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Molecular Characterization and DNA Fingerprinting of *Xanthomonas oryzae* pv. *oryzae* Isolates from Climate Change Prone Areas in East Africa

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ABSTRACT

Genomic DNA fingerprinting is a useful tool for effective and reliable identification and differentiation of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) pathogen from rice. The study aimed to conduct molecular characterization and DNA fingerprinting of 23 *Xoo* isolates from East Africa and two *Xoo* isolates from IRRI (Philippines) as control. PCR analysis was carryout on genomic DNA of 25 *Xoo* isolates using 6 *Xoo* specific primer pairs. Cluster analyses of genetic data obtained from 25 *Xoo* DNA fingerprints revealed two major genotypes (GrpA and GrpB) among the 25 *Xoo* isolates. GrpA has three subgroups (GrpA1; GrpA2; GrpA3) and GrpB (GrpB1; GrpB2; GrpB3). GrpA genotype consists of 20 *Xoo* isolates from Uganda, Rwanda and Philippines while GrpB genotype has 5 *Xoo* isolates from Rwanda. Some *Xoo* isolates were identical (PX-1, PX-2; UX621, RX2101; RX554, UX623, RX4113; UX211, UX213, UX214, RX4112, UX215). The emergence of subgroup genotypes could possibly be due to mutations and interactions among isolates and strains in host cells. Some *Xoo* isolates from Rwanda and Uganda were identical suggesting possible pathogen migration between these countries and long-term survival. Durable resistance rice cultivars would need to overcome both GrpA and GrpB *Xoo* genotypes in order to survive after their deployment into different rice ecologies in East Africa.

Key words: *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), DNA fingerprinting, *Xoo* genotype, *Xoo* pathogen migration, Rice cultivars, East Africa

INTRODUCTION

Rice Bacterial Leaf Blight (BLB) disease is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). This BLB disease is highly destructive and found mostly in Asia, America and Africa (Zeigler and Alvarez, 1990; Adhikari *et al*., 1995; Sere *et al*., 2005; Onasanya *et al*., 2009). BLB disease

incidence and yield loss in Africa ranged from 40-85 and 25-65%, respectively depending on variety type, resistance level and climatic conditions (Sere *et al*., 2005; Onasanya *et al*., 2009).

Developing resistant varieties against BLB disease would help to increase rice production in BLB disease endemic areas. But the durability of these resistant varieties in farmers' fields is being hampered by unstable *Xoo* pathogen variation (Sere *et al*., 2005). Understanding the extent of *Xoo* pathogen variations in BLB endemic areas would greatly help to maintain durable resistant varieties at field levels in different rice ecologies (Sere *et al*., 2005). Different *Xoo* genotypes and pathotypes newly identified could be used to identify durable resistant improved rice varieties (Shanti *et al*., 2010).

Several techniques have been used for analysis of *Xoo* pathogen variation and characterization. Some of these techniques include proteomics, genomics and pathomics (Onasanya *et al*., 2007-2010). Any of these techniques could be used reliably to characterize and differentiate *Xoo* pathogen and isolates. The *Xoo* genotypes and pathotypes identified could be used to screen improved rice cultivars to identify durable resistance type. Using proteomics to characterize *Xoo* isolates involves the use of isozymes and specific *Xoo* monoclonal and polyclonal antibodies using enzyme-linked immuno sorbent assay (ELISA) (Gnanamanickam *et al*., 1999; Onasanya *et al*., 2007, 2008). Genomics has wider applications into using *Xoo* genomic DNA in different Polymerase Chain Reaction (PCR) techniques to reveal variations and different genotypes among *Xoo* pathogen and isolates (Sakthivel *et al*., 2001; Onasanya *et al*., 2003, 2010).

The present study aimed to carryout molecular characterization and DNA fingerprinting of *Xanthomonas oryzae* pv *oryzae* isolates from climate change prone areas in East Africa using *Xoo* specific primers previously developed by Onasanya *et al*. (2010). Besides, the study aimed to revealed different *Xoo* genotypes, distribution and movement in East Africa.

MATERIALS AND METHODS

Research location: Bacterial isolate propagation and molecular PCR analysis were carried out at Central Biotechnology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The study was conducted in May 2009.

Isolate propagation: The 25 *Xoo* isolates (Table 1) used in this study were first propagated using a modified procedure developed by Onasanya *et al*. (2003). Nutrient broth (25 mL; pH 7.5) was prepared inside a 100 mL conical flask. *Xoo* isolate (200 μL) from storage was transferred into 25 mL of nutrient broth and kept under constant shaking at 28°C for 24 h for bacterial growth. The bacterial cells were removed by centrifugation, washed with 0.1 mM Tris-EDTA (pH 8.0) and kept at -20°C for DNA extraction.

Genomic DNA preparation: *Xoo* genomic DNA extraction and DNA quality checking were according to Onasanya *et al*. (2003). Genomic DNA extraction for all the 25 *Xoo* isolates was carried out.

Genetic analysis: The Polymerase Chain Reaction (PCR) analysis and thermal cycling program were according to Onasanya *et al*. (2010). The *Xoo* specific primers used (Table 2) in the PCR analysis were obtained from Onasanya *et al*. (2010). All the genomic DNA of the 25 *Xoo*

isolates (Table 1) was analyzed. PCR products electrophoresis, agarose gel staining and gel documentation were according to Onasanya *et al*. (2003).

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

Isolate	Host plant	Country
PX-1	Cultivated rice	Philippines
PX-2	Cultivated rice	Philippines
UX621	*Pusa Basmati*	Uganda
UX211	WITA9	Uganda
RX2101	IR64	Rwanda
UX213	WITA9	Uganda
RX2103	IR64	Rwanda
RX551	Cultivated rice	Rwanda
RX112	Cultivated rice	Rwanda
RX4114	Cultivated rice	Rwanda
RX113	Cultivated rice	Rwanda
UX214	WITA9	Uganda
UX212	WITA9	Uganda
RX554	Cultivated rice	Rwanda
RX2104	IR64	Rwanda
RX552	Cultivated rice	Rwanda
RX555	Cultivated rice	Rwanda
UX622	Pusa Basmati	Uganda
RX553	Cultivated rice	Rwanda
UX623	Pusa Basmati	Uganda
UX215	WITA9	Uganda
RX4112	Cultivated rice	Rwanda
RX4113	Cultivated rice	Rwanda
RX4111	Cultivated rice	Rwanda
UX216	WITA9	Uganda

Table 2: Identity of *Xanthomonas oryzae* pv. *oryzae* fingerprinting primers used for the study

Primer	Sequence 5'-3'
XooF1	TGGTAGTCCACGCCCTAAAC
XooR1	CCTGAGCTACAGACCCGAAG
XooF2	ATGCGCAGAAGCAGATAGGT
XooR2	TGTCGCTTCCTGTGCTATTG
XooF3	GACCACCGTGAACTCCTTGT
XooR3	GCTTCGGCTTCTCGTATGAC
XooF4	TGTGCCTAGCCATCAGACAG
XooR4	AGCCGCGACAATTTCTTCTA
XooF5	ACAAGGCGATGGATCAGTTC
XooR5	AGCTTGGTGGTGATGGTAGG
XooF6	GACAAGGCGATGGATCAGTT
XooR6	GATCTGGTGGCAAAACCTGT
XooF5	ACAAGGCGATGGATCAGTTC
XooR6	GATCTGGTGGCAAAACCTGT
XooF7	CAGATTGATGCGTTGCTGAT
XooR5	AGCTTGGTGGTGATGGTAGG

F: Forward direction, R: Reverse direction

Cluster analysis: Positions of scorable amplified DNA bands were transformed into a binary character matrix ("1" for the presence and "0" for the absence of a band at a particular position). Pairwise distance matrices were compiled by the Numerical Taxonomy System (NTSYS) 2.0 software (Rohlf, 2000) using the Jaccard coefficient of similarity (Ivchenko and Honov, 1998). Cluster dendrogram was created by unweighted pair-group method arithmetic (UPGMA) cluster analysis (Jako *et al.*, 2009). Principal component analysis with GGEbiplot was carryout on 25 *Xoo* isolates using genetic data generated from seven *Xoo* specific primers (Ebdon and Gauch, 2002).

RESULTS

Molecular characterization and DNA fingerprinting of 23 *Xanthomonas oryzae* pv *oryzae* (*Xoo*) isolates from East Africa have been carried out. The two *Xoo* isolates from IRRI (Philippines) were used as control isolates. Distinct DNA fingerprints were obtained for all the 25 *Xoo* isolates genomic DNA analyzed (Fig. 1). Cluster analyses of genetic data obtained from 25 *Xoo* DNA fingerprints revealed two major genotypes (GrpA and GrpB) among the 25 *Xoo* isolates (Fig. 2). However, GrpA has three subgroups (GrpA1; GrpA2; GrpA3) as well as GrpB (GrpB1; GrpB2; GrpB3) (Fig. 3). GrpA genotype consists of 20 *Xoo* isolates from Uganda, Rwanda and Philippines while GrpB genotype has 5 *Xoo* isolates from Rwanda (Fig. 2). At 100% similarity coefficient some *Xoo* isolates were identical (PX-1, PX-2; UX621 and RX2101; RX554, UX623, RX4113; UX211, UX213, UX214, RX4112, UX215) (Fig. 2). GrpA genotype has 80% occurrence and distribution in among countries while GrpB has only 20% occurrence and distribution in one country (Table 3).

Table 3: *Xanthomonas oryzae* pv. *oryzae* genotype, occurrence and distribution

		Isolate origin and distribution			
Genotype	No. of isolate	Rwanda	Uganda	Philippines	Occurrence (%)
GrpA	20	9	9	2	80
GrpB	5	5	0	0	20

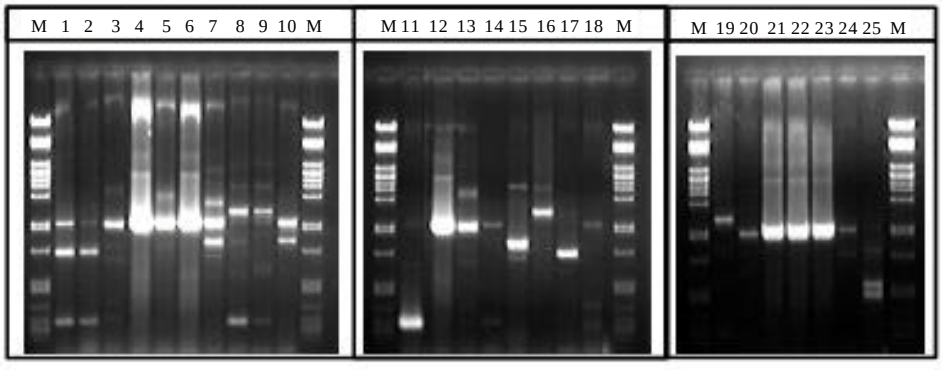

Fig. 1: *Xanthomonas oryzae* pv. *oryzae* DNA fingerprint as revealed PCR analysis using XooF1 and XooR1 *Xoo* specific primers. M: Molecular size marker

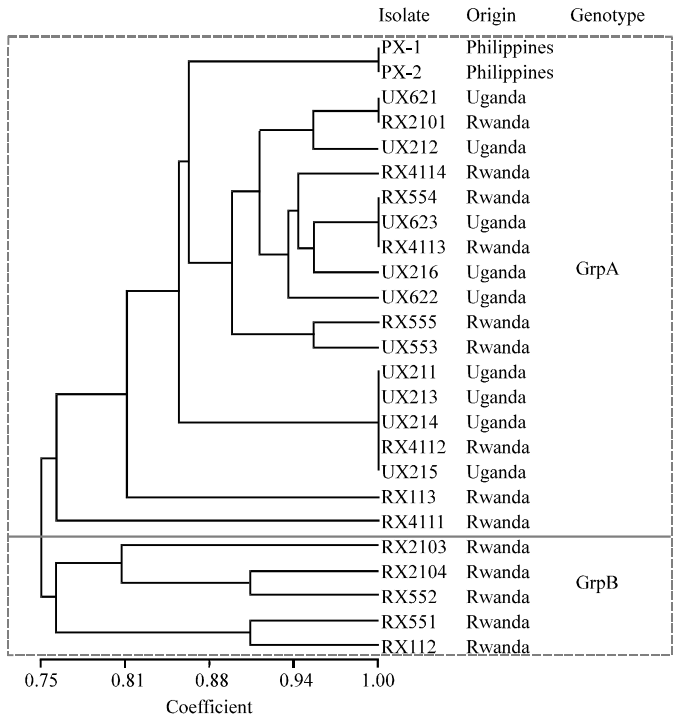

Fig. 2: Dendrogram showing genetic diversity among 25 *Xanthomonas oryzae* pv. *oryzae* isolates as revealed PCR analysis using seven *Xoo* specific primers

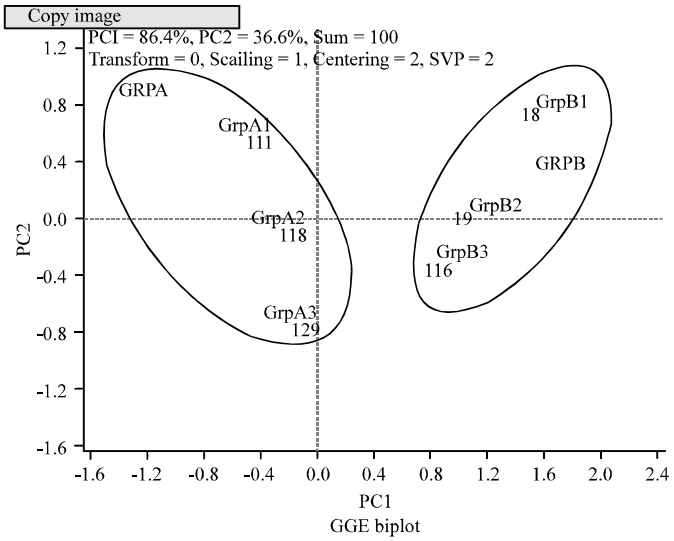

Fig. 3: Principal component analysis that revealed subgroup genotypes among 25 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates using genetic data generated from seven *Xoo* specific primers

DISCUSSION

Genomic DNA fingerprinting is a useful tool for effective and reliable identification and differentiation of *Xoo* pathogen from rice (Onasanya *et al.*, 2010). The present study identified two major *Xoo* genotypes (GrpA and GrpB) and six subgroups (GrpA1; GrpA2; GrpA3; GrpB1; GrpB2; GrpB3) in East Africa. Similarly, two major *Xoo* genotypes genetic groups (*Xoo-A* and *Xoo-B*) with

five subgroups have been identified in previously studies in West Africa (Onasanya *et al.*, 2007, 2008). The present genetic study suggests the emergence of subgroup genotypes could possibly be due to mutations and interactions among isolates and strains that originally made up GrpA and GrpB genotypes (Onasanya *et al.*, 2003, 2007, 2008, 2010).

The use of a reliable molecular technique to differentiate *Xoo* isolates is a prerequisite into understanding the genetics and population structure of *Xoo* pathogen in Africa and identification of durable resistance cultivars (Adhikari *et al.*, 1995, 1999; Onasanya *et al.*, 2009. 2010). The present study has revealed the occurrence two *Xoo* genotypes, movement and distribution in East Africa. For example, GrpA genotype has 80% occurrence among countries while GrpB has 20%. It is very critical that both GrpA and GrpB genotypes are found in Rwanda and the implication would be that durable resistance rice cultivars must overcome these *Xoo* genotypes in order to survive (Onasanya *et al.*, 2009). Similar findings were obtained in West Africa where two or more *Xoo* genotypes known exist in one country (Onasanya *et al.*, 2007, 2008).

The high level of genetic variation among *Xoo* isolates in this study suggests could be as a result of frequent occurrence of mutants in *Xoo* isolates in different host cells (Mongkolsuk *et al.*, 2000; Innes *et al.*, 2001; Onasanya *et al.*, 2003, 2010). The current genetic study revealed six subgroups *Xoo* genotypes (GrpA1; GrpA2; GrpA3; GrpB1; GrpB2; GrpB3) and were all present in Rwanda which could possibly be responsible for most sporadic cultivars infestation and epidemics in this country. Additional findings from the present study showed that some *Xoo* isolates from Rwanda and Uganda were identical (UX621, RX2101; RX554, UX623, RX4113; UX211, UX213, UX214, RX4112, UX215), suggesting possible pathogen migration between these countries and long-term survival (Adhikari *et al.*, 1995; Onasanya *et al.*, 2008, 2010). Identification of identical *Xoo* isolates as obtained by the present genetic study would be difficult to achieve using cultural and morphological techniques (Bonde *et al.*, 1993; Onasanya *et al.*, 2010). This has further revealed the importance and application of DNA fingerprinting techniques in the identification of *Xoo* isolates in situations where identification of *Xoo* isolates using cultural and morphological techniques often lack consistency and precision.

CONCLUSION

The present study on molecular characterization and DNA fingerprinting of *Xoo* isolates from East Africa identified two major Xoo genotypes (GrpA and GrpB) and six subgroups (GrpA1; GrpA2; GrpA3; GrpB1; GrpB2; GrpB3). There was evidence of *Xoo* pathogen migration between countries. Durable resistance rice cultivars would need to overcome both GrpA and GrpB *Xoo* genotypes in order to survive after their deployment into different rice ecologies in East Africa.

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REFERENCES

Adhikari, T.B., C. Cruz, Q. Zhang, R.J. Nelson, D.Z. Skinner, T.W. Mew and J.E. Leach, 1995. Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. Applied Environ. Microbiol., 61: 966-971.

Adhikari, T.B., R.C. Basnyat and T.W. Mew, 1999. Virulence of *Xanthomonas oryzae* pv. *Oryzae* on rice lines containing single resistance genes and gene combinations. Plant Dis., 83: 46-50.

Bonde, M.R., J.A. Micales and G.L. Peterson, 1993. The use of isozyme analysis for identification of plant pathogenic fungi. Plant Dis., 77: 961-968.

Ebdon, J.S. and H.G. Gauch, 2002. AMMI analysis of national turfgrass performance trials. I. Interpretation of genotype by environment interaction. Crop Sci., 42: 489-496.

Gnanamanickam, V.B. Priyadarisini, N.N. Naryanan, P. Vasudevan and S. Kavitha, 1999. An overview of bacterial blight disease of rice and strategies for its management. Curr. Sci., 77: 1435-1444.

Innes, D., I.R. Beacham, C.A. Beven, M. Douglas, M.W. Laird, J.C. Joly and D.M. Burns, 2001. The cryptic ushA gene (ushAc) in natural isolates of *Salmonella enterica* (serotype typhimurium) has been inactivated by a single missense mutation. Microbiology, 147: 1887-1896.

Ivchenko, G.I. and S.A. Honov, 1998. On the jaccard similarity test. J. Math. Sci., 88: 789-794.

Jako, E., E. Ari, P. Ittzes, A. Horvath and J. Podani, 2009. BOOL-AN: A method for comparative sequence analysis and phylogenetic reconstruction. Mol. Phylogenet. Evol., 52: 887-897.

Mongkolsuk, S., W. Whangsuk, M. Fuangthong and S. Loprasert, 2000. Mutations in oxyR resulting in peroxide resistance in *Xanthomonas campestris*. J. Bacteriol., 182: 3846-3849.

Onasanya, A., H.D Mignouna and G. Thottappilly, 2003. Genetic fingerprinting and phylogenetic diversity of isolates of *Staphylococcus aureus* from Nigeria. Afr. J. Biotechnol., 2: 246-250.

Onasanya, A., M.M. Ekperigin, Y. Sere, F.E. Nwilene, J.O. Ajele and G. Oboh, 2007. Isozyme fingerprinting and genetic differentiation of *Xanthomonas oryzae* pv. *oryzae* isolates as revealed by glucose 6-phosphate dehydrogenase (G6PH) analysis. Biotechnology, 6: 357-363.

Onasanya, A., M.M. Ekperigin, Y. Sere and J.O. Ajele, 2008. Enzyme polymorphism and genetic diversityin *Xanthomonas oryzae* pv. *oryzae* isolates causing rice bacterial leaf blight disease in West Africa. Int. J. Agric. Res., 3: 227-236.

Onasanya, A., M.M. Ekperigin, F.E. Nwilene, Y. Sere and R.O. Onasanya, 2009. Two pathotypes of *Xanthomonas oryzae* pv. *oryzae* virulence identified in West Africa. Curr. Res. Bacteriol., 2: 22-35.

Onasanya, A., A. Basso, E. Somado, E.R. Gasore and F.E. Nwilene *et al.*, 2010. Development of a combined molecular diagnostic and DNA fingerprinting technique for rice bacteria pathogens in Africa. Biotechnology, 9: 89-105.

Rohlf, F.J., 2000. NTSys pc, Version 2.02j, Exeter Software. Setauket, New York, USA.

Sakthivel, N., C.N. Mortensen and S.B. Mathur, 2001. Detection of *Xanthomonas oryzae* pv. *oryzae* in artificially inoculated and naturally infected rice seeds and plants by molecular techniques. Applied Microbiol. Biotechnol., 56: 435-441.

Sere, Y., A. Onasanya, V. Verdier, K. Akator and L.S. Ouedraogo *et al.*, 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.

Shanti, M.L., C.M.K. Varma, P. Premalatha, G.L. Devi, U. Zehr and W. Freeman, 2010. Understanding the bacterial blight pathogen-combining pathotyping and molecular marker studies. Int. J. Plant Pathol., 1: 58-68.

Zeigler, R.S. and E. Alvarez, 1990. Characteristics of *Pseudomonas* spp. Causing grain discoloration and sheath rot of rice and associated *Pseudomonad epiphytes*. Plant Dis., 74: 917-922.