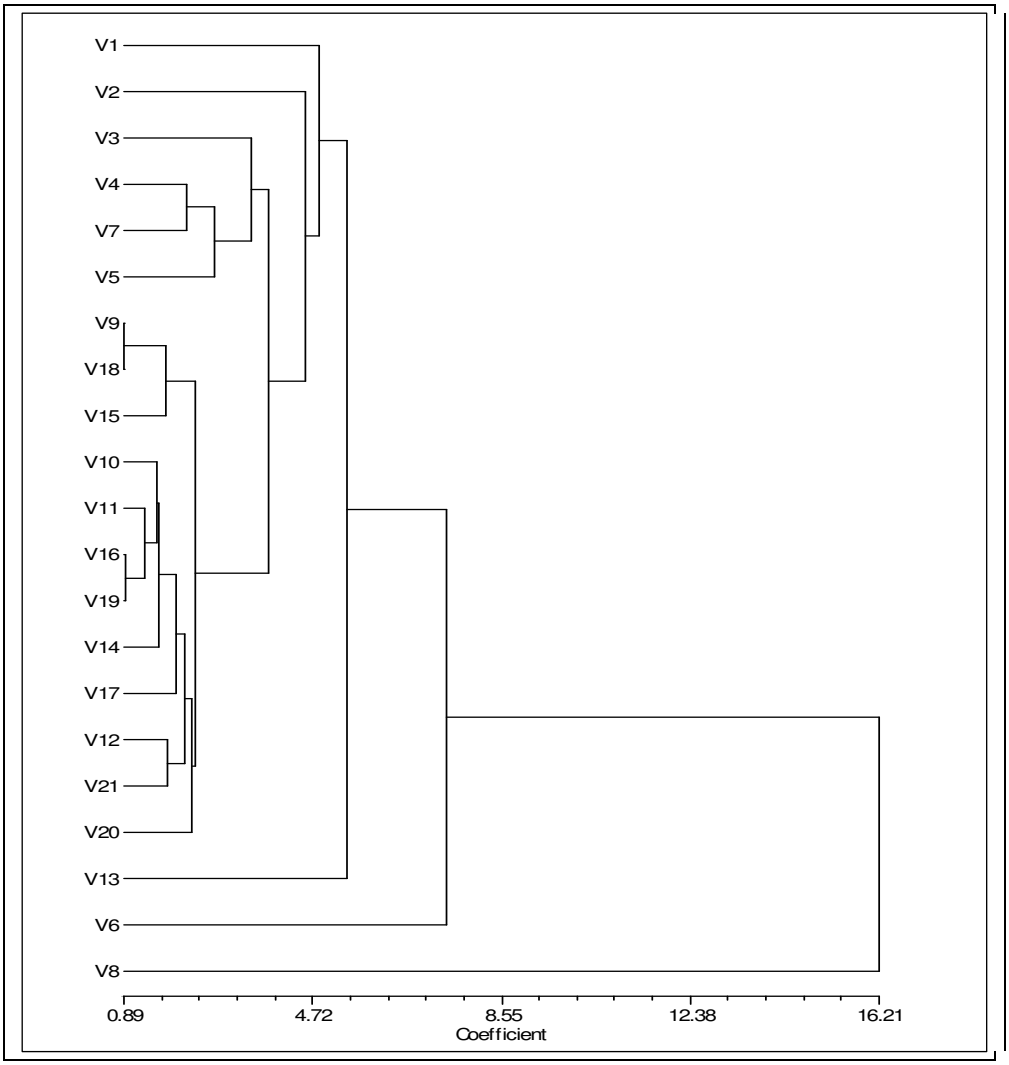

Figure 1: Dendogram of rice genotypes resistance to bacterial leaf blight using AMMI analysis

Coefficient = Fusion level; genotypes: V1 = TGR203; V2 = IR841; V3 = NERICA4; V4 = NERICA8; V5 = NERICA14; V6 = NERICA19; V7 = Giganté; V8 = TOG5681; V9 = IRBB1; V10 = IRBB2; V11 = IRBB3; V12 = IRBB4; V13 = IRBB5; V14 = IRBB7; V15 = IRBB8; V16 = IRBB10; V17 = IRBB11; V18 = IRBB13; V19 = IRBB14; V20 = IRBB21; V21 = IR24.

DISCUSSION

Bacterial leaf blight of rice was reported in several rice-growing ecozones of Togo with high incidence and severities (Déwa *et al*., 2011). Therefore, a control strategies adapted to environment must be developed to avoid possible epidemics. Among strategies in controlling bacterial diseases such as bacterial leaf blight of rice, host-plant resistance is an important control means. For this knowledge on variety resistance is important for selecting cultivars with durable resistance to the disease (Nelson *et al*., 1994; Choi *et al*., 1998; Banito *et al*., 2010). In this study, rice cultivars widely grown in Togo were screened together with near isogenic lines under greenhouse for resistance to bacterial leaf blight by inoculating 13 strains from various localities in Togo, and cultivar x strain interactions were analysed and cultivars were ranked following their level of disease expression.

Differential reactions of cultivars in the expression of bacterial leaf blight due to inoculation were observed. These reactions revealed differences in characteristics of cultivars. Several research works on the interactions between strains of *X. oryzae* pv. *oryzae* and rice cultivars have been widely documented (Mew *et al*., 1992; Zhu *et al*., 1998; Belkhadir *et al*., 2004; Lim & Kunkel, 2004; Gu *et al*., 2005). Ouedraogo *et al*. (2007) found differential reactions of rice lines to *X. oryzae* pv. *oryzae* strains in Burkina Faso. In the present study, AMMI analysis revealed that all grown cultivars tested were medium resistant to susceptible, whereas the near isogenic lines were resistant except IRBB5 which was susceptible. Some strains overcome the resistance of the near isogenic line IRBB5 with gene Xa-5. This confirms results reported by Onasanya *et al*. (2009) who found IRBB5 susceptible to bacteria strains from 7 African countries. Hoang *et al*. (2008) observed also differential reactions of rice lines to strains in Mekong Delta. These authors found that rice lines IRBB1, IRBB3, IRBB4, IRBB10, IRBB11 and IRBB14 with resistance genes were susceptible to all strains of *X. oryzae* pv. *oryzae* used. Also, studies to assess differential characteristics of 24 near isogenic rice lines with resistance gene to strains from China have been reported (Liu *et al*., 2007). The authors found 21 days after inoculation that IRBB1, IRBB2, IRBB3, IRBB10, IRBB11 and IRBB14 were the most susceptible of the lines tested. Genotype x strain analysis to find out bacteria pathotypes revealed differential reactions of 18 rice lines to 50 *X. oryzae* pv. *oryzae* strains from 7 African countries (Onasanya *et al*., 2009). The studies revealed that rice lines IRBB2, IRBB5, IRBB11 and IRBB21 were susceptible. However, in the present studies these rice lines were resistant to bacteria strains from Togo 14 days after inoculation. Additionally, rice line IR24 was found to be one of the resistant lines tested as it was reported by Onasanya *et al*. (2009). The later authors found that the genotypes TOG5681 and Gigantée from Africa Rice (WARDA) were among the most susceptible genotypes tested. In the present results these genotypes were classified susceptible and medium resistant, respectively. Liu *et al*. (2007) and Hoang *et al*. (2008) reported that the rice line IR24 was one of the most susceptible genotypes tested; however in the present studies IR24 revealed resistance to the 13 strains used, confirming the results found by Onasanya *et al*. (2009) who reported that IR24 was one of the resistant lines tested against 50 *X. oryzae* pv. *oryzae* strains from different countries.

This study revealed preliminary useful information on rice genotypes including the widely grown cultivars in Togo and *X. oryzae* pv. *oryzae* strains interactions. The widely grown genotypes were only medium resistant to susceptible, being the potential means of dissemination of bacterial leaf blight of rice. This information is useful for selection of genotypes with durable resistance to the disease in order to prevent farmers' fields from epidemics. However, further studies including trials under field conditions must be undertaken.

REFERENCES

Aleong J and Howard D, 1985. Extensions of the Duncan's multiple range test for unbalanced data. *J. Appl. Stat.*, 61: 966-971.

Banito A, Kpémoua KE, Wydra K, 2010. Screening of cassava genotypes for resistance to bacterial blight using strain x genotype interactions. *J. Plant Pathol.*, 92: 181-186.

Basso A, Onasanya A, Issaka S, Sido AY, Haougui A, 2011. Bacterial leaf blight of rice in Niger: Pathological diversity of isolates collected on irrigated lands. *J. Appl. Biosci.*, 38: 2551-2563.

Belkhadir Y, Nimchuk Z, Hubert DA, Mackey D, Dangl JL, 2004. Arabidopsis RIN4 negatively regulates disease resistance mediated by RPS2 and RPM1 downstream or independent of the NDR1 signal modulator and is not required for the virulence functions of bacterial

type III effectors AvrRpt2 or AvrRpm1. *Plant Cell* 16: 2822-2835.

Bruckner CM and Slanger WD, 1986. Symmetric differences squared and analysis of variance procedures for estimating genetic and environmental variances and covariances for beef cattle weaning weight: I. comparison via simulation. *J. Anim. Sci.*, 63: 1779-1793.

Choi SH, Casiana MVC, Jan EL, 1998. Distribution of *Xanthomonas oryzae* pv. *oryzae* DNA modification systems in Asia. *Appl. Environ. Microbiol.*, 64: 1663-1668.

Déwa KMK, Banito A, Onasanya A, Kpémoua KE, Séré Y, 2011. Rice bacterial blight in Togo: Importance of the Disease and Virulence of the pathogen. *Curr. Res. Bacteriol.* 4(3): 94-100.

Ebdon JS and Gauch HG. 2002. AMMI analysis of national turfgrass performance trials. I. Interpretation of genotype by environment interaction. *Crop Sci.*, 42: 489-496.

Gu KY, Yang B, Tian DS, 2005. R gene expression induced by a type-III effector triggers disease resistance in rice. *Nature,* 435: 1122-1125.

Hoang DD, Nghi KO, Nguyen DT, Pham VD, Le Cam L, 2008. Pathotype profile of *Xanthomonas oryzae* pv. *Oryzae* isolates from the rice ecosystem in Cuulong River Delta. *Omorice,* 16: 34-40.

Kadai EA, 2010. Criblage des varieties améliorées de riz du Togo pour la résistance au flétrissement bactérien en conditions d'infestation artificielles. Mémoire d'ingénieur Agronome, Université de Lomé, 72 p.

Kauffman HE, Reddya PK, Hiesh SPY, Merca SD, 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Dis. Rep.*, 57: 537-541.

Lee KS, Rasabandith S, Angeles ER, Khush GS. 2003. Inheritance of resistance to bacterial blight in 21 cultivars of rice. *Phytopathology* 93: 147-152.

Lim MT and Kunkel BN, 2004. The Pseudomonas syringae type III effector AvrRpt2 promotes virulence independently of RIN4, a predicted virulence target in Arabidopsis thaliana. *Plant J.* 40: 790-798.

Liu H, Yang W, Hu B, Liu F, 2007. Virulence Analysis and Race Classification of Xanthomonas oryzae pv. oryzae in China. *J. Phytopathol.*, 155: 129–135.

Mew TW, Vera Cruz CM, Medalla ES, 1992. Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in Philippines. *Plant Dis,.* 76: 1029-1032.

Mew TW, 1987. Current status and future prospects of research on bacterial blight of rice. *Ann. Rev. Phytopathol.,* 25: 359-382.

Mew TW, 1989. An overview of the world bacterial blight situation. In: *Bacterial Blight of Rice*, Ogawa T, Khush GS (eds)., International Rice Research Institute, Manila, Philippines, pp. 7-12.

Mew TW, Alvarez AM, Leach JE, Swings J, 1993. Focus on bacterial blight of rice. *Plant Dis.*, 77: 5-12.

Nelson RJ, Baraoidan MR, Vera Cruz CM, Yap IV, Leach JE, Mew TW, Leung H, 1994. Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. *Appl. Environ. Microbiol.*, 60: 3275-3283.

Onasanya A, Ekperigin MM, Nwilene FE, Séré Y, Onasanya RO, 2009. Two pathotypes of *Xanthomonas oryzae* pv. *oryzae* Virulence Identified in West Africa. *Curr. Res. Bacteriol.*, 2: 22-35.

Ou SH, 1985. Rice Diseases. Commonwealth Mycological Institute, Kew, Survey, England.

Ouedraogo SL, Somda I, Wonni I and Sere Y, 2007. Etude de la résistance au flétrissement bactérien de lignées inter- et intraspécifiques de riz de bas-fonds en conditions d'infestation artificilles. *Afr. Crop Sci. J.*, 15: 191-199.

Sanchez AC, Brar DS, Huang N, Li Z, Khush GS, 2000. Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. *Crop Sci.*, 40: 792-797.

Séré Y, Onasanya A, Verdier V, Akator K, Ouedraogo LS, Segda Z, Mbare MM, Sido AY, Basso A, 2005. Rice bacterial leaf blight in West Africa: Preliminary studies on disease in farmers' fields and screening released varieties for resistance to the bacteria. *Asian J. Plant Sci.*, 4: 577-579.

Swings J, Mooter MV, Vauterin L, Hoste B, Gillis M, Mew TW, Kersters K, 1990. Reclassification of the causal agents of bacterial blight of rice (*Xanthomonas campestris* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) of rice as pathovars of *Xanthomonas oryzae*. *Int. J. Syst. Bacteriol.*, 40: 309-311.

Xiaoping Z and Ognjen K, 2005. A short preview of free statistical software packages for teaching statistics to industrial technology majors. *J. Ind. Technol.*, 21: 1-6.

Zhu W, Yang B, Chittoor JM, Johnson LB, White FF, 1998. AvrXa10 contains an acidic transcriptional activation domain in the functionally conserved C terminus. Mol Plant Microbe Interact 11: 824-832.