POPULATION STRUCTURE OF *Xanthomonas oryzae* pv. *oryzae* AND RICE CULTIVAR RESISTANCE IN THREE REGIONS IN TANZANIA

MESHACK M. MWENDA

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

MOROGORO, TANZANIA.

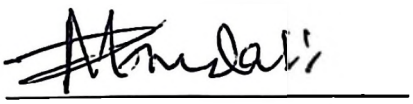
2014

ABSTRACT

This study determined the pathogen population structure and the reaction of rice cultivars against *Xanthomonas oryzae* pv. *oryzae*. Field trapping nurseries were established at Dakawa, Bunda and Kyela and inoculations for pot experiment were conducted at Agricultural Research Institute-Uyole from December, 2012 – July, 2013. Twenty two rice cultivars obtained from Agricultural Research Institute-Uyole and twenty one near isogenic lines from AfricaRice Program, Dar-es-Salaam, Tanzania were arranged in a completely randomized design and randomized complete block design in screenhouse and field experiments, respectively. Nineteen isolates of the pathogen were obtained from the study areas and used for physiological and biochemical tests. Data on incidence and severity were subjected to analysis of variance (ANOVA). Duncan's Multiple Range Test was used to separate means. Six pathotypes namely; TK3CD, TK4E, TM2C, TM2B, TB2F and TB5A were identified from the presumed nineteen isolates of the pathogen. Results showed that IR-BB4 and IR-BB52 were resistant to *Xanthomonas oryzae* pv. *oryzae* with significantly ($P = 0.05$) low disease incidence (8.18 % and 6.72 %) and severity (0.5 cm and 0.65 cm), respectively. IR-BB 14 was highly susceptible with 61.55 % disease incidence and 6.12 cm disease severity. The cultivars Domo la Fisi, Rangi Mbili, Tule na Bwana, Jicho la Samola, Rufiji, SARO 5, Mtalima Wangu, Zambia and Supa showed intermediate resistance compared to the IR24 (control). The IR-BB4 and IR-BB52 are therefore, possible promising rice lines that can be incorporated into rice breeding programs in Tanzania.

DECLARATION

I, **MESHACK M. MWENDA,** do hereby declare to the Senate of the Sokoine University of Agriculture (SUA) that the work presented here is my original work and has not been submitted for a higher degree in any other Institution.



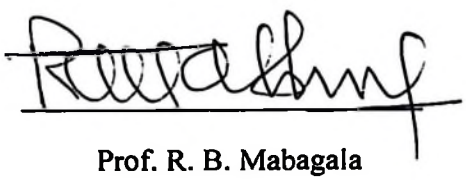
Meshack M. Mwenda

(MSc. Crop Science Candidate)

04 | 11 | 2014

Date

The above declaration is confirmed by



Prof. R. B. Mabagala

(Supervisor)

04/011/2014

Date

COPYRIGHT

ACKNOWLEDGEMENTS

This study would never have been completed without the guidance, interest and encouragement that were generously extended to me by my supervisor Prof. R. B. Mabagala throughout the study, to whom I am mostly grateful.

Special thanks to Drs. Y. Sere, A. Onasanya, S. Silue, C. Vera Cruz of AfricaRice Program and Mrs. F. Mwalyego of ARI-Uyole for suggesting many constructive amendments during the study and writing up of the dissertation. I also aknowledge the support given by Dr. E. Mbega of ARI-Ilonga.

I am indebted to my colleague, Mr. E. Kisetu, of Sokoine University of Agriculture (SUA) for his guidance and nice exposure to the computer work during data analysis.
I would like to thank the farmers of Nyatwali- Bunda, Dakawa-Morogoro and Kyela-Mbeya for providing me land to conduct my field trials and allowing me to collect diseased rice samples in their fields. The support given by Dr. Z. Malley and B. Kiwovele of ARI-Uyole by allowing me to use the institute facilities is also acknowledged.

The financial support given by AfricaRice Program under the regional project MICCORDEA for my study is greatly acknowledged. Support given by my employer, the Ministry of Agriculture, Food Security and Cooperatives is highly acknowledged for the permission granted to me to pursue this study.

My acknowledgement is also extended to Judith Hubert of AfricaRice Program and all staff of the Department of Crop Science and Production at SUA and all my fellow students for their useful advice, discussions and support during my study.

My heartfelt appreciation are extended to my wife Witness Mtweve for her academic and moral support, encouragement, guidance and patience extended to the bountifully throughout my study. My dear children Irene and Glory are highly commended for their high tolerance they showed when they missed unreturnable fatherly love and company during my long stay away from home.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AfSHC	African seed health centre
ANOVA	Analysis of variance
ARI-Uyole	Agricultural research institute-Uyole
ATDA	2-amino 1,3,4 – thiadiazole
BLB	Bacterial leaf blight disease
Cfu	Colon forming unit
CMPA	3-chloro-1*H*-pyrazole-5-carboxylic acid
CRD	Completely randomized design
CV (%)	Percent coefficient of variation
DAS	Days after sowing
DMRT	Duncan's Multiple Range Test
EPS	Extracellular polysaccharide
GPS	Geographical positioning system
IRRI	International Rice Research Institute
KOH	Potassium hydroxide
LSD	Least significant difference
NA	Nutrient agar
NILs	Near isogenic lines
Pv	Pathovar
PBDI	Percentage bacterial blight disease incidence
PDS	Percent disease severity
Xoo	*Xanthomonas oryzae* pv. *oryzae*
YGA	Yeast glucose agar

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Rice, *Oryza sativa* L. is an important food crop cultivated worldwide (Mahadevappa, 2004). It is a staple food for more than half of the world population (Salim *et al.*, 2003). Rice was introduced in Tanzania between 1890 and 1920, and has become an important second staple food after maize (MAFSC, 2009). However, in East African countries, bacterial leaf blight (BLB) has become an endemic disease on rice following repeated cultivation (Onasanya *et al.*, 2011). The disease was first noticed by the rice farmers in Japan in 1984. Since then, its incidence has been reported in different parts of the world, including Northern Australia, United States of America and Africa (Ashura *et al.*, 1999; Sere *et al.*, 2005). In the 1960s BLB became prevalent in rice growing regions worldwide with the introduction of improved cultivars such as TNI, IR 6 and IR8 which were high yielding but susceptible to BLB disease (Ali *et al.*, 2009; Bashir *et al.*, 2010).

1.2 Justification

Bacterial leaf blight of rice (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive bacterial diseases of rice throughout the world (Mew, 1993). The disease causes reduction in quantity and quality of rice. In Tanzania, the average rice yield is 1.5 t/ha compared to the estimated potential yields of 4–5 t/ha (Bucheyeki *et al.*, 2011). Economically, the disease has had the greatest impact in Asia, where several epidemics have occurred in the past four decades, and in West Africa, particularly in Niger, where irrigated rice was reported to be extensively damaged in 1982 (Mew *et al.*, 1992).

Yield losses of 10-50 % from bacterial leaf blight have been reported (Webster and Gunnel, 1992). In Pakistan in1985, the disease incidence on farmers' field was recorded to reach 10–25 % (Akhtar, 2003). Bacterial leaf blight is difficult to manage and can cause up to 50– 90 % yield reduction (Sere *et al.*, 2005). In East Africa, yield losses have been reported to range between 45 – 90 % on susceptible rice cultivars in farmers' fields (Onasanya *et al.*, 2011).

Management of BLB in rice focuses on the use of botanical, bactericides, cultural measures and synthetic chemical methods (Rukhsana, 2007). However, none of these approaches is considered effective and economical for control of this disease (Devadath, 1998). Varietal resistance is considered as a key BLB management tool under tropical conditions. This approach is comparatively economical and more convenient to control the disease in large scale areas (Onasanya *et al.*, 2011). Information on pathogen population structure from the current study will be used in gene pyramiding, identifying and characterizing rice germplasm for the biotic stress and hence, formulation of long-term strategies to manage the disease.

Little information is available on the virulence and population structure of *X. oryzae* pv. *oryzae* in Tanzania. Therefore, the present study aimed at identifying pathotype structure of *X. oryzae* pv. *oryzae* isolates and screen rice varieties for resistance against *X. oryzae* pv. *oryzae* in Morogoro, Mbeya and Bunda, Tanzania.

1.3 Objectives

1.3.1 Overall objective

To improve yield through the use of rice varieties resistant to *Xanthomonas oryzae* pv. *oryzae* in Tanzania.

1.3.2 Specific objectives

i) To assess pathotype structure of *Xanthomonas oryzae* pv. *oryzae* populations at Dakawa, Kyela and Bunda.

ii) To screen rice varieties grown at Dakawa, Kyela and Bunda for resistance to *Xanthomonas oryzae* pv. *oryzae*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 An overview

Bacterial leaf blight is a vascular disease of rice caused by *X. oryzae* pv. *oryzae* (Sanchez *et al.*, 2000). The bacterium normally enters the host through wounds or natural openings such as hydathodes and multiplies in the tissues of the epitheme, into which xylem vessels open (Gonzalez *et al.*, 2007). Some of the bacterial cells have been reported to reach the xylem vessels of the vascular system. When a bacterial colony has been established in the xylem vessels, the bacterial cells grow vigorously and translocation occurs through the network of the vascular system (Ezuka and Kaku, 2000).

2.2 Symptomatology

On affected young rice plants the outer leaves wither and become grayish-green or light brown. Some of the younger leaves roll up and wither. Bacterial exudates can be seen coming from cut leaf blades or sheaths (Goto, 1992). The symptoms become visible one week after infection and it is characterized by drying of the leaf tip, and inward rolling and twisting of the leaf blade. Linear yellowish stripes may develop along the midrib or on the blade and marginal necrosis may also occur.

The disease extends to the leaf sheaths and culms, killing single tiller or the whole plant. Panicle sterility and unfilling has been reported under severe conditions. Older leaves do not show symptoms (IRRI and Queensland University, 2001). According to Rukhsana (2007) the kresk phase of the disease is characterized by systematic infection. The symptoms usually appear one to two weeks after transplanting. The leaves become grayish green, suddenly withers and roll up (Webster and Gunnell, 1992).

2.3 Epidemiology

The presence of weeds around the field, the rice stubbles, and ratoons of infected rice plants sustain survival of the disease and become sources of initial inocula. The bacteria can survive for 30-40 days in the soil (Burdon, 1993). Warm temperature of 25-30 °C, high relative humidity (80 %) and waterlogged conditions also favour BLB, but the spread is rapid under heavy rainfall, strong wind and shade (Klement, Rudolph and Sands, 1990). Wetland areas also encourage the presence of the disease. Severe winds cause wounds which become entry points of the pathogen. Excessive application of nitrogen, close spacing and deficiency of potassium are factors that favour the development of BLB (IRRI, 2002). Irrigation water and splashing or windblown rain can disseminate *X. oryzae* pv. *oryzae* from plant to plant. The use of trimming tools for transplanting and by handling during transplanting can also trigger new infection (Mcgrath, *et al.*, 1999).

2.4 Diversity and genetic resistance of *Xanthomonas oryzae* pv. *oryzae*

There are over 30 reported races of *X. oryzae* pv. *oryzae* isolates worldwide (Noda *et al.*, 2001). A set of races identified in the Philippines using differential rice cultivars has been used worldwide for identifying and classifying resistance to BLB in other cultivars (Lee *et al.*, 2003). *Xanthomonas oryzae* pv. *oryzae* also has a high degree of genetic diversity among different isolates, based on restriction fragment length (RFLP) and pathotype analyses of more than 300 strains from different parts of the world, using a repetitive insertion sequence element as the RFLP probe (Ryba-White, 1995).

Xanthomonas oryzae pv. *oryzae* haplotypes were reported in the collection of strains from China, 13 were detected from India, 16 were detected from Indonesia, 17 were detected from Korea, 7 were detected from Malaysia, 30 were detected from Nepal, and 38 were detected from the Philippines (Noda *et al.*, 2001). A wide distribution of lineages

over time and location, suggests movement and long-term survival of *X. oryzae* pv. *oryzae* (Ogawa *et al.*, 1991). In the Philippines, prevalent of *X. oryzae* pv. *oryzae* was detected in the lowland areas prior to the deployment of the *Xa-4* gene for resistance to bacterial blight. After the deployment of *Xa-4*, new virulent pathotypes were detected in upland areas.

Rice differential cultivars carrying the *Xa-4*, *xa-5*, *Xa-7*, *Xa-10* and *Xa-11* genes were tested for resistance in the Philippines. Differential cultivars with the *xa-5* gene were regionally differentiated (Ryba-White, 1995). Many South Asian rice cultivars possess the *xa-5* gene and most of the *X. oryzae* pv. *oryzae* strains from Nepal and India were virulent to cultivars carrying the *xa-5* gene for resistance (Sidhu *et al.*, 1998).

The Philippine strains are virulent to cultivars with the *xa-5* gene (Mew *et al.*, 1993). Strains from Malaysia, Indonesia and Korea have been reported to be avirulent to cultivars carrying the *xa-5* gene while Indonesian strains were reported to be homogeneous in reactions to the rice differential cultivars (Adhikari *et al.*, 1995). In contrast, the strains from the Philippines were reported to be relatively diverse represented the six pathotypes distinguished by the rice differential set utilized (Nelson *et al.*, 1994). Furthermore, reports indicate that new pathotypes were identified among the Philippine populations of *X. oryzae* pv. *oryzae* (Vera Cruz *et al.*, 1992).

2.5 Control

Bacterial leaf blight (BLB) disease is controlled by either one or a combination of cultural, chemical spray, resistant genotype, physical, and biological methods. The cultural methods includes the use of healthy seeds, clean field which is done by removing diseased stubble and straw from the field, direct sowing, keeping the nursery not

submerged under water and avoiding excess application of nitrogenous compounds and separating irrigation canals for each field are recommended (Singh and Monga, 1985) .

Chemical control is another control measure against rice BLB disease. This method has been used for a long time. In controlling the BLB disease in the early growth phase, Bordeaux mixture showed a protective effect to some extent although this chemical was phytotoxic to rice plants (Hori, 1993). Dipping rice seedlings in antibiotics at transplanting has also been proposed (Durgapal, 1983). Systemic bactericides were developed such as Agrimycin 100, Agrimycin 500, Agric. Terramycin 17, A.S. 50 and Streptocycine, Agric. Terramycin 17, Brestanol, Agrimycin 500 and a combination of Agrimycin 100 + Fytolan have been reported to be effective against BLB disease (Takahi, 1985). An application of a stable bleaching powder to standing water to provide 19 ppm chlorine helped to reduce the disease in India. In India and Korea Nickel dimethyl dithiocarbamate (Sankel) was used to control BLB disease in rice plants (Chand *et al.*, 1997).

However, these chemicals were not effective to some extent in controlling BLB disease which led to the introduction of a novel compound, 2-amino 1, 3, 4-thiadiazole (ATDA: TF128), which was efficient in controlling the BLB disease (Webster and Gunnell, 1992). A year later Hori (1993) reported that ATDA: TF128 had acute adverse symptoms to human health. Pyrazole derivate 3-chloro-1*H*-pyrazole-5-carboxylic acid (CMPA) exhibited high anti-rice blast activity without any significant anti-microbial activity but after assessment of its mode of action of CMPA, it was found that CMPA reduced BLB disease symptoms in a dose-dependent manner (Nishioka *et al.*, 2005).

Physical control of BLB disease has been applicable by hot water treatment of seeds for 30 minutes at 52 °C preceded by pre-soaking for 8 – 10 hrs, seed disinfection, soaking the

rice seeds in 50 ppm solution of Zhongshengmycin (Zhang *et al.*, 1996). A test was conducted to investigate the effect of seed cleaning with fresh water and washing with 20 % brine solution. It was found that the seeds washed with fresh water or washed with brine solution reduced the incidence of BLB disease (Anwar-ulHaq *et al.*, 2002).

It has also been reported that the use of chemicals to control disease had adverse effects on the environment and human health (Hori, 1993). Physical and cultural methods could not be effective.

Nishioka *et al.* (2005) reported that bacterization of rice seeds with fluorescent pseudomonad, inoculation of rice plants with *X. oryzae* pv. *oryzae* mixed with *Erwinia herbicola* controlled the BLB disease. Bacterial antagonists of *X. oryzae* pv. *oryzae* have received particular attention as biological control agents, largely because of their rapid growth, easy handling and effective colonization of the rhizosphere (Bruce and Celeste 2002). In India, about 40 bacterial isolates antagonistic to *X. oryzae* pv. *oryzae* were identified through *In-vitro* and field assays. Among those antagonists' native strains of the rice-associated rhizobacteria, *Pseudomonas fluorescens* and *P. putida* strain. *Rhizoctonia solani* significantly suppressed rice bacterial blight severity when sprayed on leaves (Cook *et al.*, 1993). Anuratha and Gnanamanickam, (1987) also reported *Aspergillus* sp. and *Penicillium* sp. to be antagonistic to *X. oryzae* pv. *oryzae*. These fungal species inhibited the growth of *X. oryzae* pv. *oryzae* in rice cultivars thus, were used as biological control agents against rice BLB disease.

The use of resistant rice varieties to control BLB disease in rice has also been emphasized (IRRI, 2002). This method of disease control is applicable against bacteria, viral and fungal diseases in rice worldwide. It is the main control measure presently available since no other control method is economically effective (Bogdanove and Ronald, 2006).

CHAPTER THREE

3.0 MATERIAL AND METHODS

Pot and field experiments were conducted to analyse the population structure of *X. oryzae* pv. *oryzae* in Bunda, Kyela and Dakawa, and screen for rice against *X. oryzae* pv. *oryzae* using 21 near isogenic lines (NILs) and a local popular variety Kilombero. The field and pot experiments were conducted between January and July, 2013.

3.1 Study location

The field studies were conducted in Bunda, Morogoro and Mbeya. Geographically, Bunda lies between latitudes -2.0185322 / -2° 1' 6.7146", longitude 33.8746152 / 33° 52' 28.6134 south of the Equator. Morogoro is located at latitude 9° 10' 01" S and longitude 36° 09' 34" E and Mbeya is located at 08°54'00"S 33°27'00"E. The laboratory work was conducted at the Agricultural Research Institute – Uyole (ARI-Uyole) and the African Seed Health Centre (AfSHC), Sokoine University of Agriculture, Morogoro.

3.2 Rice cultivars, near isogenic lines and experimental designs

Twenty one rice cultivars and differential rice lines comprising of near isogenic lines (NILs) and varieties were used (Table 1). These NILs were developed as international differential rice varieties for *X. oryzae* pv. *oryzae*. These NILs were sown by drilling in trapping nurseries of 7 m x 11.6 m plot size at each location of Bunda, Morogoro and Kyela. Sowing included a local popular variety Kilombero susceptible to bacterial leaf blight disease. Near isogenic lines were provided by the AfricaRice Program, Dar-es-salaam, Tanzania while rice cultivars were provided by Agricultural Research Institute-Uyole. Each rice cultivar was replicated four times in a Randomized Complete Block Design (RCBD).

Table 1: List of near isogenic lines (NILs) and rice cultivars used in the field and screen house experiments to test against bacterial leaf blight disease

Near isogenic lines (NILs)[a]		Rice cultivars[b]
Differentials lines	**Resistant gene**	
IR-BB1	*Xa-1*	Pijo
IR-BB2	*Xa-2*	Malemata
IR-BB3	*Xa-3*	Rangi Mbili
IR-BB4	*Xa-4*	Domo la Fisi
IR-BB5	*Xa-5*	Tule na Bwana
IR-BB7	*Xa-7*	Jicho la Samola
IR-BB8	*Xa-8*	Kihogo
IR-BB10	*Xa-10*	Mwasungo
IR-BB11	*Xa-11*	Rufiji
IR-BB13	*Xa-13*	Selena
IR-BB14	*Xa-14*	SARO 5
IR24	*Xa-16*	Mtalima Wangu
IR-BB21	*Xa-21*	Zambia
IR-BB50	*Xa-4/xa-5*	Japan
IR-BB51	*Xa-4/xa-13*	Kilombero
IR-BB52	*Xa-4/xa-5*	Supa
IR-BB53	*Xa-4/xa-13*	Mwangulu
IR-BB54	*Xa-4/Xa-21*	Shingo ya Mwali
IR-BB55	*Xa-5/xa-13*	Mwaya
IR-BB59	*Xa-5/xa-13/Xa-21*	Yanga
IR-BB60	*Xa-4/xa-5/xa-13/Xa-21*	Wahiwahi
Control	-	IR24

Sources of seeds: [a] **AfricaRice Centre, Dar-es-Salaam, Tanzania.**
[b] **Agricultural Research Institute–Uyole Mbeya, Tanzania.**

In the screen house, the 21 rice cultivars were arranged in Completely Randomized Design (CRD). One plastic pot per cultivar per isolate in four replications was used.

3.2.1 Pot size and planting media

Pots of 57 cm x 40 cm were used. The soil/humus mixture of soil: humus (1:3) was first sterilized as recommended by Namai and Ehara (1986) before sowing.

3.2.2 Sowing and fertilizer application

Each differential rice line was sown at a spacing of 10 cm x 10 cm in four rows, four seeds per hill. In the screenhouse, each pot was sown with 24 seeds. Fertilizer application was done as recommended by Sere *et al.* (2005).

3.3 Sources of inocula and sample collection

According to Akhtar and Hameed (2008), active rice disease lesions are the best source of inoculum. Diseased rice leaves showing typical bacterial leaf blight symptoms were collected based on the random sampling method, on rice plants at heading to about maturity stages as the disease usually develops well in these plant growth stages (Sere, 2005). The samples were collected from NILs in the trapping nursery and indigenous cultivated varieties from various rice farmers' fields over the three study locations of Bunda, Kyela and Dakawa. Diseased leaves were detached and put into paper envelopes. These envelopes were labeled with variety name, incidence, plant growth stage, name of the collector, location and sampling date. Sampled rice leaves were put into the plastic boxes containing silica gel for absorbing moisture. The samples were then taken to the Agricultural Research Institute–Uyole (ARI-Uyole) and the African Seed Health Centre (AfSHC) laboratories and kept in the refrigerator for further processing.

3.4 Isolation of *Xanthomonas oryzae* pv. *oryzae*.

Infected leaves were cleaned with tap water and sterilized with 70 % alcohol, washed five times in sterile distilled water and cut into small pieces about 5 mm x 5 mm in size and put into the test tubes containing 1ml of sterilized distilled water (Plate 1). The samples were comminuted with a glass rod and allowed to stand for about 5 to 10 minutes to allow the bacteria to ooze out from the leaf tissues. Using the sterilized loop needle, bacterial suspensions were streaked onto petri dishes containing nutrient agar (NA) medium. The inoculated plates were incubated at 28-30 ^{0}C for 72 hrs (Nayak *et al.*, 2008). Single yellow colonies that emerged were selected and transferred onto yeast glucose agar medium for purification and incubated at 28 ^{0}C for 72 hrs (Stead, 1990).

Plate 1: Isolation of *Xanthomonas oryzae* pv. *oryzae* from infected rice leaf samples collected in Mbeya, Morogoro and Bunda.

3.5 Identification and storage of *Xanthomonas oryzae* pv. *oryzae* isolates

3.5.1 Gram reaction

This test aimed at differentiating bacteria into two broad groups: Gram–positive and Gram-negative bacteria. A thinly spread air-dried bacterial film was fixed on clean glass slide by a light flame. The specimen were treated with 0.5 % aqueous crystal violet

solution for 30 seconds and afterwards washed with running tap water for one minute, rinsed in water and decolorized with 95 % ethanol. The specimens were again rinsed with tap water and counter-stained for 10 seconds. (Akhtar *et al.*, 2008). It was eventually washed with sterile distilled water and observed under the microscope for colouration.

3.5.2 Potassium hydroxide (3 % KOH) test

Gram staining results were confirmed by 3 % potassium hydroxide test. The bacteria were aseptically removed from Petri plates with a wire loop, placed on glass slide in a drop of 3 % KOH solution and stirred by hand for 10 seconds (Lelliott and Stead, 1987). Presence of slimy mucous was considered positive and absence of mucous was considered negative.

3.5.3 Starch hydrolysis test

Powdered nutrient agar (NA) was dissolved in water by progressive heating. Starch was then dissolved in distilled water separately and added to the molten agar with constant stirring. An aliquot of this basal medium was dispensed in conical flask and sterilized at 121 ^{0}C for 10 minutes. The medium was then poured in petri plates into which each isolate was transferred aseptically and incubated at 28 ^{0}C for 72 hours. After scraping the superfluous growth, the plates were flooded with Lugol's iodine, which was prepared by dissolving iodine along with potassium iodide in distilled water, and stirred for four hours (Schaad, 1988). Yellowish colour, clear zones around the bacterial growth indicated amylase activity revealing a positive reaction.

3.5.4 Copper nitrate test

Copper nitrate test was done using *X. oryzae* pv. *oryzae* selective medium (cupric nitrate medium). The composition of this medium was 30 g nutrient agar and 0.01 g copper nitrate, $Cu(NO_3)_2$ in 1000 ml sterile distilled water. This medium was autoclaved for 20

minutes, cooled at 50 ^{O}C and poured into the petri plates. Bacterial colonies from yeast glucose agar (YGA) medium were transferred into the petri-dishes containing cuppuric nitrate medium, and incubated at 28 ^{O}C for 72 hours. Yellow and viscous colonies revealed growth of *X. oryzae* pv. *oryzae* isolates (Xie and Mew, 1998). Bacterial strains were maintained at the AfSHC laboratory at +4 ^{O}C for immediate use and at -20 ^{O}C for long storage in 50 % glycerol (Stead, 1990).

3.5.5 Pathogenicity test

Xathomonas oryzae pv. *oryzae* isolated from infected rice leaf samples were tested for pathogenicity by clip inoculation (Akhthar *et al.*, 2008; Sere *et al*, 2005). This was done on 21-day-old rice seedlings of susceptible cultivars Kilombero and IR24 grown in the screenhouse. A 72-hour-old culture of each isolate incubated at 28 ^{O}C in yeast glucose agar medium was used as inoculum. The inoculum was prepared by scraping the bacterial colonies in petri-dishes and suspended in 10 ml of distilled water. The spectrophotometer was used to adjust the absorbance (A) of the inoculum to A= 0.05 to contain a concentration value of 10^{8} cells per ml. (Habarurema *et al.*, 2013). To avoid loss of viability of *X. oryzae* pv. *oryzae*, the inoculum was used within 60 minutes after preparation.

Rice leaves of each cultivar in each plastic tray were clip inoculated 1-3 inches on the fully expanded leaf of 21-day-old rice plants (Liu *et al.*, 2007). This was done using a pair of scissors every time dipped into the *X. oryzae* pv. *oryzae* suspension. Inoculum was prepared from a 72-hr-old actively growing culture of each isolate grown on NA and YGA media. Inoculation was conducted in the evening and inoculated leaves were immediately covered with polyethylene bags, to reduce possible noxious effects of high temperature and entry of bacteria into infection courts in the presence of sufficient moisture on the leaf

surface. Inoculated plants were maintained at temperature ranging 25 to 33 ^{o}C and 60 to 80 % relative humidity as recommended by Singh *et al.* (2001). Plants were examined for BLB disease symptoms 3 and 7 days after inoculation. Therefore, elongation of a yellowish-grey necrotic zone of tissue away from the point of inoculation was scored as a positive reaction. To confirm that the symptoms that appeared on inoculated rice plants were caused by *X. oryzae* pv. *oryzae*, diseased leaves showing typical BLB symptoms were detached and bacteria were re-isolated as in section 3.3 of the current study. The pathogens were identified on the basis of consistency in causing disease reaction on the two susceptible inoculated varieties. Isolates were considered pathogens because all rice plants inoculated showed positive reactions.

3.6 Data collection

Rating of bacterial leaf blight disease incidence and severity in nurseries was done progressively at 21, 28 and 35 days after disease symptoms appearance while in the screen house; BLB disease rating was done at 7,14 and 21days after inoculation. For each disease rating, plants were carefully examined for development of typical rice BLB disease symptoms. Three infected rice plants were assessed randomly per plot and pot in field and screenhouse experiments, respectively for BLB disease incidence and severity (Plate 2) as recommended by Gnanamanickam (1999).

Bacterial leaf blight disease severity rating was done using a scale of 0-9 Where: 0 = immune or highly resistant (HR), 1= resistant (R), 3 = moderate resistant (MR), 5= moderately susceptible (MS), 7 = susceptible (S) and 9 = highly susceptible. (Dewa *et al.*, 2011; Gnanamanickam, 1999).

Plate 2: Rice bacterial leaf blight disease severity rating

3.7 Data analysis

The data included BLB disease lesion length and disease incidence. Disease incidence was estimated by counting the number of BLB symptomatic rice leaves, expressed as percentage of the total number of plants per plot. The mean BLB disease lesion length (MDLL) was expressed as the sum of numerical rating/Total number of leaves observed x Maximum rating as recommended by (Wheeler, 1969; Gnanamanickam, 1999). Where; MDLL = mean bacterial blight disease lesion length (severity) in centimetre. The calculated lesion length data for each differential line were transformed at $(X+1)^{1/2}$ as recommended by (Snedecor and Cochran, 1989; Herbarulema *et al.*, 2013). Transformation increased the precision with which the differences between small calculated means were measured. The transformed data were subjected to analysis of variance to test the homogeneity between plots and the significant differential lines using GenStat. Duncan's Multiple Range Test (DMRT) was used to determine differences between means.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Bacterial blight incidence and severity to near isogenic lines (NILs)

The results obtained from the NILs test against bacterial leaf blight disease indicated that rice lines IR-BB4 and IR-BB52 were resistant (Table 2). Bacterial leaf blight disease severity and incidence on rice lines IR-BB4 and IR-BB52 were as low as 0.5 cm and 6.72 %, respectively at Kyela, Morogoro and Bunda. High bacterial leaf blight disease incidence and severity of 61.55 % and 6.12 cm, respectively on differential rice line IR-BB14 was recorded at Dakawa (Table 3). This indicates that lines with low rating values of incidence and severity are promising resistant material and those lines with high rating values are susceptible against the BLB pathogen (Madden and Hughes, 1999). In addition, responses of NILs revealed variation in BLB pathogen populations in this study.

These findings of resistance and susceptibility of differential rice lines IR-BB52 and IR-BB14, respectively, agree with those of Onasanya *et al.* (2009) who demonstrated population diversity of *X. oryzae* pv. *oryzae* and resistance of 24 near isogenic lines against bacterial leaf blight disease in three countries. Therefore, these lines can be used as genetic sources for resistance against bacterial leaf blight disease (Banito, *et al.*, 2012). All multi-gene lines with the exception of IRBB4 and IR-BB52 showed moderate resistant while others were susceptible against bacterial leaf blight disease. Line IR-BB4 with a single gene *xa-4* for resistance was observed to be resistant to BLB across the two locations at Dakawa and Bunda.

Table 2: Percentage of bacterial leaf blight disease incidence for near isogenic lines (NILs) with their respective responses to strains of *Xanthomonas oryzae* pv. *oryzae* in Mbeya, Morogoro and Bunda

Near isogenic lines	[x]Mean disease incidence (%)			Response[y]		
	Kyela	Moro goro	Bunda	Kyela	Morogoro	Bunda
IR-BB1	34.96g-o	47.02m-s	24.77c-j	MS	MS	MR
IR-BB2	29.16e-k	50.41n-s	18.49a-g	MS	MS	MR
IR-BB3	36.90h-p	49.67n-s	29.49e-k	MS	MS	MR
IR-BB4	19.02a-g	10.41a-c	8.18ab	MR	R	R
IR-BB5	34.53g-n	51.86p-s	20.06a-h	MS	S	MR
IR-BB7	31.38f-m	51.00o-s	26.38c-j	MS	S	MR
IR-BB8	23.26b-i	49.86n-s	23.34b-i	MR	MS	MR
IR-BB10	31.20f-n	56.10rs	40.21j-r	MS	S	MS
IR-BB11	40.80j-r	46.40l-s	23.21b-i	MS	MS	MR
IR-BB13	31.73f-m	51.63p-s	11.35a-d	MS	S	MR
IR-BB14	29.01e-k	53.74q-s	27.62d-j	MR	S	MR
IR24	28.41e-j	45.31k-s	29.99e-k	MR	MS	MR
IR-BB21	26.36b-i	61.55s	30.84f-m	MR	S	MS
IR-BB50	31.46f-m	52.36p-s	18.62a-g	MS	S	MR
IR-BB51	27.56d-j	50.93n-s	22.21a-h	MR	S	MR
IR-BB52	21.88a-h	13.18a-e	6.72a	MR	MR	R
IR-BB53	39.27i-q	52.28p-s	24.60c-j	MS	S	MR
IR-BB54	34.55g-n	49.24n-s	19.36a-g	MS	MS	MR
IR-BB55	29.67r-k	52.58p-s	29.19e-k	MR	S	MR
IR-BB59	27.33d-j	54.02q-s	24.86c-j	MR	S	MR
IR-BB60	30.52f-l	57.76s	15.68a-f	MS	S	MR
Control	17.50a-f	52.38p-s	55.21qrs	MR	S	S
LSD						2.838
SE ±						9.544
CV (%)						28.0

[x]Within a column, means followed by the same letter (s) are not significantly different ($P \leq 0.05$) by Duncan's multiple range test. Data are means of four replications.

[y]Scale of 0 - 9 rice bacterial blight disease assessment in which 0 % = 0 = highly resistant (HR), > 1 - 10 % = 1 = resistant (R), > 10 - 30 % = 3 = moderate resistant (MR), > 30 - 50 % = 5 = moderate susceptible (MS), > 50 - 75 % = 7 = Susceptible, > 75 - 100 % = 9 = highly susceptible.

However, previous researchers indicate that lines with combined genes for resistance exhibit more effective and more durable resistance against *X. oryzae* pv. *oryzae* than the resistance conferred by single genes (Singh *et al.*, 2001). Different responses to BLB were observed on the other rice lines and were grouped as moderate resistant, intermediate and susceptible. Earlier findings also indicate that, under field conditions *X. oryzae* pv. *oryzae* can infect leaves of both resistant and susceptible rice NILs at different infection responses when native inocula is naturally introduced into young rice plants (Dewa *et al.*, 2011), suggesting breakdown of resistance or plant age specific resistance.

Under conditions of the current study, the first BLB disease symptoms were observed 34 days after seed germination with the exception of differential lines IR-BB4 and IR-BB52 in Morogoro and Bunda, respectively. On these lines, BLB disease symptoms were first observed 39 days after seed germination. Of the 21 near isogenic lines tested, ten did not differ significantly ($P \leq 0.05$) in response to the BLB disease in Morogoro. However, highly significant differences between lines were observed in all lines grown at Bunda and Kyela.

In the present study, BLB symptoms in resistant near isogenic lines IR-BB4 and IR-BB52 took longer time to appear than on susceptible lines IR-BB1, IR-BB5, IR-BB7, IR-BB10, IR-BB14, IR-BB21, IR-BB50, IR-BB51, IR-BB53, IR-BB55, IR-BB59 and IR-BB60 (Table 3).This implies that, infection by the BLB pathogen in resistant plant tissues was slower than in susceptible plants (Tabei, 1997). In addition, a higher pathogen populations is required in resistant than in susceptible rice plants to produce BLB symptoms (Leach *et al.*, 1992). It was also noted that vascular tissues of susceptible NILs were highly infected by *X. oryzae* pv. *oryzae* with mean severity of 6.12 cm (Table 3) thus, supported higher populations of *X. oryzae* pv. *oryzae* than those of resistant genotypes (Abbasi *et al.*, 2011).

Table 3: Bacterial blight disease severity for Near Isogenic Lines (NILs) and rice cultivars with their respective responses to *Xanthomonas oryzae* pv. *oryzae* in Morogoro, Kyela, Bunda and ARI-Uyole

Differentials	Field experiments severity (cm)[s]						Screenhouse experiments			
	[q]Site			Response[t]						
	Kyela	Morogoro	Bunda	Kyela	Morogoro	Bunda	Rice cultivars	Isolates[r]	Severity (cm)	Response
IR-BB1	3.0ab	4.38c	2.625a	MR	MS	MR	Pijo	TB2F	5.0ab	MS
IR-BB2	3.875ab	5.34cd	2.2a	MR	MS	MR	Malemata	TK3CD	5.375b	MS
IR-BB3	3.2ab	4.97cd	2.375a	MR	MS	MR	Rangi Mbili	TK4E	3.25ab	MR
IR-BB4	1.925ab	1.3ab	0.5a	MR	R	R	Domo la Fisi	TM2C	2.875a	MR
IR-BB5	4.375ab	5.42cd	2.75a	MS	S	MR	Tule na Bwana	TK4E	3.75ab	MR
IR-BB7	3.75ab	5.27cd	3.875a	MR	MS	MR	Jicho la Samola	TK3CD	3.625ab	MR
IR-BB8	2.375ab	4.71cd	2.375a	MR	MS	MR	Kihogo	TB5A	4.625	MR
IR-BB10	2.75ab	5.62cd	5.4c	MR	MS	MS	Mwasungo	TK4E	3.45ab	MR
IR-BB11	3.625ab	5.67cd	2.3a	MR	MS	MR	Rufiji	TM2C	3.625ab	MR
IR-BB13	3.375ab	4.16c	1.875a	MR	MS	R	Selena	TM2B	4.25ab	MR
IR-BB14	2.5ab	6.12d	4.5a	MR	S	MR	SARO 5	TB2F	2.875a	MR
IR24	3.5ab	5.4cd	3.375a	MR	MS	MR	MtalimaWangu	TM2B	3.625ab	MR
IR-BB21	2.375ab	5.45cd	4.125a	MR	MS	MS	Zambia	TB5A	4.125ab	MR
IR-BB50	2.375ab	4.98cd	2.375a	MR	MS	MR	Japan	TM2B	5.125ab	MS
IR-BB51	3.375ab	4.608cd	2.0a	MR	MS	R	Kilombero	TM2C	3.625ab	MR
IR-BB52	2.0ab	1.25ab	0.65a	R	R	R	Supa	TB5A	4.12ab	MR
IR-BB53	4.375ab	4.983cd	4.25a	MS	MS	MS	Mwangulu	TM2B	3.5ab	MR
IR-BB54	4.4ab	4.166cd	2.125a	MS	MS	MR	Shingo ya Mwali	TK3CD	3.875ab	MR
IR-BB55	3.125ab	5.66cd	2.75a	MR	MS	MR	Mwaya	TB2F	3.75ab	MR
IR-BB59	3.5ab	5.625cd	2.75a	MR	MS	MR	Yanga	TM2C	5.0ab	MS
IR-BB60	3.5ab	6.075d	2.925a	MR	S	MR	Wahiwahi	TB5E	4.125ab	MR
Control	4.27ab	5.558cd	11.15ab	MS	MS	S	IR24	TK4E	8.25b	S

[q]Within a column, means followed by the same letter (s) are not significantly different ($P \leq 0.05$) by Duncan's multiple range test. Coefficient of variation = 38.8, standard error = 1.356. Data are means of four replications. [r]T = Tanzania, K = Kyela isolate, M = Morogoro isolate, B = Bunda isolate, Numbers = Plates.
[s]Mean lesion length for each differential line were transformed at $(X+1)^{1/2}$
[t]Scale of 0 - 9 rice bacterial blight disease assessment in which 0 % = 0 = highly resistant (HR), > 1 - 10 % = 1 = resistant (R), > 10 - 30 % = 3 = moderate resistant (MR), > 30 - 50 % = 5 = moderate susceptible (MS), > 50 - 75 % = 7 = Susceptible, > 75 - 100 % = 9 = highly susceptible.

Different authors have reported that resistant rice plants normally habour population of plant pathogenic bacteria with no distinguishable BLB necrotic lesions (Baso *et al.*, 2011; Akhthar, 2003). Such reports also exist for other host-pathogen systems (Mabagala, 1997; Bruce and Seleste, 2002). Therefore, the use of seeds from resistant rice plants grown in areas where BLB occurs may be a source of inoculum. This is because *X. oryzae* pv. *oryzae* is among the seed borne pathogens in rice thus, it can over season in rice seeds and cause BLB disease in other rice fields when such rice seeds are planted without being treated with chemicals.

It should also be noted that even resistant rice plants grown under contaminated fields can produce both BLB infested and infected rice seeds. Mortensen (2000) demonstrated that seeds of resistant plants can become infected with plant pathogenic bacteria in the same way as seeds from susceptible rice plants under artificial inoculation. Under natural conditions, this may not be the case because the response of a plant in the field is a combination of genotype and environmental factors (Shanti *et al.*, 2001).

Performance of rice lines containing the same gene for resistance to *X. oryzae* pv. *oryzae* is a major concern in breeding programs and rice production. This is because rice seeds from resistant rice plants may transmit *X. oryzae* pv. *oryzae* to new rice growing areas. Rice seeds from these plants may also introduce the pathogen randomly throughout the field and provide numerous foci for primary infection of rice leaves. Such inoculum is more important than that spreading from rice plant leaves in the field (Lee *et al.*, 2003). In addition, detection of symptomless rice seed in resistant rice plants is difficult due to the low frequency in seed lots and sometimes less number of *X. oryzae* pv. *oryzae* populations within such seeds (Di *et al.*, 1991; Jeung *et al.*, 2006).

Banito *et al.* (2012) using differential lines also observed variation on BLB pathogen populations in seven African countries. These authors found that rice lines IR-BB10, IR-BB13 and IR-BB14 produced BLB disease symptoms under field inoculation. Studies on variation between locations on populations of *X. oryzae* pv. *oryzae* using 24 near isogenic lines with known resistance genes of *X. oryzae* pv. *oryzae* in China have also been reported (Liu *et al.*, 2007). The authors found that IRBB13, IRBB10, IRBB11 and IRBB14 were the most susceptible of the 24 lines tested against bacterial leaf blight disease. Such findings imply that these lines harbor high pathogen populations of BLB disease. Furthermore, these findings suggest that the genes/gene combinations involved are ineffective against *X. oryzae* pv. *oryzae* populations in such localities (Shanti and Shenoy, 2005). The line which was resistant across the two locations used in the current study, Bunda and Morogoro (IR-BB4) was moderate resistant at Kyela. Furthermore, lines IR-BB13 and IR-BB51 were resistant against BLB disease at Bunda but moderate resistant at Kyela and Morogoro.

Shenoy (2005) reported that rice lines may respond differently against the BLB pathogen populations across locations because of diversity in population structure across locations. This implies that a wide variability in virulence among the bacterial strains is a major constraint in breeding programs for BLB resistance (Shanti *et al.*, 2001). However, attempts have been made worldwide to study the population structure of *X. oryzae* pv. *oryzae* and to group bacterial strains into virulence categories using a set of differential lines (Kaku, 1993). These studies suggest that, further studies are needed to assess the pathogenic variation using differential lines in Tanzania; and provide the necessary information needed for the control of bacterial leaf blight disease and strengthen our understanding of the population structure of *X. oryzae* pv. *oryzae* in rice genotypes.

4.2 Isolation and characterization

Isolation of *X. oryzae* pv. *oryzae* was done from infected rice leaf samples. On NA and YGA media after 48 hours of incubation yellow, smooth, convex and viscous colonies were observed. These colonies became irregular at 72 hours due to viscous fluids secreted by the bacteria. Khan (2000) also observed similar colonies in these culture media (Plate3). Yellow color and mucoid colonies of *X. oryzae* pv. *oryzae* are cultural characteristics of Xanthomonads and are due to the production of extracellular polysaccharides; this is the EPS in media containing sugar (Roberts, 1996).

A total of six *X. oryzae* pv. *oryzae* strains namely; TK3CD, TK4E, TM2C, TM2B, TB2F and TB5A were isolated and identified from 110 samples collected in Morogoro, Mbeya and Bunda. These isolates exhibited slow growth in NA and YGA medium, Gram-negative, oxidase negative, reduced nitrate, L-alanine negative and produced a mucoid substance in 3 % Potassium hydroxide. Liu *et al.* (2007) reported similar results on NA medium when he isolated and characterized 50 *X. oryzae* pv. *oryzae* strains in China.

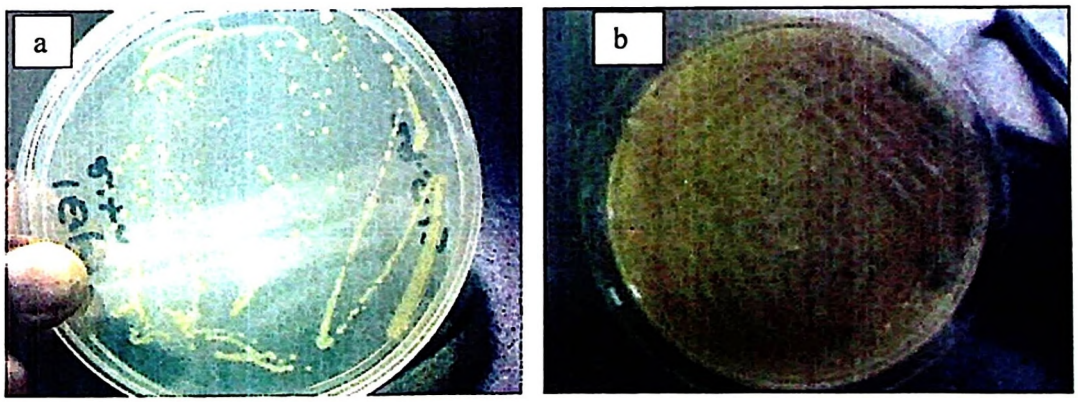


Plate 3: *Xanthomonas oryzae* pv. *oryzae* colonies on (a) nutrient agar medium (b) yeast glucose agar on petri-plates before and after purification, respectively.

4.2.1 Physiological and biochemical characters

Different biochemical tests were used to characterize the isolated pathogen. The identity of *X. oryzae* pv. *oryzae* was confirmed by physiological tests including Gram staining which demonstrated that the pathogen was a Gram - negative rod, producing yellow, convex and viscous colonies in nutrient and yeast glucose agar media which are also in agreement with the characteristics of *X. oryzae* pv. *oryzae* reported by other authors (Lelliott and Stead, 1987). All the isolates consistently gave similar results (Table 4). Three percent Potassium hydroxide test was performed in order to confirm the Gram staining results (Plate 4). Presence of mucous was considered positive and absence of mucous was considered negative. The selected isolates showed positive reaction and confirmed that they were Gram - negative.

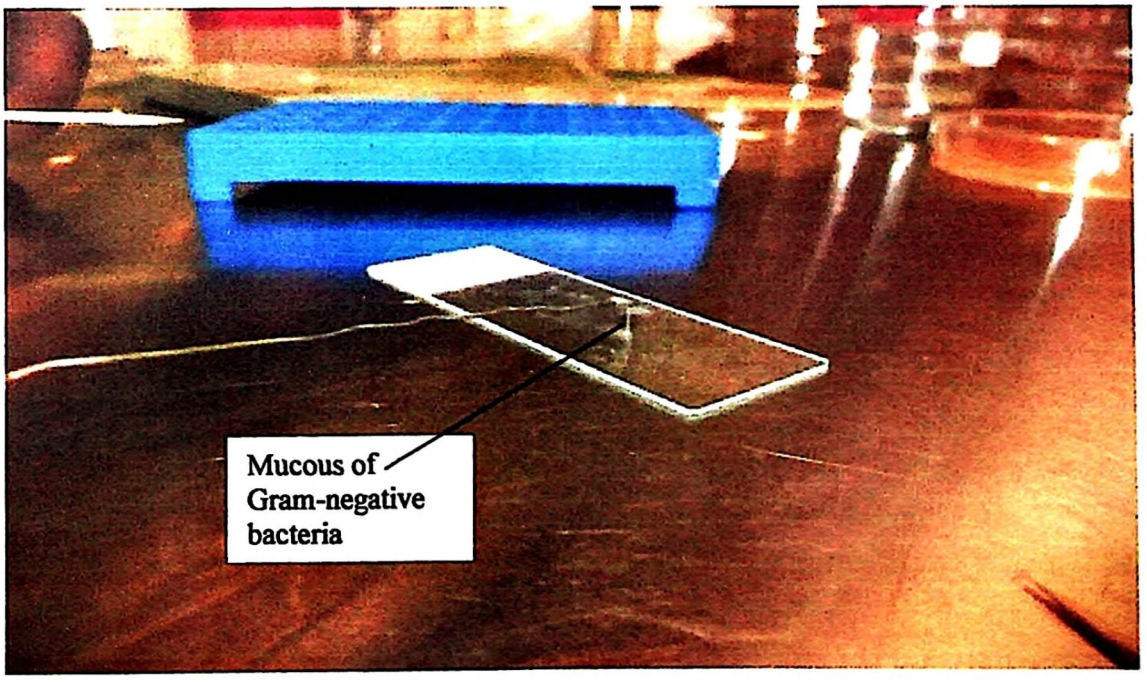


Plate 4: Mucous thread of Gram-negative *Xanthomonas oryzae* pv. *oryzae* on three percent Potassium hydroxide test

Table 4: Comparison of physiological and biochemical tests for isolates of *Xanthomonas oryzae* pv. *oryzae* isolated in the current study

Serial Number	Selected Isolate[x]	Biochemical test				
		Gram Reaction	Potassium hydroxide Test	Starch Hydrolysis	Copper Nitrate	L-alanine
1	TK1F	-	+	+	+	+
2	TK6CD	-	-	+	+	+
3	TK5E	-	+	+	+	+
4	TK5CD	-	+	+	-	+
5	TK3CD	-	+	-	+	-
6	TK4E	-	+	-	+	-
7	TK2E	-	+	+	-	+
8	TM1EF	-	+	+	+	+
9	TM2C	-	+	-	+	-
10	TM4CD	-	+	+	+	+
11	TM5CD	-	+	+	+	+
12	TM3F	-	+	+	+	+
13	TM2E	-	+	+	+	+
14	TM2B	-	+	-	+	-
15	TB2C	-	-	+	+	+
16	TB2F	-	+	-	+	-
17	TB2EF	-	+	+	+	+
18	TB5A	-	+	-	+	-
19	TB3B	-	+	+	+	+

[x]T = Tanzania, K = Kyela isolate, M = Morogoro isolate, B = Bunda isolate, Numbers = plate number, + = positive reaction and - = negative reaction.

4.2.2 Pathogenicity

Previous studies indicate that pathogenicity of *X. oryzae* pv. *oryzae* is studied using BLB susceptible rice varieties IR24 and Kilombero (Dewa *et al.*, 2011; Kihupi *et al.*, 2011). The isolated strains consistently incited lesions on susceptible IR24 and Kilombero test plants that were inoculated in the screen house.

Rice seedlings showed typical BLB symptoms which included slight curling near the clipped leaf tip 3-4 days after inoculation. Water-soaked symptoms also appeared at the point of inoculation. Leaf infection was evident 7 days of inoculation when the vascular tissues developed yellowish–grey symptoms on leaves. Thirty eight days after inoculation infected leaves of susceptible rice plants developed wilting symptoms. Infection with *X. oryzae* pv. *oryzae* isolates led to death of infected panicle or the entire rice plant. These symptoms correspond to a typical rice bacterial leaf blight necrosis of the vascular tissues which have been reported by several authors (Singh *et al.*, 2001).

Grayish-green or light brown symptoms on the vascular tissues, indicated the presence of *X. oryzae* pv. *oryzae* in rice seedlings (Baso *et al.*, 2011). All isolates of *X. oryzae* pv. *oryzae* tested were pathogenic because they caused typical BLB symptoms on susceptible rice varieties IR24 and Kilombero used in the current study. Such symptoms were similar to those on the susceptible control IR24 used. In the present study, the use of clip inoculation was found to be a very appropriate method in assessing pathogenicity of *X. oryzae* pv. *oryzae* isolates in susceptible rice plants because the isolates induced typical BLB symptoms. Akhtar *et al.* (2008) also reported similar findings.

4.3 Reaction of rice cultivars

There were no significant differences ($P \leq 0.05$) between rice cultivars on their reaction to BLB disease (Table 5). All the rice cultivars used in the study and also inoculated with *X. oryzae* pv. *oryzae* isolates produced typical BLB disease symptoms (Plate 5).

Plate 5: Rice plants thirty eight days after clip inoculation with *Xanthomonas oryzae* pv. *oryzae* isolates in the screenhouse (a) Domo la Fisi and (b) Wahiwahi.

Rice cultivars with mean score of > 10-30 % were grouped as moderate resistant and included "Domo la Fisi, Rangi Mbili, Tule na Bwana, Jicho la Samola, Rufiji, SARO 5, Mtalima Wangu, Zambia and Supa."Also rice cultivars with mean score of > 30-50 % were grouped as moderate susceptible and included "Mwangulu, Mwasungo, Kihogo, Pijo, Malemata, Selena, Japan, Kilombero, Mwaya, Shingo ya Mwali, Yanga and Wahiwahi."

Rice cultivars which gave a moderate resistant reaction to BLB disease, their leaves did not wilt at 38 days after inoculation. Such findings reflect partial resistance against *X. oryzae* pv. *oryzae* in rice cultivars Domo la Fisi, Rangi Mbili, Tule na Bwana, Jicho la Samola, Rufiji, SARO 5, Mtalima Wangu, Zambia and Supa (Banito *et al.*, 2012). Thus, these rice cultivars may be used in breeding against BLB disease.

Table 5: Percentage of disease incidence caused by *Xanthomonas oryzae* pv. *oryzae* isolates on the leaves of twenty one rice cultivars under artificial inoculations in the screen house at Agricultural Research Institute-Uyole (ARI-Uyole).

Rice Cultivars	Isolate[z]	[x]Disease Incidence[y] (%)	Response
Pijo	TB2F	36.45ab	MS
Malemata	TK3CD	37.09ab	MS
Rangi Mbili	TK4E	30.28ab	MR
Domo la Fisi	TM2C	23.34a	MR
Tule na Bwana	TK4E	30.21ab	MR
Jicho la Samola	TK3CD	29.60ab	MR
Kihogo	TB5A	32.10ab	MS
Mwasungo	TK4E	33.19ab	MS
Rufiji	TM2C	28.55ab	MR
Selena	TM2B	36.29ab	MS
SARO 5	TB2F	30.06ab	MR
Mtalima Wangu	TM2B	25.97a	MR
Zambia	TB5A	29.57ab	MR
Japan	TM2B	34.05ab	MS
Kilombero	TM2C	32.86ab	MS
Supa	TB5A	26.97a	MR
Mwangulu	TM2B	30.70a	MS
Shingo ya Mwali	TK3CD	37.86ab	MS
Mwaya	TB2F	36.57ab	MS
Yanga	TM2C	33.61ab	MS
Wahiwahi	TB5E	45.33b	MS
Control	TK4E	30.47ab	MR
LSD			N.S
SE ±			8.457
CV (%)			25.6

[x]Within a column, means followed by the same letter (s) are not significantly different ($P \leq 0.05$) by Duncan's multiple range test. Data were transformed by the relationship;
[y]Means percent rice bacterial blight disease incidence used to assess infection response were based on the scale of 0 – 9 giving values of categories; 0 % = highly resistant (HR), > 1 - 10 % = resistant (R), > 10 - 30 % = moderate resistant, > 30 - 50 % = moderate susceptible (MS), > 50 - 75 % = Susceptible, > 75 - 100 % = highly susceptible.
[z]T = Tanzania, K = Kyela isolate, M = Morogoro isolate, B = Bunda isolate, Numbers = plate number, + = positive reaction and - = negative.

However, early studies indicate that infection on susceptible rice cultivars by *X. oryzae* pv. *oryzae* produce clear and visible symptoms while intermediate reactions produced only limited symptoms or hypersensitive–like reaction localized at the point of inoculation (Cottyn *et al.*, 2001).

Other reports indicate that line IR-BB52 also tested resistant against the *X. oryzae* pv. *oryzae* race A3 in Mali and Burkina Faso (Baso *et al.*, 2010). However, previous findings indicate that some of *X. oryzae* pv. *oryzae* isolates from Japan and Korea were capable of inducing lesions longer than 7 cm to line IR-BB4 in the donor and engineered lines indicating susceptibility (Lin *et al.*, 1996).

This suggests that Tanzanian strains possess an avirulent gene that would be specifically recognized by IR-BB4. These findings reflect a different pathogenicity pattern between the Asian and Tanzanian isolates of *X. oryzae* pv. *oryzae*. It also highlights the diversity of *X. oryzae* pv. *oryzae* worldwide. Different reports also indicate the dissimilarities between African and Asian *X. oryzae* pv. *oryzae* strains and give further requirement for regional and local efforts for breeding against BLB disease (Gonzalez *et al.*, 2007).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The current investigation on population structure of *X. oryzae* pv. *oryzae* and screening of rice cultivars against this pathogen revealed that;

Six virulent pathotypes of *Xanthomonas oryzae* pv. *oryzae* namely; TK3CD, TK4E, TM2C, TM2B, TB2F and TB5A were available at Dakawa, Bunda and Kyela, which induced bacterial leaf blight disease of rice.

The rice lines IR-BB4 and IR-BB52 which showed resistance against *Xanthomonas oryzae* pv. *oryzae* pathotypes, are potential material for including in breeding programs for resistance against *Xanthomonas oryzae* pv. *oryzae*. However, IR-BB14 was significantly susceptible to the pathogen while intermediate resistance was observed in Domo la Fisi, Rangi Mbili, Tule na Bwana, Jicho la Samola, Rufiji, SARO 5, Mtalima Wangu, Zambia and Supa.

5.2 Recommendations

(i) Rice lines IR-BB4 and IR-BB52 which showed resistance to *Xanthomonas oryzae* pv. *oryzae* are potential sources of resistance for rice breeding against this pathogen in Bunda, Morogoro and Kyela. Intermediate rice cultivars namely; Domo la Fisi, Rangi Mbili, Tule na Bwana, Jicho la Samola, Rufiji, SARO 5, Mtalima Wangu, Zambia and Supa should further be used by farmers at Dakawa, Bunda and Kyela. This will reduce the possible occurance of epidemics of bacterial leaf blight disease in rice.

(ii) Further studies on molecular characterization of the pathotypes should be carried out to determine the genetic variation of the pathogen in rice in Tanzania.

REFERENCES

Abbasi, F. M., Masood, H., Ahmed, U., Khan, A. J. and Afzal, M. (2011). Molecular screening of Pakstani rice germplasm for *xa-5* gene resistance to bacterial blight. *African Journal of Biotechnology* 10:2833-2837.

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

Adhikari, T. B., Vera-Cruz, C. M., Zhang, Q., Nelson, R.J., Skinner, D.Z., Mew, T.W. and Leach, J. E. (1995). Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in *Asian Applied Environmental Microbiology* 61: 966–971.

Akhtar, M. A. (2003). Study on genetic variation in *Xanthomonas oryzae* pv. *oryzae* in relation to resistance in rice. First Annual Progress Report. Institute of Plant and Environmental Protection, National Agricultural Research Centre, Islamabad, Pakistan 110pp.

Akhtar, M. A., Rafi, A. and Hameed, A. (2008). Comparison of methods of inoculation of *Xanthomonas oryzae* pv. *oryzae* in rice cultivars. *Pakistan Journal of Botany* 40(5): 2171-2175.

Akthar, M. A., Zakria M., Abbasi, F. M. and Masood, M.A. (2003). Incidence of bacterial blight of rice in Pakistan during 2002. *Pakistan Journal of Botany* 35(5): 993-997.

Ali, A., Khan, M. H., Bano, R., Rashid, H., Raja, N.I. and Chaudhry, Z. (2009). Screening of Pakistan rice (*Oryzae sativa*) cultivars against *Xanthomonas oryzae* pv. *oryzae*. *Pakistan Journal of Botany* 41(5): 2595-2604.

Anonymous, G. (1996). Standard Evaluation System for Rice. Fourth edition. Inger Genetic Resources Centre, International Rice Research Institute, Manila, Philippines pp 20-21.

Anuratha, C. S. and Gnanamanickam, S. S. (1987). *Pseudomonas fluorescens* suppresses development of bacterial blight symptoms. *International Rice Research Newsletter* 12, 1, 17.

Anwar-ulHaq, A. K. M., aLMm, R., Nazrul, I. M. D., Anam, M.K., Asad-ul-dollah and Hossain, M. M. (2002). Effects of seed cleaning and washing on the incidence of bacterial blight of rice. Islamabad, Pakistan. 89pp.

Ashura, L. K., Mabagala., R.B. and Mortensen, C.N. (1999). Isolation and characterization of seed-borne pathogenic bacteria from rice (*Oryza sativa* L.) in Tanzania. *Tanzania Journal of Agricultural Sciences* 2(1):71-80.

Bashir, M. U., Akbar, N., Iqbal, A. and Zaman, H. (2010). Effect of different sowing dates on yield and yield components of direct seeded coarse rice (*Oryza sativa* L). *Pakistan Journal of Agricultural Science* 47: 361-365.

Baso, A. A., Onasanya., A., Issaka, S., Sido, A.Y., Haogui, A. (2011). Bacterial leaf blight of rice in Niger: Pathological diversity of isolates collected on irrigated lands. *Journal of Applied Bioscience* 38:2551-2563.

Banito, A., Kadai, A. E. and Sere, Y. (2012). Screening of rice cultivars for resistance to bacterial leaf blight disease. *Journal of Applied Bioscience* 53:3742-3748.

Bogdanove, A. J. and Martin, G. B. (2000). AvrProdependent Pto-interacting proteins and AvrPto-dependent interacting proteins in tomato. *National Academy of Sciences,* United States of America 97:8836-8840.

Bruce, A. M. and Celeste, L. (2002). Pathogen population genetics, evolutionary potential and durable resistance. *Annual Review Phytopathology* 40: 349-379.

Bucheyeki, T. L., Shennkalwa, E., Kadadi, D. and Lobulu, J. (2011). *Assessment of Rice Production Constraints and Farmers Preferences in Nzega and Igunga Districts.* Journal of Advances in Developmental Research 2 (1): 30-37.

Burdon, J.J. (1993). Genetic variation in pathogen populations and its implications for adaptation to host resistance, p. 41-56. In T. Jacobs and J. E. Parlevliet (editors), Durability of Disease Resistance. Kluwer, Dordrecht.

Chand,T., Singh, N., Sing, H. and Third, B.S. (1997). Field efficacy of stable bleaching powder to control bacterial blight of rice. International Rice Research Newsletter 4(4): 12-13.

Cook, R. J. (1993). Making greater use of introduced micro-organisms for biological control of plant pathogens. *Annual Review Phytopathology* 31:53-80.

Cottyn, B., Regalado, E., Lanoot, B., De Cleene, M., Mew, T.W., and Swings, J. (2001). Bacterial populations associated with rice seed in the tropical environment. Entomology and plant pathology division, International Rice Research Institute, Manila, Philippines. *Phytopathology* 91:282-292.

Devadath, S. (1998). Chemical control of bacterial blight of rice. Pages 89-98 in: Bacterial blight of rice. International Rice Research Institute, Manila, Philippines.

Dewa, K. M. K., Banito A., Onasanya A., Kpemoua, K. E. and Sere, Y. (2011). Rice bacterial blight in Togo: Importance of the disease and virulence of the pathogen. *Current Research Bacteriology* 4: 94-100.

Di, M. U., Schaad, N.W. and Roth, D. A. (1991). Selective recovery of *Xanthomonas species* from rice seed. *Phytopathology* 81: 1358-1363.

Durgapal, J. C. (1983). Management of bacterial blight of rice by nursery treatment - preliminary evaluation. *Indian Phytopathology* 36: 146-149.

Durgapal, J. C. (1998). Maintanance of virurence in *Xanthomonas campestris* pv. *oryzae* cultures. International rice research newsletter 13:13.

Ezuka, A. and Kaku, H. (2000). A historical review of bacterial blight of rice.Department ofgenetic resources II and I. *Bull National Institute Agrobiology Resource* 15:1-207.

Gnanamanickam, S.S., Priyadarisini, V.B., Narayanan, N.N, Vasudevan, P. and . Kavitha, S. (1999). An overview of bacterial blight disease of rice and strategies for its management. Centre for advanced studies in botany, University of Madras, Guindy Campus, Chennai 600 025, India. *Current Science* 77 (11): 1435-1444.

Gonzalez, C., Szurek, B., Manceau C., Mathieu, T., Sere, Y. and Verdier, V. (2007) Molecular and pathotypic characterization of new *Xanthomonas* strains from West Africa. *Molecular Plant-Microbe Interactions* 20:534-546.

Goto, M. (1992). *Fundamentals of Bacterial Plant Pathology*. Academic Press, New Yolk. London. 342pp.

Gu., K., Sangha, J.S., Li, Y. and Yin, Z.C. (2008). High-solution genetic mapping of bacterial blight resistance gene *Xa-10*. *Theory of Applied Genetics* 116:155-163.

Habarurema, I., Edema, R., Gibson, P., Lamo, J., Asea, G., Sere, Y. and Gasore, R.E. (2013). Population diversity of bacterial leaf blight isolates in Uganda. *Asian Journal of Plant Science Research* 3(1):1-9.

Hori, M. (1993). Nipon- noyaku Monogatari. Japan plant protection association, Tokyo, Japan. 622pp.

IRRI and Queensland University. (2001). *Rice Intergraded Pest Management.* An interactive information and identification system for integrated pest management in rice. University of Queensland and International Rice Research Institute. Accessed on 29/12/2013.

IRRI, (2002). Standard evaluation system for rice. International Rice Research Institute, Los Banos, Manila, Philippines 38pp.

Jabeen, R., Iftikhar., T. and Batool , H. (2012). Isolation, characterization, preservation and pathogenicity test of *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight disease in rice. *Pakistan Journal of Botany* 44(1): 261-26.

Jeung, J.U., Heu., S. G., Shin, M.S., Vera Cruz, C.M. and Jena, K.K. (2006). Dynamics of *Xanthomonas oryzae* pv. *oryzae* populations in Korea and their relationship to known bacterial blight resistance genes. *Phytopathology* 96: 867–875.

Kaku, H. (1993). Infection types in rice – *Xanthomonasoryzae* pv. *oryzae* interaction. *Journal of Agricultural Research* 27: 81-87.

Kauffman., H. E., Reddy, A.P.K., Hsieh, S.P.Y. and Merca, S. D. (1973). An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae* pv. *oryzae*. *Plant Diseases* 57: 537-541.

Khan, A. J., Jamil, F. F. and Gill, M. A. (2000). Screening of rice varieties/lines against bakanae and bacterial leaf blight (BLB). *Pakistan Journal Phytopathol*ogy. 12(1): 6-11.

Kihupi, A.N., Angeles, E.R and Khush G.S, (2001). Genetic analysis of resistance to bacterial blight, *Xanthomonas oryzae* pv. *oryzae*, in rice, *Oryza sativa* L. *Euphytica* 117 : 39-46.

Klement, Z. K., Rudoph, Z. K. and Sands, (Editors). (1990). Methods in phytobacteriology. Academiai Kiado, Budapest.

Leach, J.E., Rhodes, M.L., Vera, M.C.C., White, F.F., Mew, W.T. and Leung, H. (1992). Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* with a repetitive DNA element. *Appllied Environmental Microbiology* 58 (7): 2188-2195.

Lelliott, R.A. and Stead, D.E. (1987). Methods for the diagnosis of bacterial diseases of plants. Blackwell Scientific Publications, Oxford, United Kingdom 216 pp.

Leung, H., Nelson, R. J. and Leach J. E. (1997). Population structure of plant pathogenic bacteria and fungi. *Advanced Plant Pathology* 10: 157-205.

Lin, X.H., Zhang, D.P., Xie, Y.F., Gao, H.P. and Zhang, Q. (1996). Identifying and mapping a new genefor bacterial blight resistance in rice based on RFLP markers. *Phytopathology* 86:1156-1159.

Liu, H., Yang, W., Hu, B. and Liu, F. (2007). Virulence analysis and race classification of *Xanthomonas oryzae* pv. *oryzae* in China. *Journal Phytopathology* 155: 129–135.

Mabagala, R. B. (1997). The effect of populations of *Xanthomonas campestris* pv. *phaseoli* in bean reproductive tissues on seed infection of resistant and susceptible bean genotypes. *European Journal of Plant Pathology* 103:175-181.

Madden, L. V. and Hughes, G. (1999). Sampling for plant disease incidence. *Phytopathology* 89:1088-1103.

MAFC. (2009). Agricultural sector review and public expenditure review 2009/10 *draft report.*

Mahadevappa, M. (2004). Rice production in India-relevance of hybrid and transgenic technologies. *Indian Journal Genetic Plant breeding.* International Journal Genetics Plant Breeding Biotechnology 64(1): 1-4.

Mew, T.W., Alvarez A. M., Leach, J. E. and Swings, J. (1993). Focus on bacterial blight of rice. *Plant Diseases* 77: 5-12.

Mew, T. W., Vera Cruz C. M. and Medalla, E.S. (1992). Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in the Philippines. *Plant Diseases* 76:1029–32.

Mortensen, C. N. (2000). Seed health testing for bacterial pathogens, Copenhagen, Denmark. 70pp.

Namai, T. and Ehara, Y. (1986). Studies on variation in virulence of Rice blast fungus *Pyricularia oryzae* Cavara. *Journal of Agricultural Research.* 36:3-4.

Narayanan, N.N., Baisakh N., Vera-Cruz C. M., Gnanamanickam, S.S., Datta, K. and Datta S.K. (2002).Molecular breeding for the development of blast and bacterial blight resistance in rice cultivar, IR50. *Crop Sci*ence 42: 2072–2079.

Nayak, D., Shanti, M. L., Bose, L.K., Singh, U.D. and Nayak, P. (2008). Pathogenicity association in *Xanthomonas oryzae* pv. *oryzae. Journal Agriculture Biological Science.* Cuttack, India 3(1):1990 -6145.

Nelson, R. J., Baraoidan, M. R., Vera Cruz, C. M., Yap, I.V., Leach, J. E., Mew, T.W. and Leung, H. (1994). Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. *Applied Environmental Microbilogy* 60:3275–3283.

Niño-Liu, D. O., Ronald, P. C. and Bogdanove, A. J. (2006). Pathogen profile. *Xanthomonas oryzae* pathovars: model pathogens of a model crop. *Molecular Plant Pathology* 7:303-324.

Nishioka, M., Suzuki, H., Akiyama, S., Yoshida, S. and Yamaguchi, I. (2005). Interaction of resistance against rice bacterial blight by 3-chloro-1-methyl-1 H-pyrazole-5-carboxylic acid. *Jounal of Pesticide Science* 30 (1): 47-49.

Noda, T., Li, C., Li, J., Ochiai, H., Ise, K. and Kaku, H. (2001). Pathogenic diversity of *Xanthomonas oryzae* pv. *oryzae* strains from Yunnan Province, China. *Japan Agricultural Research* 35: 97–103.

Ogawa, T., Busto, G. A. J., Tabien, R. E., Romero, G. O., Endo, N. and Khush, G.S. (1991). Grouping of rice cultivars based on reaction pattern of Philippine races of bacterial blight pathogen *Xanthomonas campestris* pv. *oryzae*. *Japan Journal of Breeding* 41:109–120.

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

Onasanya, A., Kiepe, P., Basso A., Nkima, G., Nwilene F.E., Ingelbrecht, I., Lamo J., Ekperigin M. M., Onasanya, R. O., Oyelakin, O., Winter, S., Sere.,Y. (2011). Molecular characterization and DNA fingerprinting of *Xanthomonas oryzae* pv. *oryzae*isolates from climate change prone areas in East Africa. *Research Journal Environmental Sciences* 5(9): 772-778.

Ou S.H. (1985). Rice diseases. Second edition. Commonwealth Mycological Institute, C.A.B. International, Farnham 380pp.

Roberts, I.S. (1996). The biochemistry and genetics of carpusular polysaccharide production in bacteria. Annual Review Microbiology 50: 285-315.

Rukhsana, J. (2007). Evaluation of Botanicals against *Xanthomonas oryzae* pv. *oryzae* and characterization of Bioactive compounds (Thesis), Quaid-i Azam Island, Islamabadi, Pakistan 195 pp.

Ryba-White, M., Notteghem, J. L. and Leach, J. E. (1995). Comparison of *Xanthomonas oryzae* pv. *oryzae* strains from Africa, North America, and Asia by restriction fragment length polymorphism analysis. International Rice Research Institute Notes 20: 25–26.

Salim, M., Akram, M., Akhtar, M.E. and Ashraf, M. (2003). *Rice Production handbook, balanced fertilization for maximizing economic crop yields.* Pakistan Agricultural Research Council, Islamabad 123pp.

Sanchez, A. C., Brar, D. S., Huang N., Li Z. and Khush, G. S. (2000). Sequence tagged site marker assisted selection for three bacterial blight resistance genes in rice. *Crop Science* 40:792 - 797.

Schaad, N.W. (1988). Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd edition. The American Phytopathology Society, Saint Paul, Minchigan. 164 pp.

Sere, Y., Onasanya, A., Verdier V., Akator, K., Ouedraogo, L. S., Segda, Z., Mbare, M. M., Sido, A.Y. and 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 Journal of Plant Science*, 4: 577-579.

Shanti, M. L., George, M. C. L. C., Vera Cruz, C. M., Bernardo, M. A., Nelson, R.J., Leung, H., Reddy, J. N., and Sridhar, R. (2001). Identification of resistance genes effective against rice bacterial blight pathogen in eastern India. *Plant Disease* 85: 506-512.

Shanti, M. L. and Shenoy, V. V. (2005). Evaluation of resistance genes and their pyramids against rice bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae*. 24: 169-173.

Sidhu, G. S., G. S. Khush, and T. W. Mew. (1998). Genetic analysis of bacterial blight resistance in seventy-four cultivars of rice, *Oryza sativa* L. *Theory of Applied Genetics* 53:105–111.

Singh, R. A. and Monga, D. (1985). New methods of seed treatment for eliminating *Xanthomonas campestris* pv. *oryzae* from infected rice seed. *Indian Phytopathology* 38: 629-631.

Singh, S., Sidhu, N., Huang, G., Vikal, Y. and Li, Z. (2001). Pyramiding three bacterial blight resistance genes (*xa5, xa13* and *xa21*) using marker assisted selection into indica rice cultivar PR106. *Theory of Applied Genetics* 102:1011-1015.

Snedecor, G. W. and Cochran, W.G. (1989). *Statistical Methods*, eighth edition. Iowa State University Press. Ames, Iowa. 97pp.

Stead, D. E. (1990). Preservation of bacteria. In: Methods in Phytobacteriology, Chapter. 1.8. Klement, Z., Rudolph, K. and Sands, D.C. (editors). Akadémiai Kiadó, Budapest. pp 275-278.

Tabei, L. (1997). Anatomical studies of rice plant affected with bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae* (Uyeda and Ishinyama) Dowson. *Bull Kyushu Agriculture Experiment Station* 19(2):193-257.

Takahi, Y. (1985). Shirahagen R-S (tecloftalam). *Japan Pesticide Information* No. 46, pp 25-30.

Vera Cruz, C. M., Nelson, R., Leung, H., Leach, J.E. and Mew, T.W. (1992). Reaction of rice cultivars from Ifugao Province, Philippines to indigenous strains of the bacterial blight pathogen. International Rice Research Institute, Newsletter. 17:8.

Webster and Gunnell. (1992). *Compendium of rice Diseases.* University of California, Davis American Phytopathology Society. Press America. pp10.

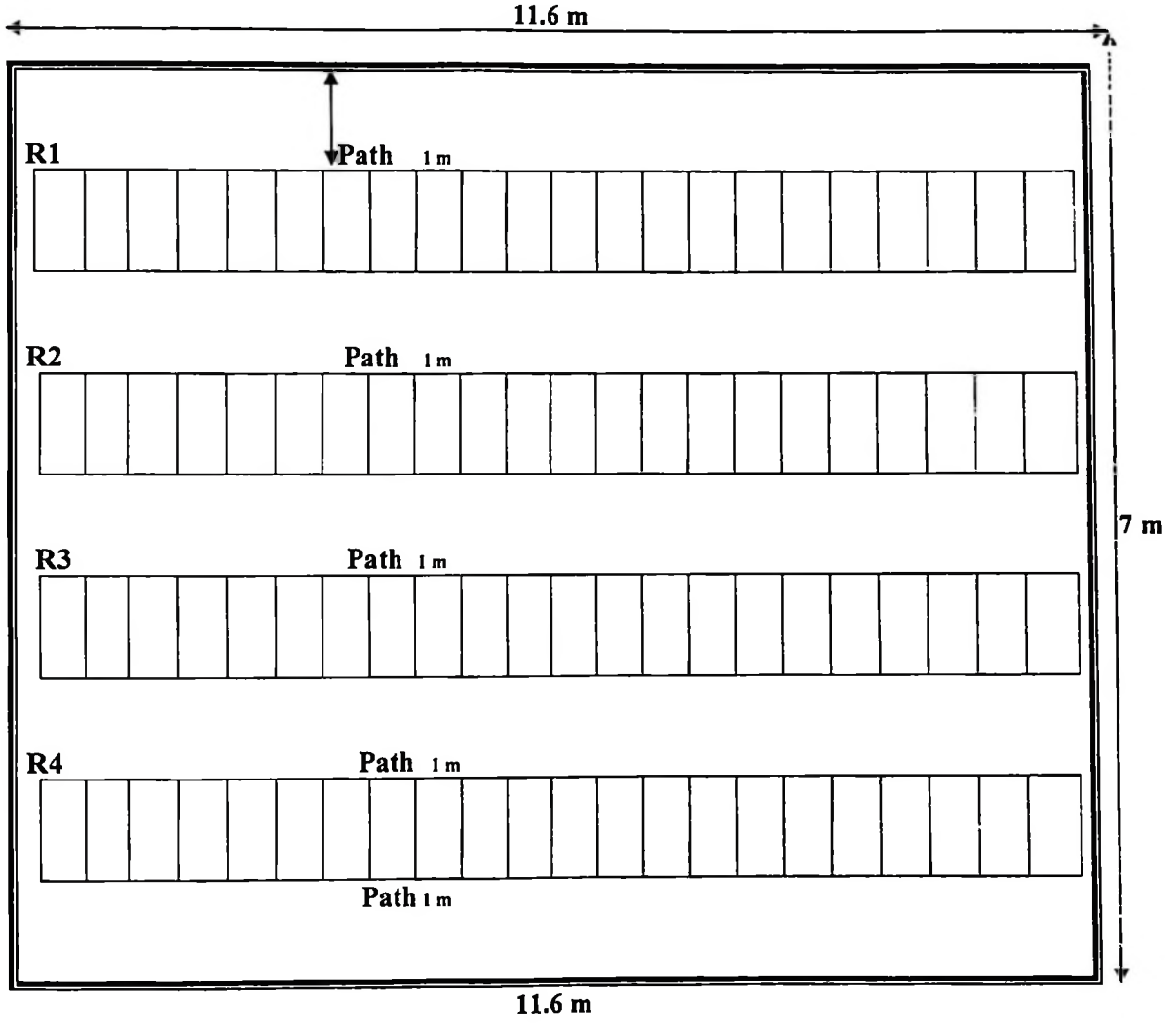
Wheeler, B.E.J. (1969). An Introduction to Plant Disease. John Wiley and Sons, London. 301pp.

Xie, G. L. and Mew, T. W. (1998). A leaf inoculation method for detection of *Xanthomonas oryzae* pv. *oryzicola* from rice seed. *Plant Diseases* 82:1007-1011.

Zhang, G. G., Lu, C. T., Shang, Z. C., Kong, J. (1996). Application methods of Zhongshangmycin in controlling rice bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) progress research on plant protection in China. Proceedings of the Third National Conference of Integrated Pest Management. pp 167-169.

APPENDICES

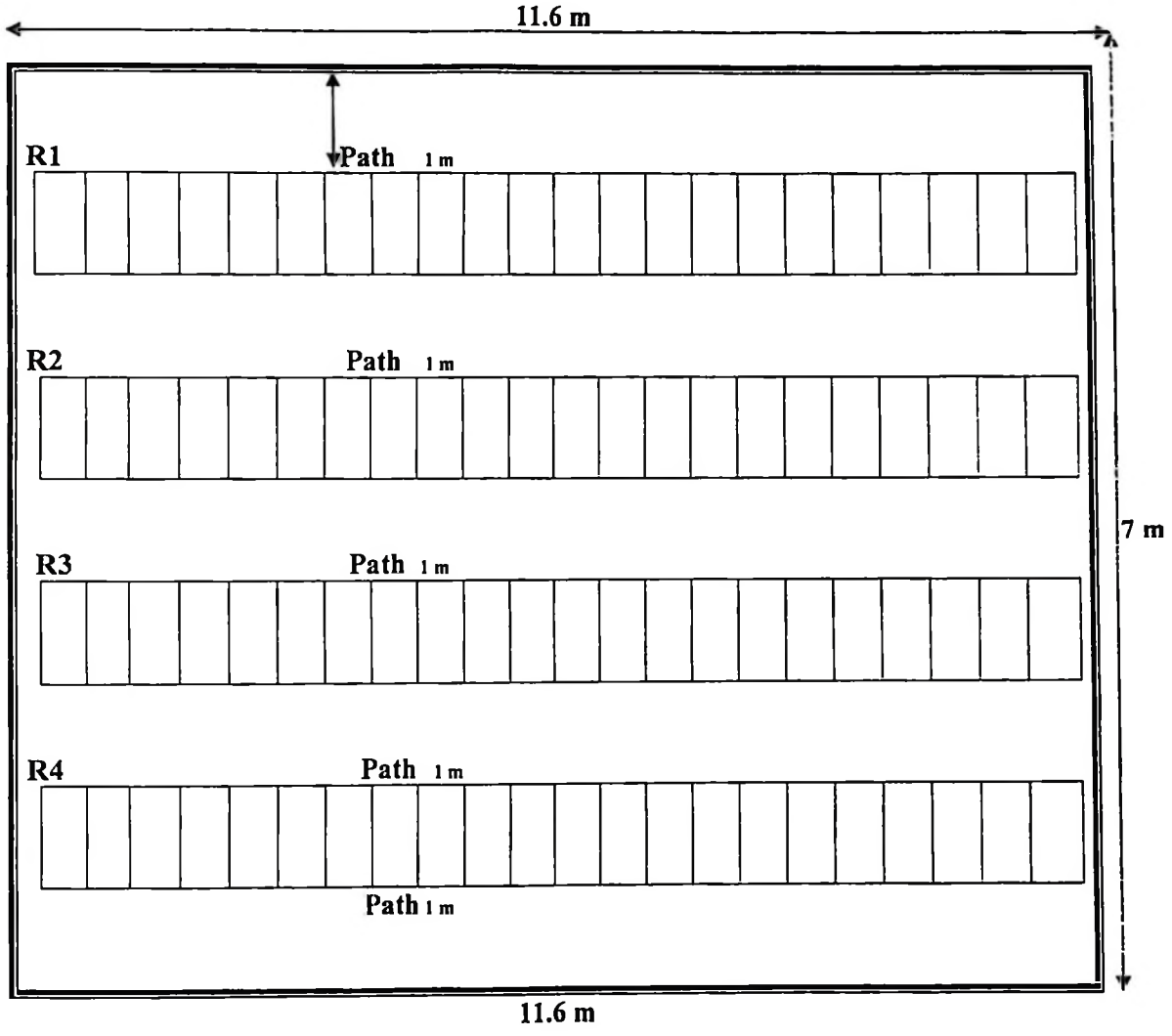
Appendix 1: Field experimental layout



R= Replecation

APPENDICES

Appendix 1: Field experimental layout



R= Replecation

Appendix 2: List of near isogenic lines (NILs) and rice cultivars used in the field and screen house experiments to test against bacterial leaf blight disease

Near isogenic lines (NILs)[a]		Rice cultivars[b]
Differentials lines	**Resistant gene**	
IR-BB1	*Xa-1*	Pijo
IR-BB2	*Xa-2*	Malemata
IR-BB3	*Xa-3*	Rangi Mbili
IR-BB4	*Xa-4*	Domo la Fisi
IR-BB5	*Xa-5*	Tule na Bwana
IR-BB7	*Xa-7*	Jicho la Samola
IR-BB8	*Xa-8*	Kihogo
IR-BB10	*Xa-10*	Mwasungo
IR-BB11	*Xa-11*	Rufiji
IR-BB13	*Xa-13*	Selena
IR-BB14	*Xa-14*	SARO 5
IR24	*Xa-16*	Mtalima Wangu
IR-BB21	*Xa-21*	Zambia
IR-BB50	*Xa-4/xa-5*	Japan
IR-BB51	*Xa-4/xa-13*	Kilombero
IR-BB52	*Xa-4/xa-5*	Supa
IR-BB53	*Xa-4/xa-13*	Mwangulu
IR-BB54	*Xa-4/Xa-21*	Shingo ya Mwali
IR-BB55	*Xa-5/xa-13*	Mwaya
IR-BB59	*Xa-5/xa-13/Xa-21*	Yanga
IR-BB60	*Xa-4/xa-5/xa-13/Xa-21*	Wahiwahi
Control		IR24

Sources of seeds: [a] **AfricaRice Centre, Dar-es-Salaam, Tanzania.**

[b] **Agricultural Research Institute–Uyole Mbeya, Tanzania.**

Appendix 3: Analysis of variance for incidence of near isogenic lines (NILs) to strains of *Xanthomonas oryzae* pv. *oryzae* at Dakawa, Kyela and Bunda

Source of variation	Degrees of freedom	Mean squares
Replication	3	828.28
Site	2	13898.44***
Variety	21	639.99***
Interaction	42	270.91***
Residual/error	195	91.08
Total		263
F pr.	Site	<.001
	Variety	<.001
	Interaction	<.001

Key: *= Very highly significant and Fpr = F probability**

Appendix 4: Analysis of variance for incidence of rice cultivars and their response to infection by *Xanthomonas oryzae* pv. *oryzae* isolates under artificial inoculation.

Source of variation	Degrees of freedom	Mean squares
Replication	3	35.87
Variety	21	99.57 N.S
Residual/error	63	71.52
Total	87	
F pr.		0.157

Key: N.S = Not significant different P ≤0.05

Fpr.= F probability