RICE BLAST DISEASE CAUSED BY *PYRICULARIA ORYZAE*: EPIDEMIOLOGY, CHARACTERIZATION AND YIELD LOSS IN MAJOR RICE GROWING AREAS OF TANZANIA

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CROP SCIENCE

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MOROGORO, TANZANIA.

2016

EXTENDED ABSTRACT

This study was conducted in Mbeya, Morogoro, Kilimanjaro, Shinyanga and Dar es Salaam regions. The overall objective of the study was to establish the magnitude of rice blast disease in Tanzania and its contribution to yield losses. Rice is an important staple food crop and is affected by blast disease with suspected high yield losses of up to 100%. Surveys were conducted in two years (2012 and 2014) to observe disease prevalence of rice blast disease in two different ecosystems in Mbeya, Morogoro, Kilimanjaro and Shinyanga regions of Tanzania. Results showed that the incidence and severity of blast disease varied considerably across the surveyed regions. The highest rice blast incidence (74.38%) and severity (87.62%) were observed in the rainfed ecosystem in Mbeya and Morogoro, respectively. The lowest rice blast incidence (19.38%) and severity (41.5%) occurred in the irrigated ecosystem in Kilimanjaro region. Another study was conducted in the screen-house to assess yield loss caused by *Pyricularia oryzae* using ten rice varieties Jaribu 220, Supa, Kalamata, Shingo ya mwali, Mwarabu, Mbawambili, Kihogo, IR 64, TXD 306 and TXD 85. The varieties were sown in completely randomised design and inoculated to evaluate the effect of rice blast disease on yield. Results showed that most of the rice varieties were susceptible to *P. oryzae* and caused grain yield losses of between 11.9 and 37.8% per hectare. Identification and characterization of pathogenic variation of *P. oryzae* causing rice blast disease were conducted in the laboratory and screen-house at Sokoine University of Agriculture, Morogoro. Ten *P. oryzae* isolates collected from Shinyanga, Kilimanjaro, Mbeya and Morogoro were tested with a set of ten rice blast differentials viz; IRBLk-Ka, IRBLkm-Ts, IRBLb-w/co, IRBLkp-K60, IRBLz-Fu, IRBLa-C, IRBLi-F5, IRBLta2-Pi), St and IRBLt-K59. The experiment was laid out in a completely randomized design with three replications. Twenty one-day-old seedlings grown in pots were inoculated with a *P. oryzae* spore suspension (2×10^5 ml $^{-1}$) with the

hand sprayer in the evening. Disease assessment was done in seven to ten days after inoculation. Considerable pathogenic variations among the tested isolates were observed. The International rice blast disease differentials IRBLk-Ka, IRBLkp-K60, IRBLa-C, IRBLi-F5, IRBLta2-Pi, St and IRBLt-K59 were resistant to all isolates, except that IRBLb-w/co, IRBLkm-Ts and IRBLz-Fu (*Piz*) were susceptible. Further studies were conducted in the biotechnology laboratory at Mikocheni Research Institute in Dar es Salaam to characterize *P. oryzae* using molecular techniques. DNA was extracted from seven isolates of *P. oryzae*. Polymerase chain reaction (PCR) amplification was carried out in a thermocycler. The results of DNA amplification showed that the isolates collected from Morogoro, Shinyanga, Mbeya and Kilimanjaro regions were genetically similar. The study recommends that research on diversity of *P. oryzae* pathotype should be conducted further in order to develop rice varieties resistant to the disease.

DECLARATION

I, Charles Joseph Chuwa, do hereby declare to the Senate of Sokoine University of Agriculture that, this thesis is my own original work done within the period of registration and that it has neither been submitted nor concurrently being submitted in any other institution.

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ACKNOWLEDGEMENTS

I would like to express my deep gratitude to Professor Robert B. Mabagala and Professor Reuben, S. O. W. Mnyuku, my research supervisors, for their patient guidance, encouragement and useful critiques in this study. I also acknowledge the Eastern African Agricultural Productivity Programme (EAAPP) Tanzania for providing funds for this work. I would also like to thank the District Agricultural and Livestock Development Officers (DALDO) of Kahama, Kyela, Moshi and Mvomero for their help during the survey and collection of samples for the study.

Thanks are due to AfricaRice and the International Rice Research Institute (IRRI) Tanzania for supplying rice plant materials. The assistance from Mr. Jackson Nahson, Sokoine University of Agriculture (SUA) laboratory technician, Mr Linus Paul, technician Mikocheni Molecular Biotechnology Laboratory, Dar es Salaam is also acknowledged.

I also owe a debt of gratitude to the Head, lecturers and supporting staff of the Department of Crop Science and Production, SUA, for helping me in many ways during my studies. Finally, I wish to thank my family and my parents for their prayers and encouragement throughout my studies.

DEDICATION

This thesis is dedicated first and foremost to God, my beloved wife; Aurelia, my children; Busara-Teddy, Goodson and Grace, and last but not least my parents Casmir Joseph and Theresia.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
AUDPC	Area under disease progress curves
bp	Base pair
CIMMYT	International Maize and Wheat Improvement Centre
DALDO	District Agricultural and Livestock Development Officers
DMRT	Duncan Multiple Range Test
EAAPP	Eastern African Agricultural Productivity Programme
IRRI	International Rice Research Institute
ISTA	International Seed Testing Association
LSD	Least Significant Difference
MARI	Mikocheni Agricultural Research Institute
N	Nitrogen
ng	Nano-gram
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
RH	Relative humidity
SUA	Sokoine University of Agriculture
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
µl	Micro-litre
<	Greater than
>	Smaller than

CHAPTER ONE

1.0 INTRODUCTION

Rice (*Oryza sativa* L.) is the second most important staple cereal grain in Tanzania after maize. The crop is mainly produced by smallholder farmers. The majority of rice farmers depend on rice both for food and cash. Rice production and processing provide vital employment and generate income for a relatively large cross-section of the population (Dar *et al.,* 2010). In Tanzania, however, production efforts are made to meet domestic demand for food. Despite the efforts employed, rice production and yields are still low (Luzi-Kihupi, *et al*., 2009). The major constraints contributing to low yields include biotic factors (diseases, insect pests, weeds and birds), abiotic stresses (inadequate rainfall, low soil fertility), poor field management and lack of resources.

Rice blast caused by the fungus *Pyricularia oryzae* [anamorph] Cavara (Kato, 2001; Nutsugah *et al*., 2008; Rodrigues *et al*., 2005) is one of the major biotic constraints to rice productivity in Tanzania. It is widely distributed and can cause severe crop destruction under favourable conditions. Blast is the most common and serious disease of rice and can attack the plant at all stages of growth from seedling stage through grain formation (Onwueme and Sinha, 1991). It is also associated with leaf blast, collar rot, nodal blast, neck blast and panicle blast (Jia, *et al.,* 2008). *Pyricularia oryzae* spreads through multiple asexual spore production cycles throughout the cropping season (Castejon-Munoz *et al.*, 2007). Leaves and panicles are most affected organs and reduce the photosynthetic area of the rice plants. Lesion formation on leaves is followed by premature leaf senescence of infected tissue, especially in case of heavy infections. Moreover, panicle blast causes direct yield losses, since filling of the grains in infected panicles is poor (Castejon-Munoz *et al.*, 2007).

Rice blast disease was reported for the first time in China as early as 1637, and it was known as rice fever disease (IRRI and CIMMYT, 2008). The International Rice Research Institute and CIMMYT (2008) also reported rice blast in Japan in 1704, Italy in 1828, United States of America as early as 1876 and India in 1913. As the rice production expanded through Asia, Latin America and Africa over the last few centuries, rice blast disease followed and it is now found in over 85 countries worldwide (Bussaban *et al*., 2005).

The genus *Pyricularia* (Cooke) Sacc (anamorphic) Magnaporthaceae was originally described on crabgrass (*Digitaria sanguinalis* L.) (Bussaban *et al*., 2005). Cavara (1892) reported that *P. oryzae* was described from rice (*Oryza sativa* L.), a taxon with similar morphology to *P. grisea*. However, Couch and Kohn (2002a) using molecular approaches based on three genes (actin, beta-tubulin and calmodulin), noted the difference between the teleomorph *Magnaporthe oryzae* B. Couch (associated with *Oryza sativa* and other cultivated grasses) and *M. grisea* (associated with the grass genus *Digitaria* Haller). *Pyricularia oryzae* also has other scientific names, including *Pyricularia grisea* [anamorph] Cooke Saccardo, and *Magnaporthe grisea* [teleomorph] Hebert (El-kazzaz *et al*., 2009; Jia *et al*., 2008).

Pyricularia oryzae forms conidiophores single or in fascicles, simple, rarely branched, showing sympodial growth (Cother *et al*., 2004). Conidia are pyriform (pear-shaped) with two septa (sometimes 1 or 3 septa) (Dar *et al.*, 2011). Rice blast fungus infects all growth stages of the rice plant and it attacks different parts except roots (Hajano *et al.*, 2011). Lesion shape, colour and size vary depending on variety, resistance, environmental conditions and age of the lesions (Lanoiselet and Cother, 2011). On leaves of susceptible rice varieties, the disease initially appears as whitish or grayish specks along the leaf

margins (Galhano and Talbot, 2011). Under favourable conditions, they turn into elliptical spots that are elongated and diamond shaped with pointed ends. The spots become necrotic in the center with brown or reddish-brown margins (Wilson *et al.*, 2010). The spots join to form large lesions. Wilson *et al*. (2010) demonstrated that the fungus produces elongated, grayish to black colour lesions on stems. The disease symptoms may also appear on rice seeds as brown diamond shaped spots (Hajano *et al.*, 2011).

Seed, crop residue and secondary hosts have been reported as possible sources of *P. oryzae* primary inocula (Greer and Webster, 2001). Manandhar *et al*. (1998) demonstrated that rice seeds transmit *P. oryzae* to seedling. However, transmission of *P. oryzae* from seed to seedlings is difficult under the water-seeded conditions (Chung and Lee, 1983). Apart from seeds, rice crop residues have also been reported to be important source of inoculum for rice blast disease (Lee, 1994; Soderlund *et al.*, 2006).

Rice blast disease causes severe crop losses in rice where environmental conditions are favourable for disease development. Environmental conditions play a very important role on rice blast disease incidence (Bonman, 1992). Greer and Webster (2001) reported that the favourable temperature for blast development is between 17 to 28°C and relative humidity of 90% or above. Bonman (1992) stated that the key environmental factors favouring rice blast are; 1) night temperatures of between 17 and 25°C, 2) long duration of leaf wetness, 3) Nitrogen application, 4) aerobic soil conditions, 5) soil water deficit and 6) still air at night. Furthermore, Bonman (1992) added that environmental influence is primarily through the effects on the physiology of the host, the pathogen, or the host-pathogen interaction.

Pyricularia oryzae can infect the host at optimum temperature of 25ºC after 6-8 hours of leaf wetness (Greer and Webster, 2001). However, high temperatures (above 28ºC) stimulate host resistance while lower temperatures (about 20ºC) make plants more susceptible to blast disease (Bonman, 1992). Furthermore, varieties released as resistant have been reported to became susceptible after only few seasons, or few years of cultivation due to pathogenic variation through evolution over time and adaptation to cultivated varieties (El-kazzaz *et al.*, 2009). Thus, screening for blast resistance is a continuous challenge to rice pathologists and breeders. There is a need therefore, for developing rice varieties with durable resistance.

Pyricularia oryzae infects rice plants by producing spores which lie on the leaf, panicle and nodes and penetrate the plant using the appressorium (Galhano and Talbot, 2011). The pathogen then sporulates from the diseased rice tissue and is dispersed as conidiospores (Hajano *et al.*, 2011). However, the cycle of rice blast disease involves infection, colonization and sporulation. All three distinct phases interact to determine the incidence and severity of the disease. Infection is initiated by a conidium landing on the leaf surface of a rice plant. The conidium produces a germ tube which forms an appressorium (Kim and Yoshino, 1995). In a compatible interaction between susceptible host and pathogen under favourable conditions, the appressorium produces an infection peg which penetrates the epidermis (Galhano and Talbot, 2011). Subsequent hyphal growth inside the host cells results in the development of a lesion. Under high relative humidity, a lesion on a susceptible rice plant produces conidia for 3-4 days (Hajano *et al.*, 2011). These conidia are easily dislodged and dispersed and can be the source of inoculum for the next infection cycle. The cycle can be repeated if rice straw, stubble, weed hosts and favourable

conditions are present. Under favourable conditions a single disease cycle can be completed within a week (Kim and Yoshino, 1995).

Rice blast occurs in all rice growing areas worldwide (Nutsugah *et al.*, 2008). The disease is endemic in Southern United States, Southern Carolina and in African countries. Blast is highly destructive in lowland rice in tropical Asia, Latin America and Africa (IRRI and CIMMYT, 2008). The geographic distribution, incidence and severity of rice blast have been much greater in recent years. Such a situation has been contributed by several factors including susceptible host, favorable environment, and the presence of the pathogen for disease to occur (Ahmad *et al.*, 2011).

In Tanzania, rice blast has been reported in all rice-growing areas (Teri and Ali, 1983). Spores are usually distributed within the rice – growing areas by wind. However, Ahmad *et al*. (2011) demonstrated that weather and soil conditions like temperature, soil moisture, soil nutrients, light, air humidity, soil pollutants and soil pH, influence the seasonal development and geographical distribution of plant diseases, including rice blast.

Rice blast has the potential to cause severe crop losses in both irrigated and upland rice where environmental conditions are favourable for disease development (Greer and Webster, 2001; Naik *et al*., 2012; Picco and Rodolfi, 2002). However, rice is most susceptible to blast in the seedbed, and at the tillering stage of the crop (Castejon-Munoz *et al.*, 2007). The importance of blast could be justified through different factors; such as geographical distribution, its epidemiology and associated yield losses. Although rice blast disease has been observed to cause severe yield losses of up to 100% in some locations, little information exists on the extent and intensity of actual losses in farmers' fields (IRRI and CIMMYT, 2008).

Grain yield losses due to blast alone have not yet been quantified in Tanzania (Teri and Ali, 1983). However, substantial losses of more than 50% in susceptible cultivars have been reported in Taiwan (Yung-chieh and Huang, 2010), about 60% in Southern United States (Greer and Webster, 2001) and 3.2 – 77% in Ghana (Nutsugah *et al.,* 2008). In Tanzania, rice blast has been increasingly observed in various regions with reports of high incidences in Morogoro (Luzi-Kihupi *et al.*, 2009b). The disease potentially threatens the regional food security (Strange and Scott, 2005).

Therefore, given favourable conditions for rice blast development, the disease is likely to cause rice yield losses in significant magnitudes. Published information on rice blast disease in Tanzania is limited and numerous attempts to isolate the pathogen on media have proven difficult. This has made specific studies on rice blast disease including basic epidemiology and artificial inoculation difficult to undertake.

Currently, specific cultural control measures do not exist for rice blast disease. Control using fungicides has been effective (Ghazanfar *et al.*, 2009b; Kato, 2001), however, they are too expensive and not affordable by smallholder farmers. There is also a concern for environmental pollution when using fungicides (Picco and Rodolfi, 2002).

Preliminary evaluations for resistance to rice blast disease have indicated cultivar variation (Soderlund *et al.*, 2006). This implies that development of inbred lines with adequate levels of resistance to blast disease should be possible through selection. The occurrence of rice blast epidemic, pathogen evolution toward virulence and pathogenic variability often contribute to breakdown of resistance of newly released rice varieties (Ballini *et al*., 2008; Prabhu *et al.*, 2002). The pathogenic variation within strains of *P. oryzae* has been

reported (Correa-victoria and Zeigler, 1993). However, there is no information in Tanzania on pathogenic variation of *P. oryzae* under natural conditions in farmers' fields. Understanding of such variation, epidemiology and yield loss caused by rice blast disease in the field is imperative. Therefore, it is highly important to assess the disease because infected plants serve as sources of inocula.

Farmers can control rice blast disease in their fields by planting on time, appropriate use of nitrogen fertilizers, tillage, weed control and crop rotation are important to break continuity in the disease cycle and reduction in disease severity in areas where the disease is endemic. The use of chemicals, resistant varieties, agronomic practices and biotechnological methods (Ribot *et al.*, 2008) effectively control rice blast disease. Gouramanis (1997) and Ghazanfar *et al.* (2009) reported some of the fungicides which are recommended for control of rice blast worldwide. Such chemicals include Derosal (carbendazim), Fongoren (pyroquilon), Hinosan TCP (edifenphos), Kitazin P (iprobenfos), Bla-S (blasticidin), Chlobenthiazone, Beam (tricyclazole), Orysemate (probenazole) and Neotopsin (thiophanate-methyl). Others include Rabicide 30WP, Nativo SC, Thiovit 80 WP, Cuproxit 345 SC, Score 250 EC, Filia 525 SC, Armure, Tilt 250 EC, and WSH004. Prasanna and Veerabhadraswamy (2014) investigated a combination of fungicides which were effective against leaf blast as well as neck blast disease. Such fungicides include Conika 50% WP (Kasugamycin 5% + Copper Oxychloride 45% WP), Dhanucop Team (Tricyclazole 75% WP) and RIL-068/F1 48 WG (Kresoxim methyl 40% + Hexaconazole 8% WG).

However, the use of resistant varieties is the most economical, effective and environmental friendly method of controlling rice blast (Ghazanfar *et al.*, 2009) especially

for resource-poor farmers. Although the resistance is subject to break down due to appearance of new/more virulent races of the pathogen, considerable effort should be directed towards developing and identifying blast-resistant varieties in order to provide farmers with low-cost blast management options.

1.1 Justification

The incidence of rice blast disease in Tanzania has been reported to be on the increase (Teri and Ali, 1983) although there has been no research on the disease in recent years. Luzi-Kihupi *et al.* (2009) reported that the majority of locally available varieties in Tanzania are susceptible to blast disease. Since rice is an important staple food crop and is severely affected by blast with high yield losses of up to 100% in Ghana (IRRI and CIMMYT, 2008), it was necessary that a comprehensive study on the pathogen variability, epidemiology and yield loss be conducted in major rice growing areas of Tanzania. Such studies provide insight into breeding and management strategies to improve resistance of the crop to the disease and reduce yield losses.

1.2 Objectives

1.2.1 The overall objective

The study established the magnitude of rice blast disease in Tanzania and its contribution to yield losses for purposes of improved management interventions.

1.2.2 The specific objectives

The specific objectives of the study were**:**

i. To determine incidence, severity and distribution of rice blast disease in the selected rice-growing areas of Tanzania.

ii. To evaluate pathogenic variation of *Pyricularia oryzae* strains on locally grown rice varieties

iii. To determine the grain yield loss caused by rice blast disease on selected farmer preferred rice varieties

iv. To identify and characterize pathogenic variation of *Pyricularia oryzae* using both differential host genotypes and molecular technique.

1.3 Organization of this Thesis

This thesis developed in publishable manuscripts format consisting five chapters. Chapter one is general introduction of the thesis, chapter two, three and four consisted publishable manuscripts in form of publishable papers. Chapter five is general conclusion and recommendations.

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CHAPTER TWO

2.0 INCIDENCE, SEVERITY AND DISTRIBUTION OF RICE BLAST (*PYRICULARIA ORYZAE*) DISEASE IN FOUR MAJOR RICE GROWING REGIONS IN TANZANIA.

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2.1 Abstract

Surveys were conducted in 2012 and 2014 to determine prevalence of rice blast disease in Mbeya, Morogoro, Kilimanjaro and Shinyanga regions in Tanzania. Through the surveys, 32 farmers' fields were visited and disease incidence and severity were recorded. Plant parts with rice blast disease symptoms were collected for isolation and identification of the pathogen in the laboratory. Results showed that the incidence and severity of rice blast varied considerably across the surveyed regions. The highest incidence (62.5%) and severity (66.5%) were recorded in the rainfed ecosystem in Mbeya and the lowest rice blast incidence (21.9%) and severity (33.3%) occurred in the irrigated ecosystem in Kilimanjaro region. Rice blast disease incidence and severity varied across the villages. The highest rice blast disease incidence of 85% and 80% were recorded in rainfed ecosystem at Kapwili and Kikusya villages, respectively in Mbeya. The highest disease severity was observed at Luhindo (90%) village followed by Kapwili (86.5%) villages, and the lowest incidence and severity were recorded in irrigated fields at Mabogini, Rau, Chekereni and Oria villages in Kilimanjaro region. The rice variety; TXD 306 followed by Supa, Kalamata, and Mbawambili showed the highest disease severity while the lowest

disease severity and incidence were recorded on the varieties IR 64 and Kilombero. Results revealed that rice blast disease incidence and severity were significantly high in rainfed farmers' fields. The study confirmed that rice blast remains among the most serious biotic constraints of rice production in Morogoro, Mbeya, Kilimanjaro and Shinyanga regions in Tanzania.

Key words: Incidence, *Pyricularia oryzae,* rice blast, severity

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2.2 Introduction

Rice (*Oryza sativa* L.) is the most important staple food crop for more than half of the population in the world (Koide *et al.*, 2009). The demand for rice today is increasing as the world population increases. Rice blast disease caused by the fungus *Pyricularia oryzae* [anamorph] Cavara (Ghazanfar *et al.*, 2009a; Kato, 2001; Nutsugah *et al.*, 2008; Rodrigues *et al.*, 2005) is one of the most serious and widely distributed diseases of rice. Despite efforts made by farmers to meet rice demand, rice blast disease continues to cause severe losses under favourable climatic conditions in both irrigated and rainfed rice production ecosystems. The disease attacks the plant at all stages of growth from seedling through grain formation (Onwueme and Sinha, 1991). It is also associated with leaf blast, collar rot, nodal blast, neck blast and panicle blast (Jia *et al*., 2008).

Pyricularia oryzae spreads through multiple asexual spore production cycles throughout the cropping season (Castejon-Munoz *et al.*, 2007). The damage caused by rice blast disease differs depending on the part of the plant affected and the variety. Leaves and panicles are the most affected organs thus, reducing the photosynthetic area of the rice plants (Hwang *et al*., 1987). Lesion formation on leaves is followed by premature leaf senescence of infected tissue, especially in cases of heavy infections (Castejón-Muñoz,

2008). Rice blast disease limits the plant health, seed quality and production potential of the rice crop (Mousanejad, *et al.*, 2010).

It is therefore, important to occasionally assess disease incidence and severity. Disease severity is a better predictor of crop loss than disease incidence (Brown and Keane, 2013). Rice blast disease incidence of 25% and 70% on node and leaf, respectively, caused yield losses of up to 90% (Dar *et al*., 2010). In Kenya, rice blast disease incidence of 55.5% was reported by Kihoro *et al*. (2013). Under high relative humidity (greater than 90%), the incidence and severity measure the extent of damage caused by rice blast disease depending on the physiological conditions of the *Pyricularia oryzae* strains (Obilo *et al.*, 2012).

The incidence of rice blast disease in Tanzania has been reported to be on the increase (Teri and Ali, 1983). Luzi-Kihupi *et al.* (2009) reported that the majority of locally grown varieties in Tanzania are susceptible to rice blast disease. Studies to assess the current status of rice blast disease in the country are urgently needed. The objective of this study was to determine the incidence and severity of rice blast disease caused by *P. oryzae* in the major-rice growing areas of Tanzania. Information obtained from this study will enable scientists to establish appropriate approaches for management of rice blast disease.

2.3 Materials and Methods

Rice blast disease surveys were conducted in 2012 and 2014 during the rice growing season. The incidence and severity of blast disease were studied in 64 farmers' fields in Kyela district in Mbeya, Mvomero district, Morogoro, Lower Moshi Irrigation Scheme in Kilimanjaro and Kahama district in Shinyanga (Figure 2.1). The incidence of rice blast

disease was determined by field observations and infected rice samples were collected for laboratory studies at Sokoine University of Agriculture. Before isolation, all the samples were stored in the refrigerator temperature 20ºC in the mycology laboratory at the African Seed Health Center, Sokoine University of Agriculture, Morogoro, Tanzania.

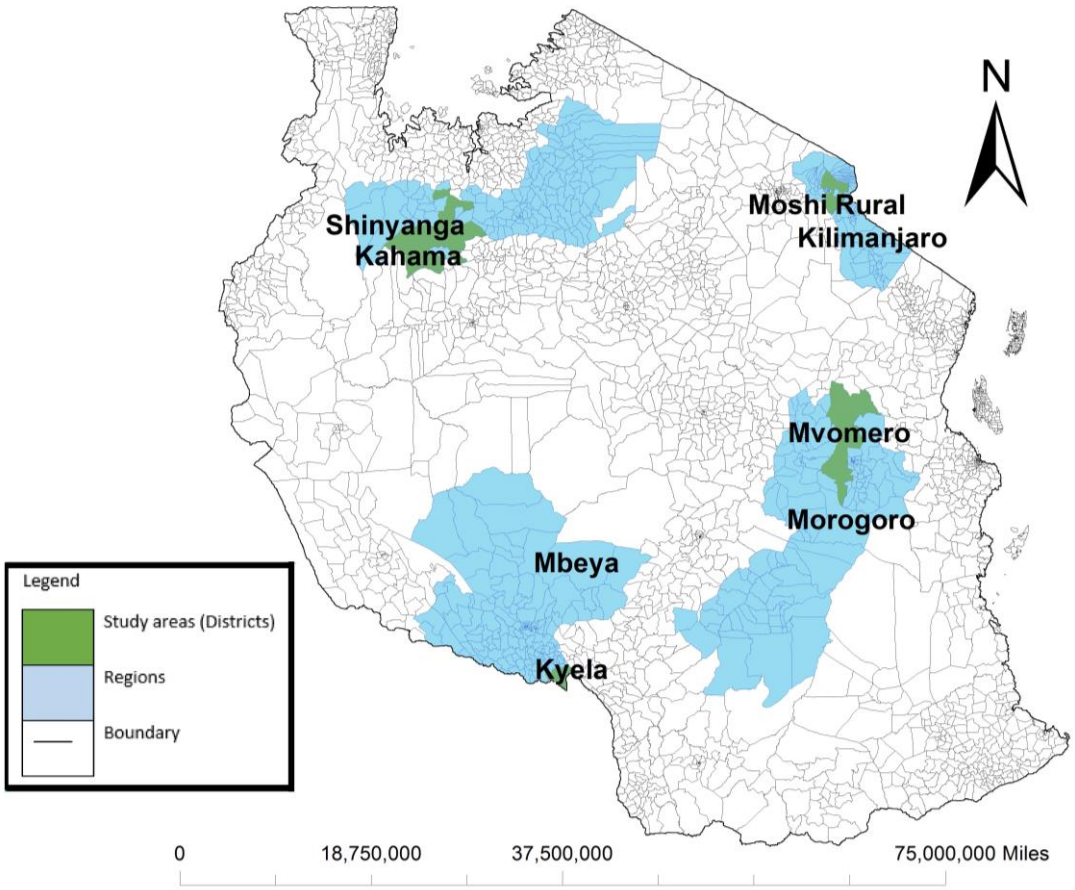

Figure 2.1: Map of Tanzania showing the study sites

2.3.1 Assessment of disease incidence and severity

The incidence and severity of rice blast disease were determined from randomly selected rice fields with typical blast disease symptoms. The regions surveyed constituted a strata. The rice growing villages Kapwili, Kikusya, Itope and Buloma in Mbeya, Hembeti, Dakawa, Luhindo and Mkindo in Morogoro, Kiyenza, Mngula, Bududa and Kagongwa in

Shinyanga and other four villages Oria, Chekereni, Rau and Mabogini in Kilimanjaro region were randomly studied. From each village, four rice fields of one hectare were randomly selected. Ten plants were examined after every ten paces using the diagonal technique across the field following the procedures of Puri *et al*. (2006). The incidence and severity of rice blast disease were recorded based on measurements (Figure 2.2) and visual assessment of symptoms arising from infection of *P. oryzae* (Obilo *et al.,* 2012). Lesion size (width and length) (Plate 2.1), number of infected plants with the disease and number of plants observed were recorded.

The incidence of blast disease across locations was determined using the following formula described by Hajano *et al*. (2011).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants with the disease}}{\text{Total number of plants studied}} \times 100$$

The severity of rice blast disease was determined using the disease scoring scale of 1-6 as described by Prasad *et al*. (2009) with modifications where: 1 = no disease symptoms; 2 = brown specks smaller than 0.5 mm in diameter, no sporulation; 3 = brown specks approximately. 0.5–1.0 mm in diameter, no sporulation; 4 = round to elliptical lesions 1–3 mm in diameter, gray center surrounded by brown margins, lesions capable of sporulation; 5 = typical spindle- shaped blast lesions capable of sporulation, 3 mm or longer in diameter; 6 = lesions as in 5, but about half of one or two leaf blades killed by coalescence of lesions. Then the disease severity scores were converted into percentages using the formula described by Ghazanfar *et al*. (2009b).

$$\text{Disease severity \%} = \frac{\text{Average of the disease score}}{6} \times 100$$

These two pathometric variables (incidence and severity) were used to assess the prevalence of the rice blast disease with respect to leaf symptoms. The disease incidence was determined by recording the percentage of affected plants. Rice blast disease severity was determined by measuring the total plant surface area affected and the number of affected panicle bases (Plate 2.1).

2.3.2 Isolation of *Pyricularia oryzae*

Infected rice tissues were incubated at 24ºC under moist conditions for 24 - 72 hours to allow for fungal sporulation on the lesions. After 72 hours (three days), the mycelium that developed were observed with the stereo microscope at magnification of 50 X. Thereafter, sterile drawing pins were dipped in potato dextrose agar (PDA) petri plates and then slightly touched on the sporulating blast lesions, followed by inoculation of the agar plates with the pin containing spores of the presumed *P. oryzae*. Conidia of *P. oryzae* were examined under a dissecting microscope and their identity confirmed by light microscopy following procedures of Levy *et al.* (1993) and Nutsugah *et al*. (2005).

2.3.3 Statistical analysis

Before analysis data were transformed using the arcsine square root transformation formula as described by Mousanejad *et al*. (2010) as shown:

$$Y = \text{Arcsin } \sqrt{P}$$

Where, Y is transformed data, p is the observed proportion.

Analysis of variance and comparison of means for rice blast disease incidence and severity were performed using GenStat® Executable release 14 Statistical Analysis Software. The means were compared by Duncan's multiple range test (DMRT) at 5% probability.

2.4 Results and Discussion

Rice blast disease incidence and severity caused by natural infection in farmers' fields varied significantly among regions surveyed ($P<0.05$). Typical symptoms of blast disease on both leaves and panicles were observed in all areas surveyed in Tanzania (Plate 2.1). The blast incidence and severity in most of the fields visited were observed at the seedling and vegetative growth stages. The lowest rice blast disease severity was observed at panicle initiation and grain-filling stages due to varietal and agro-ecological differences of the regions covered by the study.

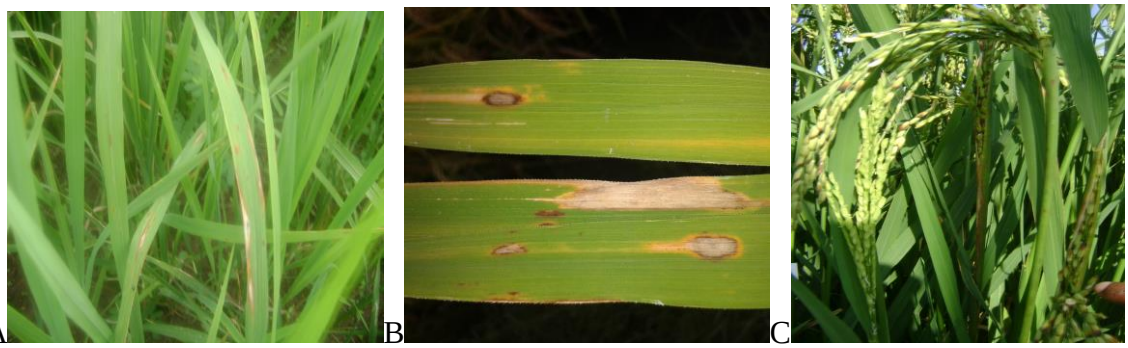

Plate 2.1: A and B) Symptoms of rice blast disease on leaves, C) on panicles in farmers' fields (photos: C. J. Chuwa, Mbeya and Morogoro).

The incidence and severity of rice blast disease in the major rice-growing regions of Tanzania during 2012 and 2014 are presented in Tables 2.1, 2.2 and 2.3. Results indicate that rice blast disease incidence in Kapwili, Mkindo and Mabogini villages were statistically significantly different ($P<0.005$). Rice blast disease severity varied significantly between villages ($P<0.05$) (Table 2.1). The highest rice blast disease severity was observed in Luhindo village (90%) in the rainfed ecosystem in Mvomero District, Morogoro Region.

On the other hand, the lowest rice blast disease severity was recorded in Oria (33%) and Mabogini (33%) villages in the Lower Moshi irrigation scheme, Kilimanjaro region (Table 2.1). Such observations were influenced by growing improved rice varieties which are resistant to rice blast disease.

Supa variety was the most widely grown commercial variety in rainfed areas where studies were conducted. Under rainfed ecosystem, most of the rice fields were dry showing water stress to rice plants. Such a stress may also predispose rice plants to high rice blast disease severity. Even slight stress may significantly destroy the plant ability to tolerate infection by *P. oryzae*.

Table 2.1: Incidence and severity of rice blast disease in major rice growing areas in Tanzania

Villages	Ecosystem	Incidence (%)	Severity (%)
Kapwili	Rainfed	85.0a	86.5ab
Kikusya	Rainfed	80.0ab	71.0c
Itope	Rainfed	72.5abc	71.0c
Buloma	Rainfed	60.0cde	76.8bc
Hembeti	Rainfed	67.5abc	78.5abc
Dakawa	Irrigation	62.5bcd	78.5abc
Luhindo	Rainfed	55.0cdef	90.0a
Mkindo	Irrigation	45.0def	78.5abc
Kiyenza	Rainfed	45.0def	71.0c
Mngula	Rainfed	42.5efg	71.0 c
Buduba	Rainfed	37.5fgh	67.0 c
Kagongwa	Rainfed	25.0ghi	75.0bc
Oria	Irrigation	25.0ghi	33.0e
Chekereni	Irrigation	20.0hi	50.0d
Rau	Irrigation	20.0hi	50.0d
Mabogini	Irrigation	12.5i	33.0e
	Fpr.	**<0.001**	**<0.001**
	CV (%)	**25.7**	**11.8**

Means in the same column followed by the same letter are not significantly different by Duncan's Multiple Range Test at $P < 0.05$.

Fpr = probability value, CV % = Percent of coefficient of variation.

Rice plants grown in deep water have been reported to exhibit increased resistance to the disease compared with those grown in shallow water depths (Greer, 2010). From an irrigation standpoint, maintaining a deep continuous flood is the best option for minimizing the risk associated with rice blast disease. Rice plants in irrigated areas were generally healthy and vigorous, enabling them to better resist attack by *P. oryzae* pathogen

than in water stressed areas. Similar findings have been reported by Puri *et al.* (2006) when they were studying the reaction of different rice lines against leaf and neck blast under field condition of Chitwan Valley. They discovered that water stress in paddy fields increased lesion size of rice blast disease, caused the highest rice blast disease incidence and severity.

Under irrigated schemes, rice blast disease incidence (30.8%) and severity (36.3%) were moderately high (Table 2.2). The highest rice blast disease incidence (56.8%) and severity (60.2%) were recoded in rain-fed ecosystems. These results indicated that the incidence and severity of rice blast disease in irrigated fields in Tanzania were lower than in the rainfed fields (Table 2.2).

Table 2.2: Incidence and severity of rice blast disease in irrigated and rainfed ecosystems in Tanzania

Ecosystem	Incidence (%)	Severity (%)
Irrigated	30.8	36.3
Rain-fed	56.8	60.2
LSD	**10.7**	**13.4**
CV (%)	**44.2**	**51.2**

LSD = Least significant differences, CV (%) = Percent of coefficient of variation.

Severe leaf and panicle rice blast disease incidence and severity were recorded in rainfed rice fields. However, general observations indicated low disease development under irrigated ecosystems. This may partly be attributed by differences in environmental conditions such as temperature and relative humidity (Table 2.3). Irrigated rice fields were large open lands, with consistent breezes during rice growing season. These environmental

conditions significantly resulted to low rice blast disease incidence and severity because such conditions do not allow retention of spores on the rice leaf surface and would not facilitate them to germinate. In addition, consistent breezing may also affects retention of water droplets on leaf surface that can facilitate blast disease development (Jones *et al.*, 1993).

Higher rice blast incidence and severity in rainfed rice fields may also be influenced by the farmers' cultivation methods. Rice fields under rainfed ecosystem were small and mostly surrounded by grasses which served as windbreaks. These caused longer retention of water droplets on the leaf surface which are necessary for successful germination of spores of *P. oryzae* and subsequent invasion. Similar findings have been reported by Jones *et al.* (1993) and Greer (2010) when they were determining occurrence, epidemiology, distribution and reaction of rice varieties to rice blast disease in Cameron and California. Another reason could be alternative sources of *P. oryzae* inoculum that may come from other hosts such as weeds.

Table 2.3: Rice blast disease incidence and severity in four major rice growing regions in Tanzania.

Region	Maximum temperature (ºC)	Minimum temperature (ºC)	Rainfall (mm)	Relative humidity (%)	Incidence (%)			Severity (%)		
					2012	2014	Mean	2012	2014	Mean
Mbeya	26.7	15.0	93.2	90.5	74.4 a	50.6 a	62.5	78.2 b	54.8 a	66.5
Morogoro	29.8	20.9	85.2	95.8	57.5 b	43.5 ab	50.5	87.6 a	35.4 ab	61.5
Shinyanga	31.0	21.5	36.9	68.5	37.5 c	33.1 bc	35.3	71.0 b	30.8 b	50.9
Kilimanjaro	28.4	22.5	65.0	60.7	19.4 d	24.4 c	21.9	41.5 c	25.1 c	33.3
Fpr.					**<.001**	**<.001**		**<.001**	**<.001**	
CV (%)					**28.5**	**42.5**		**17.2**	**38.0**	

Means with the same letter within a column are not significantly different by Duncan's Multiple Range Test at $P < 0.05$.

Fpr = probability value, CV % = Percent of coefficient of variation

Results in Table 2.3 indicate that rice blast disease incidences were statistically significantly ($P < 0.05$) different between Mbeya, Morogoro, Shinyanga and Kilimanjaro regions in 2012 and 2014. The highest rice blast disease incidence (62.5%) was observed in Mbeya region, while the lowest disease incidence (21.9%) was recorded in Kilimanjaro region. Moderate disease incidences (50.5%) were recorded in Morogoro followed by Shinyanga (35.3%). Similarly, rice blast disease severity was significantly different ($P < 0.05$) between the four regions in 2012 and 2014 (Table 2.3). The highest rice blast disease severity was recorded in Mbeya region (66.5%), followed by Morogoro (61.5%) and Shinyanga (50.9%). The lowest severity was recorded in Kilimanjaro region (33.3%). In 2012, the highest blast disease severity was recorded in Morogoro (87.6%), while in 2014, the highest disease severity was recorded in Mbeya region (54.8%) and the lowest (25.1%) in Kilimanjaro. In both seasons, Mbeya had maximum temperature of 26.7^{o}C, minimum temperature of 15^{o}C, relative humidity of 90.5% and rainfall of 93.2 mm (Table 2.3). It implies that these conditions have a strong influence on the development of rice blast disease and may have substantially favoured spore release from pathogen sources (Castejon-Munoz, 2008) and germination of spores.

The incidence and severity of rice blast disease varied significantly ($P<0.05$) between regions from 21.9 to 62.5% and 33.3 to 66.5%, respectively (Table 2.3). This variation was probably due to variation in favorable climatic factors (temperature, relative humidity and rainfall) in Mbeya and Morogoro regions. Rice blast disease incidence and severity decreased gradually as the maximum temperature increased from 26.7 to 31^{o}C. The study also investigated the relationships between temperatures, RH and rainfall with rice blast disease incidence and severity in Morogoro, Mbeya, Shinyanga and Kilimanjaro. The

highest rice blast disease incidence and severity were recorded at a minimum temperature of 15ºC, rainfall of 93.2 mm and relative humidity of 90.5% in Mbeya region, while the lowest rice blast disease incidence and severity were recorded at the minimum temperature of 22.5ºC, rainfall of 65 mm and relative humidity of 60.7% in Kilimanjaro (Table 2.3).

The present study indicated that high rainfall, temperature, and relative humidity were associated with high risk of rice blast disease incidence and severity. The highest rice blast disease incidence and severity in Mbeya and Morogoro regions were caused by more favourable temperature (26.7ºC), rainfall (85.2 to 93.2 mm) and high relative humidity (> 90%) (Table 2.3). Such conditions have been reported to favour sporulation, release and germination of rice blast conidia. Similar results have been reported by Castejon-Munoz *et al.* (2007); Castejon-Munoz (2008) and Koutroubas *et al.* (2009) that environmental conditions especially relative humidity of 90.5%, rainfall of 93.2 mm and average temperatures of 26.7°C were optimum for incidence and severity, and favored the production and dispersion of *P. oryzae* spores. In India, similar results were reported by Selveraj *et al.* (2011) and Ahmad *et al.* (2011) that high rice blast incidence and severity is influenced by minimum night temperatures of 20 to 24ºC and relative humidity above 90% during the early morning periods from 2 to 4 days after inoculation.

In this study, a minimum temperature of 15ºC was found to be more favourable for outbreaks of rice blast disease in the studied areas. Results revealed that the incidence and severity of rice blast decreased with increase in minimum temperature from 15ºC to 22.5ºC). Similar findings have been reported by Ahmad *et al.* (2011) and Shafaullah *et al.* (2011). Rice blast disease incidence and severity increased with increase in rainfall from

36.9 – 93.2 mm (Table 2.3). These results reflect that rice blast disease was mainly severe in Tanzania in areas receiving rainfalls above 36.9 mm. However, it also appeared that the rainfall of 65 mm was permissive for rice blast disease, but not optimal for epidemic development. Although rainfall was high, the development of rice blast disease was low as indicated for Kilimanjaro and Shinyanga due to low relative humidity (Table 2.3). However, Ahmad *et al.* (2011) and Shafaullah *et al.* (2011) reported the highest blast incidence and severity at 5-17 mm total rainfall in Faisalabad. This range of rainfall reported is low compared with the one recorded in the present study (65 – 93 mm), where rice blast disease incidence and severity were high. Such findings indicated that a minimum rainfall (5 – 17 mm) in Faisalabad was favourable for rice blast disease development.

In the surveys, it was found that most of the farmers applied urea fertilizer at a rate of 120 – 150 kg N/ha during rice growing season, and Diammonium phosphate (DAP) at a rate of 100 kg N/ha during sowing or transplanting. Excessive nitrogen fertilizer application has been reported to affect the accumulation of ammonia in the plant cells and serve as suitable nutrients for growth of *P. oryzae* (Ahmad *et al.*, 2011; Prabhu *et al.*, 2003).

Plant stresses such as nutrient deficiencies, salinity and shortage of water in the fields may also be associated with significant increase in rice blast disease incidence and severity in rice plants. Slight stress of water or nutrients may significantly change the plants' ability to tolerate severity by *P. oryzae* pathogen (Jones *et al.,* 1993). Jones *et al.* (1993) reported that nutrient deficiencies like potassium and silica significantly increase rice blast disease incidence and severity. Therefore, proper management of the rice crop is necessary to

avoid plant stress to minimize risk associated with rice blast disease. In Kilimanjaro under irrigated conditions the incidence and severity of rice blast disease were the lowest. Such results may be caused by unfavourable temperature and low relative humidity (60.5%) recorded in the area.

Temperatures above 28^{o}C have been reported to induce resistance to rice blast disease in rice plants (Greer and Webster, 2001). Ahmad *et al*. (2011) reported that temperatures above 30^{o}C significantly decreased mycelial growth of *P. oryzae*. Increase in maximum temperatures from 29^{o}C to 37^{o}C has been reported to decrease rice blast disease incidence and severity (Shafaullah *et al.*, 2011). Based on results of the present study, it was observed that rice blast disease can develop and spread very fast under high rainfall, RH and in low temperatures. Therefore, holistic management approach of the disease is required to reduce losses caused by rice blast disease in areas where environmental conditions are favourable for its development.

2.4.1 The effect of rice blast disease on six rice varieties

Results in Figure 2.2 indicate that the incidence and severity of rice blast disease on IR 64, Kalamata, Mbawambili, Kilombero, Supa and TXD 306 varieties varied significantly ($P < 0.05$). The highest rice blast disease incidence was observed on the variety Kilombero while the lowest rice blast disease incidence was recorded on IR 64. Medium rice blast disease incidence was observed on Kalamata, Mbawambili, Supa and TXD 306 varieties (Figure 2.2). The highest rice blast disease severity on leaves, neck and panicles was recorded on the variety TXD 306, followed by Supa, Kalamata, Mbawambili and Kilombero (Figure 2.2). Kalamata, Mbawambili, Kilombero, Supa and TXD 306 which

are widely cultivated rice varieties in the country, were highly susceptible to rice blast disease. The lowest rice blast disease severity was recorded on IR 64 indicating that the variety has good level of resistance to the disease. During the surveys, the variety IR 64 was observed to be resistant to rice blast disease in a number of locations.

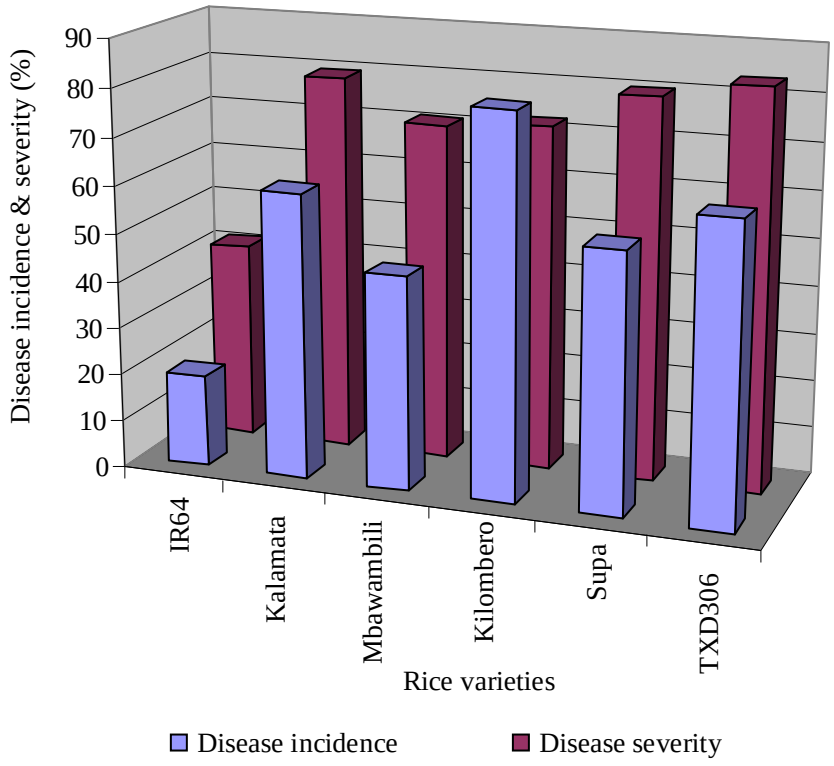

Figure 2.2: The incidence and severity of rice blast disease on six rice varieties covered in the current survey in four regions in Tanzania.

Most farmers in Kilimanjaro region preferred to plant IR 64 because it is more resistant to diseases, especially rice blast and insect pest attack. The variety IR 64 is widely grown in Lower Moshi irrigation scheme, Kilimanjaro and possesses many positive agronomic characteristics such as wide adaptability, high yield potential and tolerance to multiple

diseases and insect pests (Wu *et al.*, 2005). There is therefore, a need to formally screen IR 64 in order to confirm its level of resistance to rice blast disease so that it can be used in breeding programs against rice blast disease.

Although high level of rice blast disease severity was recorded on TXD 306 in irrigation schemes in Mvomero District, farmers in the surveyed areas have reported TXD 306 as a high yielding variety signifying tolerance. More research is needed to confirm the reaction of TXD 306 to rice blast disease under different ecological and management systems.

The differences in rice blast disease incidence and severity between regions and ecosystems were very pronounced. High yield losses of up to 60% due to blast incidence and severity were reported by farmers in Mbeya and Morogoro regions. The survey results suggest that Mbeya followed by Morogoro are hotspot areas for rice blast disease and may be good locations for evaluation of rice varieties for reaction to *P. oryzae*. Kilimanjaro and Shinyanga regions have been considered as the areas of very low rice blast disease pressure because of low incidences and severities of rice blast disease observed. Castejón-Muñoz (2008) reported similar results when studying the effect of temperature and relative humidity on the airbone concentration of *Pyricularia oryzae* spores and the development of rice blast in southern Spain.

2.5 Conclusion

Most of the rice varieties grown by farmers in Kyela, Mbeya Region (Kilombero and Supa), Kahama, Shinyanga (Kalamata and Mbawambili) and Mvomero districts in Morogoro (Kalamata, Kilombero, Mbawambili and Supa) were susceptible to rice blast

disease. However, in areas where farmers planted local varieties, rice blast disease incidence and severity were much higher than where improved rice varieties were grown. The current disease surveys have shown that rice blast disease was spreading at a moderate rate in rice fields in Tanzania. Although no effective major resistance genes for rice blast disease are known to exist in widely grown rice varieties in Tanzania, the variety IR 64 has shown tolerance to blast disease, and can therefore, be used as a source of resistance to blast in breeding programs. Variations in rice blast disease incidence and severity were apparent in the different rice growing regions in Tanzania. The incidence and severity of blast were quite high in areas under rainfed conditions. It is therefore, important that any program to develop varieties resistant to rice blast disease should consider environmental conditions and cultivation practices in different locations. More studies are needed to determine the status of rice blast disease in areas which were not covered by the current study.

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CHAPTER THREE

3.0 PATHOGENIC VARIATION OF *PYRICULARIA ORYZAE* AND GRAIN YIELD LOSSES CAUSED BY RICE BLAST DISEASE IN MAJOR RICE GROWING AREAS IN TANZANIA

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3.1 Abstract

Grain yield losses in rice (*Oryza sativa* L.) caused by *Pyricularia oryzae* Cavara, [(synonym *P. grisea* Sacc (teleomorph: *Magnaporthe grisea* (Hebert) Barr)] causal agent of rice blast disease, is a major problem facing rice growers worldwide. In Tanzania, rice blast is considered as the most serious disease, resulting in severe yield losses especially, when susceptible rice varieties are grown. In order to assess yield losses caused by *P. oryzae*, studies were conducted in the screen-house using ten rice varieties viz; Jaribu 220, Supa, Kalamata, Shingo ya Mwali, Mwarabu, Mbawambili, Kihogo, IR 64, TXD 306 and TXD 85. Results showed that rice blast disease affected rice plants at all stages of growth and resulted in reduction in number of tillers per plant, grain weight, number of seeds per panicle and grain yield. Most of the rice varieties used were susceptible to *P. oryzae* at seedling, early tillering and heading stages (reproductive stages). During early growth stages, symptoms were mainly found on leaves. Leaf blast disease severity reached maximum at tillering stage, then the disease symptoms disappeared gradually. Leaf blast development progressed significantly differently between rice varieties. The varieties

Mwarabu and Jaribu 220 were the most susceptible at 45 and 55 days after inoculation (DAI). The area under disease progress curve (AUDPC) increased with leaf age. The relationship between rice blast disease severity and grain yield loss indicated that each increase in the disease severity resulted in a simultaneous reduction in grain yield. Rice blast disease severities on both leaf and panicle were positive and highly significantly correlated with grain yield losses ($r = 0.96$, $P < 0.001$ and $r = 0.91$, $P < 0.001$, respectively). The number of tillers and seeds per panicle were negatively correlated with disease severity and grain yield weight ($r = -0.912$ and -0.958 respectively). The varieties Jaribu 220, Mbawambili, Kalamata and Supa were susceptible to blast disease. Grain yield losses of between 11.9 to 37.8% per hectare were recorded for the susceptible rice varieties.

Key words: AUDPC, *Pyricularia oryzae*, rice blast disease, yield losses

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3.2 Introduction

Rice blast disease is one of the most devastating diseases that can cause high grain yield losses in farmers' fields (Ashtiani *et al.*, 2012; Koide *et al.*, 2009; Mohapatra *et al*., 2008). It is a fungal disease caused by *Pyricularia oryzae* which belongs to the class Ascomycetes and the genus *Magnaporthe* (Gandalera *et al.*, 2013). The asexual state is called *Magnaporthe oryzae* (Couch and Kohn, 2002; Gandalera *et al.*, 2013; Noguchi *et al.*, 2007). The disease occurs worldwide where rice is grown, but its occurrence and severity vary yearly, based on location and environmental conditions (Devi and Chhetry, 2014). The disease infects all parts of the rice plant except roots, but leaves and panicles are the most seriously affected (Pinheiro *et al*., 2012b). Leaf blast lesions reduce the net

photosynthetic rate of individual leaves (Koutroubas *et al.*, 2009). Neck blast is considered the most destructive phase of the disease and can occur without being preceded by severe leaf blast (Zhu *et al*., 2005). The necrotic lesions on mature rice plants particularly in the panicle can cause major yield losses (Gandalera *et al*., 2013). Several studies have reported that leaf, panicle and neck blast disease incidences caused similar yield losses. In Japan the yield losses of 20 to 100% were reported by Khush and Jena (2009) and Pinheiro *et al.* (2012). In Brazil, yield losses as high as 100% (Prabhu *et al*., 2009) have been reported in upland rice varieties. In India, losses of 5 – 10%, were recorded (Padmanabhan, 1965), while 8% yield losses in Korea and 14% losses in China and 50 to 85% in the Philippines have been reported (Shafaullah *et al.,* 2011). Hai *et al* (2007) reported grain yield losses of susceptible rice varieties of 38.21 to 64.57% due to neck blast in Vietnam.

About 5-70% grain yield losses were reported in Kashmir depending upon the stage of the crop infected and severity of the disease (Bhat *et al.*, 2013). Inoculation of *P. oryzae* reduced grain yield from 22 to 26% in Italy (Koutroubas *et al*., 2009). Grain yield losses of 25.21 to 45.52% were recorded in Rajasthan (Maheshwari and Sharma, 2013). In Iran, low yield losses of 0.99 - 1.22% were reported by Mousaneja *et al.* (2010). Other studies in Iran reported yield reduction of 10-20% in susceptible rice varieties, but in severe cases the yield loss caused by rice blast may reach up to 80% (Pasha *et al*., 2013). Panicle blast disease severity affects grain filling (Castejon-Munoz *et al.*, 2007). Yield losses due to blast disease have a direct impact on the welfare of farm households as well as on the national economy.

The symptoms of rice blast disease on leaves, nodes and panicles may vary according to the environmental conditions, the age of the plant and the level of resistance of the host genotypes (De-xi *et al*., 2010). Climatic conditions affect greatly the disease establishment, development and severity, resulting in large genotype-by-environment interactions. The yield variability among both different rice varieties and rice growing areas, explain the effects of climate and crop management on rice yields. However, yield constraints are related to disease incidence and severity in relation to crop management and environmental conditions (Naing *et al.,* 2008). Mousaneja *et al.* (2010) demonstrated that rice blast disease incidence and severity during the reproductive stage (75 days after seeding) was most closely related to yield losses with an infected area of 1% corresponding to 3% yield loss. However, these studies reported also that most of the resistant rice varieties were severely diseased, although the level of disease was lower than that of susceptible varieties (Bonman *et al.*, 1991). In Tanzania, information on rice grain yield losses in relation to rice blast disease level is limited. Therefore, the objective of this study was to investigate the effect of rice blast disease on grain yield in major rice growing areas of Tanzania.

3.3 Materials and Methods

Ten rice varieties preferred by farmers in Tanzania (Kalamata, Mbawambili, Supa, TXD 306, TXD 85, Kihogo, Mwarabu, IR64, Shingo ya Mwali and Jaribu 220) were evaluated to determine the effect of rice blast disease on yield losses. The experiment was conducted in the screen-house at the Chinese Agricultural Technology Demonstration Centre in Dakawa, Mvomero district, Morogoro region. The seeds of each variety were sown in one square meter plot (experimental unit) at a spacing of 20 cm x 20 cm with one seed per hill

arranged in a completely randomized design, with three replications. Each replication consisted of a plot with five rows and each plot contained 25 seeds of each variety. The plots were flooded with water and such conditions were maintained until the grains reached physiological maturity. The non-inoculated plots were sprayed with Tricyclazole fungicide to prevent occurrence of rice blast disease.

3.3.1 Inoculum preparation and inoculation

Pyricularia oryzae was cultured in Petri dishes on oatmeal agar, incubated at 25 ± 1ºC to induce sporulation as described by Rathour *et al.* (2006). After sporulation, spore suspension at a concentration of about 10^4 spores/ml was prepared in sterilized distilled water with 0.1% Tween 20 to increase spore dispersion (Prasad *et al.*, 2009). The conidial concentration of 2 x 10^5 spores/ml was prepared (Namai and Ehara, 1986). Spore counts were done using a haemocytometer. When the plants reached the 4-5 leaf stage (21 days after seeding), they were inoculated with *P. oryzae* following the procedures described by Zhang *et al*. (2009). Inoculation was done in the evening using a low-pressure spray bottle on each individual rice plant. All agronomic practices including fertilizer (UREA 120 kgN/ha) were applied as recommended.

3.3.2 Disease assessment

Rice blast disease evaluation was conducted throughout the growing season, starting with observation of the first disease symptoms after inoculation. Observation of blast disease symptoms was done at 14 days after inoculation, and thereafter at 10 days intervals, based on blast disease assessment given by the standard evaluation system of IRRI (1996), using a 9 scale basis where; 0 = no lesions; 1 = small, brown, specks of pinhead size; 3 = small,

roundish to slightly elongated, necrotic, gray spots about 1-2 mm in diameter; 5 = typical blast lesions infecting < 10% of the leaf area; 7 = typical blast lesions infecting 26-50% of the leaf area; 9 = typical blast lesions infecting >51% leaf area and many dead leaves. Rice blast disease severity was then calculated using the formula described by Hajano *et al.* (2011).

$$\text{Disease severity} = \frac{\Sigma\, n \times v}{N \times V} \times 100\%$$

where, n = number of leaves infected by blast, v = value score of each category attack,

N = number of leaves observed and V = value of the highest score.

Rice blast disease development on plants was observed six times at 15, 25, 35, 45, 55, and 65 days after inoculation (DAI). Diseased leaf area was calculated by multiplying length and width of lesion.

The area under disease progress curve (AUDPC) was calculated from single ratings as described by Shaner and Finney (1977), Pasha *et al*. (2013) and Mohapatra *et al*. (2008) as follows:

$$AUDPC = \sum[(0.5)\,(Y_{i+1} + Y_i)\,(T_{i+1} - T_i)]$$

Where, Y = disease severity at time i and T = time (days) of the assessment.

The percentages of panicle blast disease severity (PBS) were obtained by rating individual panicles using the formula described by IRRI (2002) and Pasha *et al*. (2013) as shown below:

$$PBS = (A/B)\ X\ 100$$

Where, A = the number of infected panicles, and B = the number of panicles observed for each variety per plot. Based on both the leaf and panicle severities, rice varieties were

classified as resistant (R) with 0-15%; moderately resistant (MR) with 15.1-30%; moderately susceptible (MS) with 30.1-50%; or susceptible (S) with 50.1-100% disease severities (Puri *et al*., 2006).

At physiological maturity, three middle rows of each plot were harvested and kept separately. The panicles of each rice variety were threshed manually and grain weight was recorded. Rice grain yield was determined at 13% grain moisture content as recommended by Mousanejad *et al.* (2010). Percent grain yield losses caused by rice blast disease for each variety were also determined using the formula described by Mousanejad *et al.* (2010).

$$\text{Yield loss} = \frac{\text{Yield of non-inoculated} - \text{Yield of inoculated}}{\text{Yield of non-inoculated}} \times 100$$

Weather data for the months of March to July 2013 were collected from the Meteorological station at Dakawa and from Tanzania Meteorological Agency websites.

Before analysis data were transformed using arcsine square root transformation formula as described by Mousanejad *et al*. (2010) as shown:

$$Y = \text{Arcsin } \sqrt{P}$$

Where, Y is transformed data, p is the observed proportion

3.3.3 Data analysis

Data were analysed using GENSTAT computer statistical package for ANOVA to determine significant differences between ten rice varieties. Comparison between means was done using Duncan's Multiple Range Test (DMRT). A regression analysis was done to find out the correlation between the disease levels and percent loss in yield. Charts were drawn using the Microsoft Excel program (Bonman *et al.*, 1991).

3.4 Results and Discussion

Rice blast disease symptoms on leaves, neck and panicles are shown in Plate 3.1. Depending upon the genetic makeup, each of ten rice varieties reacted differently to rice blast disease. Rice blast disease symptoms were observed on leaves of all rice varieties between 14 and 20 days after inoculation. The symptoms began on the lower leaves 15 days after inoculation and progressed to the upper leaves. After heading, *P. oryzae* infected the panicles. Panicle blast caused direct yield losses, since filling of the grain on infected panicles was poor (Table 3.1). Leaf lesions began as small whitish, grayish spots and enlarged progressively. The shape, colour and size of the leaf lesions varied with plant age (Bussaban *et al.*, 2005).

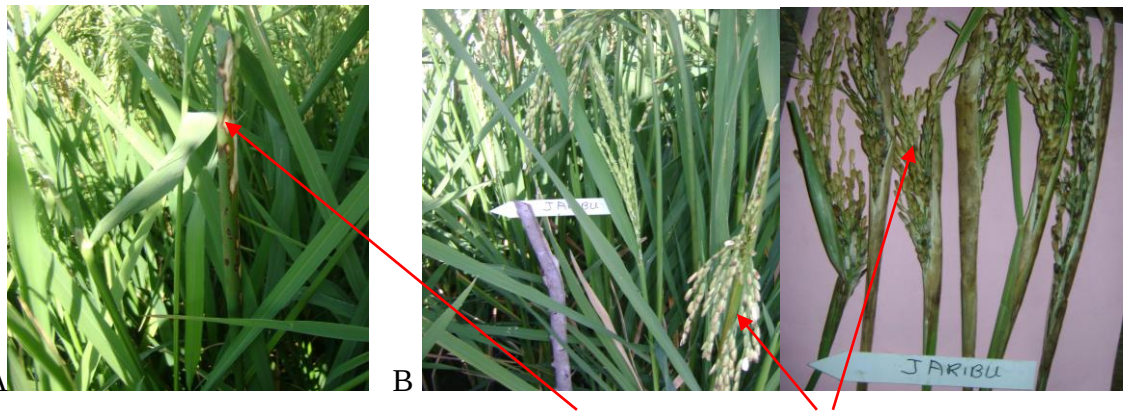

Plate 3.1: Rice blast disease symptoms on leaves (A) and panicles (B) (photos: C. J. Chuwa, Dakawa, Morogoro).

3.4.1 Rice blast disease development on leaves and panicles

Results of the current study showed that rice blast disease development on leaves was significantly ($P \leq 0.05$) different between varieties (Table 3.1). The highest rice blast disease severity was recorded on Mwarabu variety (77.78%), followed by Kalamata (66.67%), Jaribu 220 (66.67%) and Supa (55.56%). The lowest rice blast disease severity

on leaves was recorded on TXD 85 (16.67%) followed by TXD 306 (27.78%), Shingo ya mwali (33.33%) and Kihogo (38.89%). The average temperature (30.4^{o}C), precipitation (70.3 mm) and relative humidity (80.0%) during the experiment (March, April and May, 2013) were higher than at maturity period (June and July, 2013) (Table 3.3).

Table 3.1: The rice blast disease index and unfilled grains on different rice varieties in the screen-house at Dakawa, Morogoro

Variety	Disease index (%)		Unfilled grains		
	Leaf blast severity	Panicle blast severity	inoculated (%)	Non-inoculated (%)	AUDPC
1. Mbawambili	44.44abc	60.93cd	52.67bc	8.67a	456.60abc
2. TXD 306	27.78ab	26.77ab	14.00a	6.33a	276.10ab
3. Kihogo	38.89abc	39.43abc	25.67ab	13.33a	391.10abc
4. TXD 85	16.67a	17.80a	19.67a	8.67a	184.50a
5. Jaribu220	66.67cd	73.27d	74.33c	10.00a	889.00d
6. Supa	55.56bcd	45.83abcd	26.33ab	12.33a	496.70bc
7. Kalamata	66.67cd	53.33bcd	41.00ab	12.67a	590.20c
8. Shingo ya mwali	33.33ab	45.00abcd	12.33a	12.00a	564.40c
9. IR 64	44.44abc	16.80a	18.67a	8.00a	179.10a
10. Mwarabu	77.78d	28.00ab	13.00a	5.67a	652.50cd
CV %	**33.5**	**41.6**	**56.5**	**51.0**	**32.4**

Means in the same column followed by the same letter are not significantly different by Duncan's Multiple Range Test at $P < 0.05$.

CV% = Percent of coefficient of variation, (*) significantly different at the 5% by T-test, ns = not significant, AUDPC = Area under disease development progress curve.

AUDPC = Area under disease progress curve.

Table 3.2: The reaction of rice varieties to blast disease caused by *Pyricularia oryzae*

Leaf blast			Panicle blast		
Varieties	**Severity (%)**	**Host reaction**	**Varieties**	**Incidence (%)**	**Host reaction**
Nil	0-15	Resistant	Nil	0-15	Resistant
TXD 306 and TXD 85	15.1–30	Moderately resistant	TXD 306, TXD 85, IR 64 and Mwarabu	15.1– 30	Moderately resistant
Kihogo, Mbawambili, IR 64 and Shingo ya Mwali	30.1–50	Moderately susceptible	Kihogo, Supa and Shingo ya Mwali	30.1– 50	Moderately susceptible
Jaribu 220, Supa, Mwarabu and Kalamata	50.1–100	Susceptible	Mbawambili, Jaribu 220 and Kalamata	50.1– 100	Susceptible

This means that high relative humidity, rainfall but moderate temperatures, were associated with higher rice blast disease severities for Jaribu 220. Similar results on the effect of epidemiological factors on rice blast have been reported by Castejón-Muñoz, (2008), Ahmad *et al.* (2011) and Nasruddin and Amin (2013). The variety Mwarabu was susceptible to *Pyricularia oryzae* whereas, TXD 85, TXD 306, Shingo ya mwali and Kihogo gave resistant reaction (Table 3.1). The moderate resistance to rice blast was recorded on Mbawambili (44.44%), Supa (55.56%) and IR 64 (44.44%).

Rice blast disease severity on panicles was significantly different ($P \leq 0.05$) between rice varieties (Table 1). The highest rice blast disease severity on panicles was recorded on variety Jaribu 220 (73.27%) followed by Mbawambili (60.93%) and Kalamata (53.33%). These varieties also showed high panicle blast disease severity at 42.4 mm average rainfall (Table 3.3). This means that rainfall influenced development of rice blast disease severity on panicles. That rain drops provided moisture on the panicles and facilitated germination of *P. oryzae* spores.

Table 3.3: Monthly temperatures, precipitation and relative humidity at Dakawa, Morogoro Region from March to July 2013, when the current experiment was conducted

Month	Temperature			Precipitation (mm)	Relative humidity (%)
	Minimum	Maximum	Mean		
March	22.0	32.1	27.1	95.4	79.6
April	21.3	30.1	25.7	96.0	82.5
May	19.5	29.6	24.6	19.3	78.0
June	16.1	29.1	22.6	0.1	66.5
July	15.4	29.3	22.4	1.3	64.0
Average	**18.9**	**30.0**	**24.5**	**42.4**	**74.1**

Source: Meteorological Department, Mvomero District, Morogoro Region and http://www.weatheronline.co.uk/weather/maps/city?lang=en&plz=&plzn=_&wmo=63866&cont=afri&r=0&level=162& region=0009&land=tz& mod=tab&art=pre&noregion=1&fmm=3&fyy=2013&lmm=7&lyy=2013. Site visited on September 2013.

Such findings have also been reported by Koutroubas *et al*. (2009) and Shafaullah *et al*. (2011).

The lowest percentages of rice blast disease severity on panicles were obtained from varieties TXD 85 (17.8%) and IR 64 (16.8%) followed by TXD 306 (26.77%), Kihogo (39.43%), Supa (45.83%), Shingo ya Mwali (45%) and Mwarabu (28%). In this study, high rice blast disease severity on panicles caused incomplete grain filling resulting in high grain yield losses because rice blast disease destructed the rate of photosynthesis in the leaf which plays an important role in the plant body, as the result, it disturbed all the physiological processes taking place in the plant including grain filling process. Similar results have been reported by Gandalera *et al*. (2013) when working with inhibitory

activity of *Chaetomium globosum* Kunze extract against Philippine strain of *Pyricularia oryzae*.

The percentages of unfilled rice grains significantly differed ($P \leq 0.05$) between varieties (Table 3.1). Inoculated plants had higher percentages of unfilled grains than non-inoculated plants. The average of unfilled rice grains on Jaribu 220 was significantly higher (74.33%), followed by Mbawambili (52.67%). The lowest percentages of unfilled rice grains were on TXD 306 (14%), TXD 85 (19.67%), Shingo ya Mwali (12.33%), IR 64 (18.67%) and Mwarabu (13%), followed by Kihogo (25.67%), Supa (26.33%) and Kalamata (41%) rice varieties. The highest percentage of unfilled grain corresponded with high disease severity on leaves and panicles (Table 3.1). Similar findings were reported by Chaudhary *et al.* (2005) and Pasha *et al*. (2013).

The reaction of rice varieties tested to rice blast disease varied depending on the infected plant parts. Mwarabu, Kalamata, Jaribu 220 were more susceptible on leaves (Tables 3.1 and 3.2). Moreover, IR 64, TXD 85, Mwarabu and TXD306 were moderately resistant to panicle blast while Kihogo, Supa and Shingo ya Mwali were moderately susceptible (Table 3.2).

The varieties Jaribu 220, Mbawambili and Kalamata were susceptible to panicle blast (Table 3.2). This indicates that TXD 85 and TXD 306 may have partial resistance against rice blast disease. Such a phenomenon has been reported by Nasruddin and Amin (2013). Resistant varieties were not identified in this study with respect to leaf and panicle blast. Although there was no any rice blast resistant variety found in this study, this information

has provided enough descriptive and comparative information on the reaction of selected rice varieties grown in Tanzania. More research is needed in order to develop resistance rice varieties against rice blast disease.

In this study, weather factors including temperature and relative humidity (RH) have played an important role on the development of rice blast disease severity. The different levels of rice blast disease severity on rice varieties were most likely due to the corresponding differences in the weather parameters recorded during the growing period and genetic diversity of the varieties studied. The average minimum (18. 9^{o}C) and maximum (30^{o}C), temperature and RH of 74.1% (Table 3.3) were important epidemiological factors to rice blast disease development. However, high severity level of rice blast disease was due to the environmental conditions being favourable for the development of *Pyricularia oryzae*. Similar findings have been reported by Shafaullah *et al.* (2011) when they were studying the effect of epidemiological factors on the incidence of paddy blast (*Pyricularia oryzae*) disease in Pakistan. It is well known that the environmental conditions, especially moderate temperatures (25 – 30^{o}C) and high relative humidity, are important factors inducing and facilitating sporulation and growth of *P. oryzae* (Babu *et al.*, March 2014; Castejon-Munoz *et al*., 2007; Castejón-Muñoz, 2008; Koutroubas *et al*., 2009). The presence of high rice blast disease severity on leaves and panicles affected the rate of photosynthesis in the rice plants resulting into reduced grain filling and high percentages of unfilled grains (Koutroubas *et al*., 2009).

Generally, most of the rice varieties tested were susceptible to *P. oryzae* in the seedling, early tillering and heading stages of the crop. It was noted that, the symptoms of rice blast

disease on leaves were observed in the early growth stages of the rice crop. This is because rice plants are more susceptible to blast disease at young than at mature stages of development (Babu *et al*., March 2014). The trend of rice blast disease development revealed that when the plants were at maximum tillering, the disease symptoms on the leaves tended to disappear gradually. Such a situation had been reported to be attributed to adult plant resistance (Babu *et al*., March 2014).

3.4.2 The Area under Disease Progress Curve (AUDPC)

The area under disease progress curve (AUDPC) in screen-house conditions was significantly ($P \leq 0.05$) different between the ten rice varieties tested (Table 3.1). The highest AUDPC values were obtained from Jaribu 220 (889.0) followed by Mwarabu (652.5), Kalamata (590.2) and Shingo ya mwali (564.4). On the other hand, the lowest AUDPC values were recorded from TXD 85 (184.5) and IR 64 (179.1) followed by TXD 306 (276.1), Kihogo (391.1) and Mbawambili (456.6). However, TXD85 and IR64 had similar trends of rice blast disease progress (Table 3.1).

The AUDPC is considered as the best parameter to declare a variety resistant or susceptible (Kumar *et al.*, 2013). It provides more precise and practical classification of resistant and susceptible varieties than that based on the percentage disease score of each variety (Kumar *et al*., 2010; Jeger, 2004). Early occurrence of rice blast disease at young stage of rice plant growth caused high damage on the leaves and finally disturbed all physiological processes in the plant (Mousanejad *et al.*, 2010; Hwang *et al.*, 1987). The level of rice blast disease severity increased with age of rice plants during the season. The highest AUDPC values on Jaribu 220, Mwarabu, and Kalamata rice varieties showed that these varieties have high levels of susceptibility to leaf blast. The lowest AUDPC values

on TXD 85 and IR 64 showed that these varieties had high significant levels of resistance to rice blast disease. Similar results were reported by Riungu (2007) and Pasha *et al.*(2013). Therefore, rice varieties TXD 85 and IR 64 can be used as donor parents for breeding moderate resistant rice varieties to blast disease in Tanzania.

3.4.3 Leaf blast disease progress in rice varieties grown at Dakawa, Morogoro

Leaf blast disease progress curves for ten rice varieties under screen-house conditions are shown in Figure 3.2. Leaf blast development progressed differently with different rice varieties. However, for all varieties, disease started at a low level, gradually increasing in severity over time.

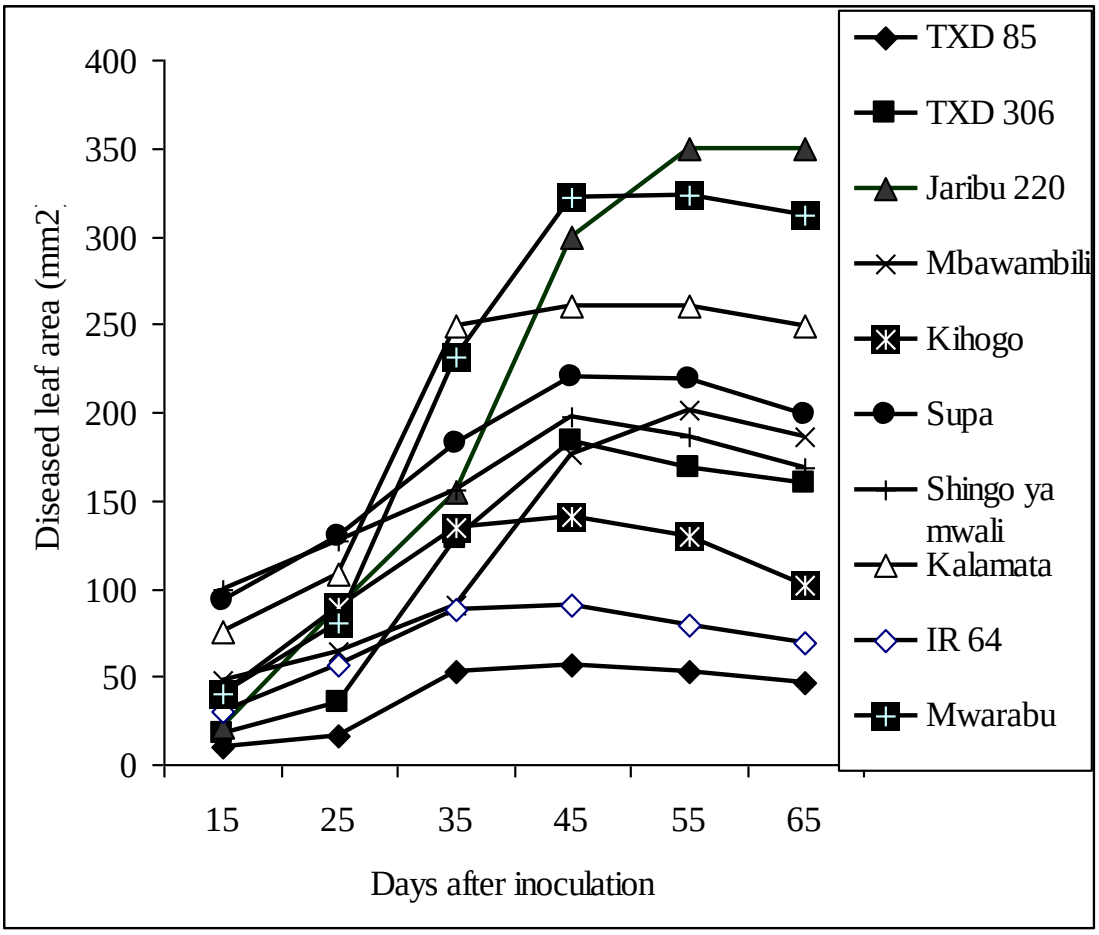

Figure 3.1: Leaf blast disease progress curves for ten rice varieties grown by farmers in Tanzania

Rice blast disease severity increased significantly ($P \leq 0.05$) from 15 to 45 days after inoculation (DAI) (Figure 3.1) indicating that at this stage all rice varieties were susceptible to rice blast disease. Results have shown that all varieties had low diseased leaf area (< 150 mm^2/leaf) at 15 DAI and 25 DAI. After 25 DAI, diseased leaf area increased drastically. Results also indicate that the highest diseased leaf area was recorded on Jaribu 220 followed by Mwarabu, whereas, the lowest diseased leaf area was obtained on TXD 85 and IR 64 followed by Kihogo. Following these results, TXD 85, IR 64 and Kihogo are the promising rice varieties for resistance to rice blast disease in Tanzania.

The lowest and the highest diseased leaf area could have been caused by genetic diversity of the varieties, age of the plant and weather conditions. The results revealed that as the plants approached maturity, the plants gained resistance to rice blast disease (Nasruddin and Amin, 2013). These findings are also supported by the study of Bonman *et al.* (1991) on the assessment of blast disease and yield loss in susceptible and partially resistant rice cultivars in two irrigated lowland environments.

The results indicated that the disease progressed and reached maximum at 45 and 55 DAI and then gradually declined. This decline in disease was attributed to adult disease resistance, leaf senescence and formation of new leaves. In quantitative resistance, where differences in level of resistance are usually less distinct, measuring disease progress is important for understanding plant–pathogen interaction (Simko and Piepho, 2012).

3.4.4 Regression and correlations between diseased leaf area and weather parameters

There was a linear relationship between increasing or decreasing environmental parameters and diseased leaf area (Figure 3.2). A negative correlation between minimum temperature, maximum temperature and disease leaf area was observed in all the ten rice

varieties. It was also observed that rice blast disease responded negatively and showed significant correlation between minimum temperature, maximum temperature and disease severity. A positive correlation between rainfall, RH and diseased leaf area was observed. The relationship between them could best be expressed by the linear regression equations: y = 544.9 – 21.84X where X is minimum temperature, y is diseased leaf area, y = 1621 - 49.53X where, X is maximum temperature and y is diseased leaf area, y = 56.4 + 14.65X where, X is rainfall and y is diseased leaf area and y = 456.6 + 7.96X where, X is RH and y is diseased leaf area.

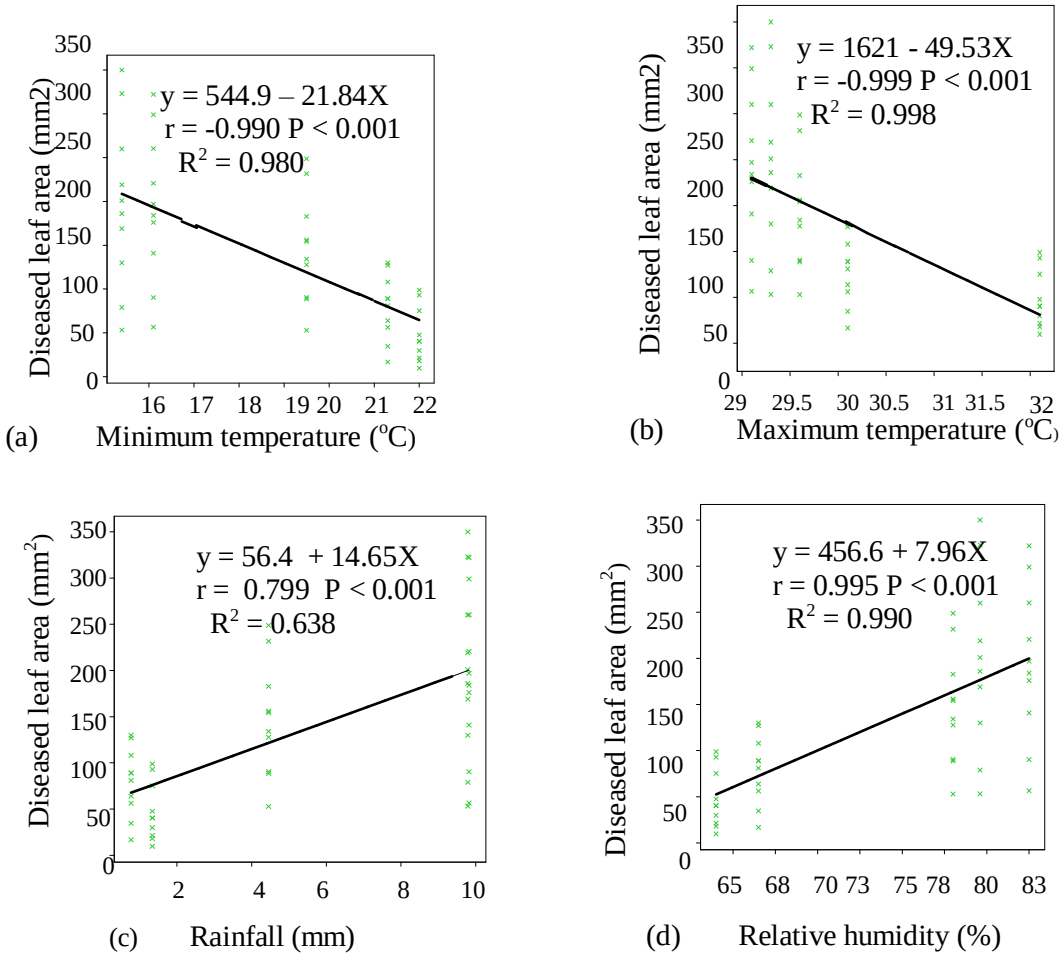

Figure 3.2: Linear correlation between rice blast diseased leaf area and (a) and (b) mean temperature, (c) rainfall during the growth period and (d) relative humidity

At 83% of RH, high rice blast disease severity on development occurred. These results indicate that temperature, rainfall and relative humidity played important role for the rice blast disease development because they influenced sporulation, release of spores and germination of blast conidia (Koutroubas *et al*., 2009).

3.4.5 Regression and correlations between leaf and panicle blast parameters and grain yield losses in rice

In Figure 3.3, the coefficient of determination (R^2) for both leaf and panicle blast disease were highly significant ($R^2 = 0.930$, $P < 0.001$ and $R^2 = 0.837$, $P < 0.001$, respectively). These results indicate that 93% of grain yield losses per ha due to leaf blast disease severity and 83.7% of grain yield losses was caused by panicle severity. The significance of the linear regression implies that some portion of the variability (93% and 83.7%) in grain yield losses were indeed explained by the linear function of leaf and panicle rice blast disease severities, respectively.

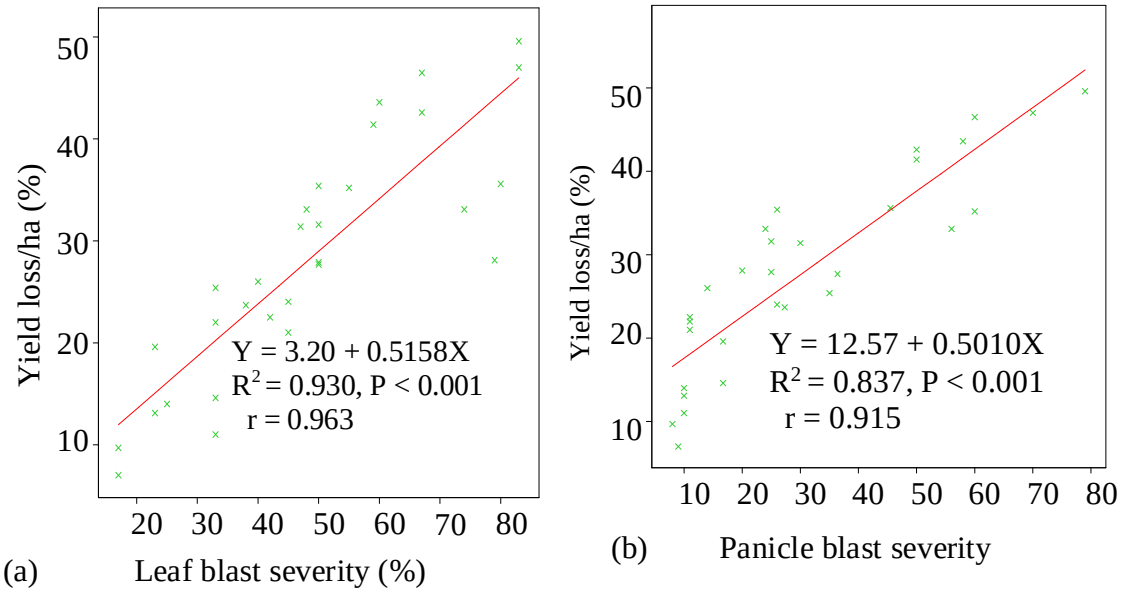

Figure 3.3: Relationship between (a) leaf blast disease severity and grain yield loss, and (b) panicle blast disease severity and grain yield loss in selected rice varieties grown in Tanzania.

However, both leaf and panicle blast disease severities were highly significantly correlated with grain yield losses ($r = 0.963$, $P < 0.001$ and $r = 0.915$, $P < 0.001$, respectively) in inoculated rice varieties (Figure 3.3). These results indicate that leaf and panicle blast disease severities were directly related to grain yield losses. The findings that an increase in leaf or panicle blast disease severity corresponded to an increase in grain yield losses have also been reported by Shim *et al*. (2005).

Based on linear regression equations related to leaf ($Y = 3.20 + 0.5158X$) and panicle ($Y = 12.57 + 0.5010X$) blast disease severities, it can be estimated that each unit increase of leaf blast severity corresponded to an increased grain yield loss of 0.5158 % per ha (Figure 3.4). The corresponding reduction in grain yield for each unit increase of panicle blast was 0.501% yield loss/ha. These models show that differences in yield losses between rice varieties were due to both leaf and panicle blast disease development. The regression model obtained can be used to estimate rice grain yield losses due to blast disease. These findings are in agreement with the findings of Bonman *et al*. (1991) and Mousanejad *et al*. (2010) that the regression model with high coefficient of determination is useful for forecasting yield losses.

3.4.6 Effect of rice blast disease on tillering and grain yield

The results in Table 3.4 indicate that, rice blast disease had a substantial impact on reduction of the number of tillers per hill, seeds per panicle and grain yield. There was significant reduction ($P<0.05$) of the number of tillers, panicle number and grain yield between rice varieties used. The tillers per hill between inoculated and non-inoculated rice

plants were highly significantly different (*t*-value = 3.55, P < 0.001) except on TXD 85 and IR 64 varieties (Table 3.4).

However, the number of tillers/hill was not significantly different (P < 0.05) between the varieties Shingo ya Mwali, Kihogo, Kalamata, Jaribu 220, Mbawambili, Supa and IR 64 for inoculated plots. The highest tillers per hill were recorded on TXD 306 and TXD 85 varieties in diseased (inoculated) plants, while in healthy (non-inoculated) plants, TXD 306, followed by Supa, Jaribu 220 and Mbawambili were recorded with the highest number of tillers per hill (Table 3.4).

The number of seeds per panicle between inoculated and non-inoculated plants were highly significantly different (*t*-value = 6.50, P<0.001) (Table 3.4) except on Shingo ya

Table 3.4: The number of tillers/hill, seeds/panicle, grain yield and yield loss from rice blast on varieties preferred by farmers at Dakawa, Morogoro

Varieties	Number of Tillers/hill		Number of seed/panicle		Yield (kg/ha)		Yield
	Inoculated	non-inoculated	Inoculated	Non-inoculated	Inoculated	Non-inoculated	loss (%)
1. Shingo ya mwali	4.7a	10.0b	203.7bc	244.7cd	8267bc	10037cd	17.7ab
2. Kihogo	5.0a	10.7b	214.3bc	296.7de	9633cd	11427de	15.5a
3. Kalamata	5.7a	18.0cd	137.7ab	330.0e	7800b	11100cde	29.6bcd
4. TXD 85	13.0c	15.0cd	215.3bc	294.7cde	7567b	9133bc	17.0ab
5. Mbawambili	6.3a	27.3de	143.3ab	288.0cde	6167a	9033b	31.0cd
6. JARIBU 220	5.7a	15.0ab	109.7a	308.3de	6130a	10027bc	37.8d
7. Mwarabu	10.0b	18.3c	189.7abc	263.0cd	7867b	9247bc	13.0a
8. Supa	7.3ab	29.0de	162.7ab	235.7c	8550bc	11033cd	22.3abc
9. TXD306	14.7c	36.7e	253.3c	352.3f	10283d	11067de	11.9a
10. IR 64	8.0ab	10.0a	158.3ab	172.0a	5967a	7097ab	16.0a
Mean	**8.0**	**19.0**	**176.6**	**278.5**	**7776.4**	**10046.1**	**22.0**
CV %	**21.9**	**19.5**	**23.4**	**11.2**	**10.9**	**10.4**	**35.1**

Means in the same column followed by the same letter are not significantly different by Duncan's Multiple Range Test at $P < 0.05$.

CV % = Percent of coefficient of variation.

Mwali, TXD 85, Mwarabu and IR 64 varieties. The highest number of seeds per panicle was recorded on TXD 306 in both inoculated and non-inoculated plants, followed by TXD 85, Kihogo and Shingo ya Mwali, varieties. The lowest seeds per panicle were recorded on, Jaribu 220 (109.7) on inoculated plants followed by Kalamata (137.7), Mbawambili (143.3), Mwarabu (189.7), Supa (162.7) and IR64 (158.3).

The rice grain yields between inoculated and non-inoculated plants were highly significantly different at *t*-value 8.04 and $P < 0.05$ on Mbawambili, Jaribu 220 and Kalamata varieties (Table 3.4). The highest grain yield was recorded on TXD 306 (10283 kg/ha and 11067 kg/ha for inoculated and non-inoculated plants, respectively) while the lowest grain yield was recorded on IR 64 in both inoculated and non-inoculated plants, followed by Jaribu 220 and Mbawambili.

Percentage rice grain yield losses due to rice blast disease were significantly different ($P < 0.05$) between the ten rice varieties (Table 3.4). Yield losses from Shingo ya Mwali, Kihogo, TXD 85, Supa, TXD 306, Mwarabu and IR 64 varieties did not differ significantly at the 5% level. Yield losses from variety Jaribu 220 differed significantly from Kihogo, Mwarabu, TXD 85, Supa, TXD 306 and IR 64 at the 5 % level (Table 4). However, the highest percentage of yield losses was recorded on Jaribu 220 (37.8%) followed by Mbawambili (31.0%) and Kalamata (29.6%) and the lowest yield losses were recorded on TXD 306 (11.9%) IR64 (16.0 %), and Kihogo (15.5%) followed by TXD 85 (17.0%).

These results indicated that yield losses due to rice blast disease caused by *P. oryzae* ranged between 11.9 and 37.8%. Similar yield losses have been reported in Egypt by Haggag and Tawfik, (2014), Iran (Mousanejad *et al.,* 2010); Korea (Bonman *et al.*, 1991;

Shim *et al.*, 2005). Early occurrence of rice blast disease at young stages of rice plant growth can cause considerable leaf and panicle damage and reduce tillering ability. Reduced tillering may result in a simultaneous reduction in grain yield because productive tillers per plant contributed to the differences in grain yield in that it is a yield attribute or component.

The number of tillers and seeds per panicle were negatively correlated with grain yield losses (r = -0.857 and -0.958, respectively) at 5% (Figure 3.4). These results revealed a direct relationship between number of tillers and seeds per panicle with grain yield. Similar coefficient of correlations (r) have been reported by Shim *et al.* (2005) in the study of damage analysis of rice panicle blast on disease occurrence time and severity. It was noted that at tillering stage, rice seedlings of Kihogo, Kalamata, Mbawambili, Jaribu 220, Supa and TXD 306 were more susceptible to blast disease than mature plants (Zhu *et al.*, 2005) and the number of tillers and seeds were reduced substantially (Koutroubas *et al.*, 2009). Whereas, late rice blast infection of plants at tillering stage had only small effect on both tillers and grain seed reduction on varieties IR 64, TXD 85 and Shingo ya Mwali (Shim *et al.*, 2005).

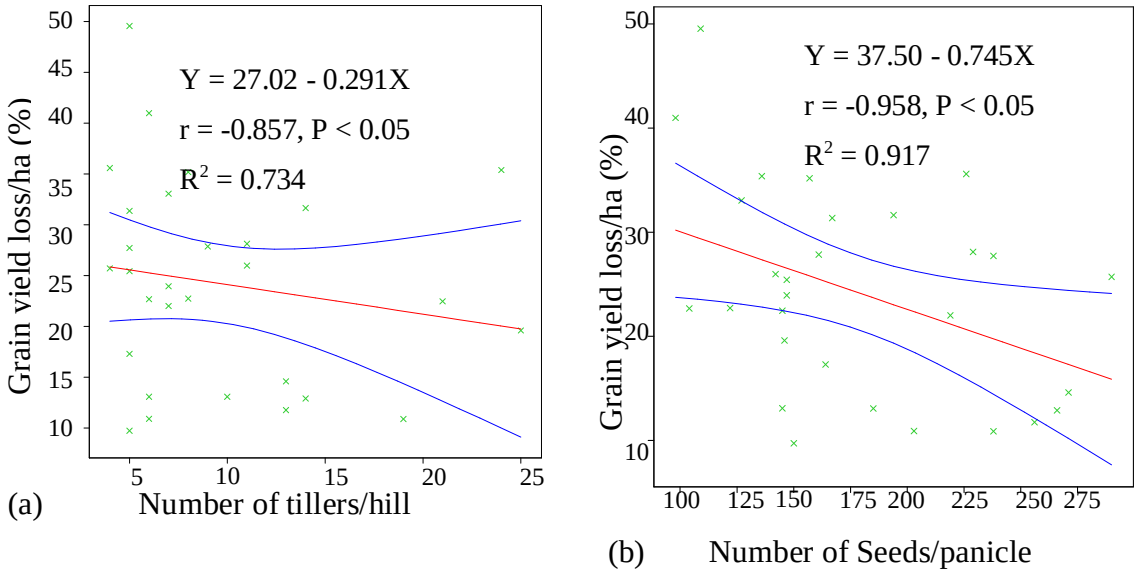

Figure 3.4: Regression and correlation coefficient between (a) number of tillers/hill and grain yield (b) seeds/panicle and grain yield losses/ha on selected rice varieties grown in Tanzania.

The regression equations of number of tillers and seeds/panicle with grain yield losses (Y = 27.02 - 0.291X, R^2 = 0.734 and Y = 37.50 - 0.745X, R^2 = 0.917, respectively) were the most appropriate models for predicting yield losses due to rice blast disease (Figure 3.5). High coefficients of determination have been reported in the analysis showing the percentage of yield losses due to the effect of rice blast disease on tillers and seeds (Mousanejad *et al.*, 2010).

3.5 Conclusion

Results of the current study revealed that rice blast disease significantly reduced tillering ability and grain yield. Yield reduction was influenced by varieties grown. Both the number of tillers and seeds per panicle were reduced by blast disease, contributing to the differences in grain yield recorded between rice varieties. Losses in grain yield due to

panicle and leaf rice blast disease ranged from 11.9% to 37.8%. Reduction in the number of tillers and seeds/panicle ranged from 19.033 to 8.033 and 278.54 to 176.6, respectively, and grain yield weight ranged from 7,776.4 kg/ha to 10,046.1 kg/ha and was influenced by both leaf and panicle rice blast disease. These results demonstrate that IR 64, TXD 85, TXD 306, Mwarabu and Kihogo were promising rice varieties resistant to rice blast disease, while Jaribu 220, Kalamata and Mbawambili varieties were susceptible to rice blast. Management measures to reduce the impact of rice blast on yield are thus, needed. Such management measures should include breeding for rice blast disease resistance.

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CHAPTER FOUR

4.0 IDENTIFICATION, MOLECULAR CHARACTERIZATION AND PATHOGENIC VARIATION OF *PYRICULARIA ORYZAE*, CAUSAL AGENT OF RICE BLAST DISEASE IN TANZANIA

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4.1 Abstract

Rice blast disease, caused by the fungus *Pyricularia oryzae*, is one of the most devastating diseases of rice. Understanding pathogenic variation and molecular characterization of *P. oryzae* is one of the most efficient ways to manage the disease. Studies on identification, characterization and pathogenic variation of *P. oryzae* were conducted in the laboratory and screen-house at Sokoine University of Agriculture, Morogoro. Ten *P. oryzae* strains collected from Shinyanga, Kilimanjaro, Mbeya and Morogoro were tested with a set of ten rice blast differentials viz; IRBLk-Ka, IRBLkm-Ts, IRBLb-w/co, IRBLkp-K60, IRBLz-Fu, IRBLa-C, IRBLi-F5, IRBLta2-Pi), St and IRBLt-K59. Twenty one-day-old seedlings grown in pots were inoculated with spore suspension of a *P. oryzae* at a concentration of 2 x 10^5 ml^{-1} using a hand-hold sprayer in the evening. Considerable pathogenic variations among the tested strains were observed. The International rice differentials IRBLk-Ka, IRBLkp-K60, IRBLa-C, IRBLi-F5, IRBLta2-Pi, St and IRBLt-K59 were resistant to all strains, however, IRBLb-w/co, IRBLkm-Ts and IRBLz-Fu (*Piz*) were susceptible. Molecular analysis using four primers Bt1a and Bt1b, CAL-228F and CAL-737R, ACT-

512F and ACT-783R, ITS1 and ITS4 were used for amplification of seven *P. oryzae* strains. The results showed no differences in banding patterns between strains, indicating that all strains analyzed were genetically homogeneous but pathogenically heterogeneous. This information however, is imperative to develop effective breeding rice varieties resistance to rice blast disease in the country.

Keywords: Characterization, pathogenic variation, *Pyricularia oryzae*, rice blast, PCR.

4.2 Introduction

Rice is one of the most important cereal crops grown worldwide. In Tanzania, more than 50% of the population depends on rice as the main food and source of income. Rice blast disease is the most important and destructive disease of rice. It is caused by the fungus *Pyricularia oryzae* (Teleomorph *Magnaporthe oryzae* Couch) formally known as *Pyricularia grisea* (Cooke) Sacc.] (Couch and Kohn, 2002; Hosseini-Moghaddam and Soltani, 2013; Urayama *et al*., 2010), [Teleomorph *Magnaporthe grisea* (Herbert)]. The incidence and severity of rice blast disease vary yearly based on location and environmental conditions (Bhat *et al*., 2013; Sukanya *et al*., 2011; Zhou *et al*., 2007). Symptoms of the disease can occur on all above ground parts of the plant and is observed at earlier growing stages up to maturity. Symptoms appear on the leaves, nodes and panicles (Ghazanfar *et al*., 2009b; Urayama *et al*., 2010). It has been reported to cause grain yield losses of up to 100% (Filippi *et al*., 2011; Prabhu *et al*., 2009a; Vanaraj *et al*., 2013).

However, *P. oryzae* [anamorph] has been reported to have high pathogenic variation with respect to host range and variety specificity (Leung *et al*., 1988). The level of pathogenic

variation of *P. oryzae* isolates differs with rice varieties (Bonman *et al*., 1987). Therefore, the studies on reaction of differential rice genotypes and molecular characterization are important in understanding pathogenic variation of *P. oryzae*. Rice blast disease causing pathogen can be classified into various pathotypes based on the infection pattern observed on a set of differential rice genotypes (Kang and Lee, 2000).

The use of resistant rice varieties is the most economical and effective means of managing blast disease in rice (Chen *et al.,* 2001; Ghazanfar *et al.,* 2009a). However, sometimes resistant varieties may become ineffective due to evolutionary changes in the pathogen population (Khadka *et al.*, 2013). Loss of resistance shortly after variety release is common in many rice growing areas (Kang and Lee, 2000). Therefore, understanding pathogenic variation of *P. oryzae* is important in overcoming constraints facing many rice breeding programs (Correa-victoria and Zeigler, 1993).

Pathogenic variation is the main cause of resistance breakdown in rice against rice blast disease (Urashima, January 2002). Several studies have reported different sources of pathogenic variation in *P. oryzae*. Great pathogenic variation has been reported in *P. oryzae* from single-spore isolates originating from single lesions and monoconidial sub-cultures (Correa-victoria and Zeigler, 1993). The isolates from the same lesion may differ in pathogenicity, and single-spore subcultures may also differ in pathogenicity from the original single-spore cultures (Gad *et al*., 2013) However, the composition of groups of isolates that are genetically different may cause variation in the pathogen (Bonman *et al*., 1987). Khadka *et al*. (2013) reported that variation in the pathogen is caused by variation in chromosome numbers or genomic rearrangements. Similarly, Ziegler *et al.* (1997) and

Ziegler (1998) reported that parasexual recombination is one of the means of variation in *P. oryzae*. Further understanding of pathogenic changes during sexual hybridization may provide evidence to pathogenic variation observed in the asexual stage of the fungus.

Pyricularia oryzae strains are not pathogenically homogenous, existence of pathogenic races has been reported from many major rice growing countries (Anwar *et al*., 2009). However, such variation in Tanzania has not yet been studied. Anwar *et al* (2009) identified about five pathogenic race groups, ID-1, ID-2, IB-4, IC-17 and IC-25. Among these groups, physiologic race group IC-17 was observed to be predominant. Padmanabhan *et al.* (1970) investigated 31 isolates of the fungus in India, of which 21 isolates belonged to a new race group designated as IJ, and the rest belonged to the international race groups. Their study identified races IC 3 and ID 1 as common in India. In Brazil, a great number of physiological races with distinct virulence characteristics have been identified based on reaction types on a set of eight standard international rice differentials (Prabhu *et al*., 2002). Races IC-1 and IB-9 were reported to be predominant in Brazil.

A culture of *P. oryzae* may change its pathogenicity but it can not be used as the criteria of assigning pathogenic race (Veeraraghavan, 1986). In a study of Veeraraghavan (1986) categorized pathogenic isolates by assessing the reaction of rice varieties with respect to blast disease, and came up with three categories namely; resistance, moderate resistance and susceptible rice varieties. The use of differential rice genotypes on blast disease is widely adapted as the technique for distinguishing pathogenic races of pathogens.

Molecular studies are currently appropriate approaches in identification and characterization of *P. oryzae* (Gad *et al.,* 2013). However, the use of DNA technologies

such as polymerase chain reaction (PCR) is the most important approach in detection of the pathogen (Babujee and Gnanamanickham, 2000; Chen *et al.*, 2001; Dar *et al.*, 2011). The PCR technique is effective for distinguishing between closely related isolates. The aim of this study was to identify, characterise and determine pathogenic variation of *P. oryzae* using a set of rice differentials as well as PCR techniques.

4.3 Materials and Methods

4.3.1 Source of isolates

Rice leaves and panicles with blast lesions were collected from Mbeya, Morogoro, Shinyanga and Kilimanjaro regions in Tanzania. A total of 320 rice blast disease samples were collected in the brown paper bags and transferred to the African Seed Health Centre laboratory at SUA for isolation, identification and characterization of the rice blast pathogen.

4.3.2 Isolation and identification of *Pyricularia oryzae*

Infected tissues (lesions) were sterilized using 1% Sodium hypochlorite for 1 minute to reduce saprophytes, and then rinsed in distilled water three times. Each lesion was placed on moistened filter papers in Petri dishes and incubated for 72 hours (3 days) at 25^{o}C to allow for fungal sporulation on the lesions (Bonman *et al.*, 1987). Identification of *P. oryzae* was done three days after incubation based on morphological features as described by Gandalera *et al*. (2013) and the International Seed Testing Association (2014). Conidia were identified from the sporulating lesions using a stereomicroscope and identity confirmed following procedures of Mathur and Kongsdal (2001).

Thereafter, sterile drawing pins were dipped in potato dextrose agar (PDA) petri plates and then slightly touched on the sporulating rice blast lesions, followed by inoculation of the agar plates with the pin containing spores of the presumed *P. oryzae*. Then the pathogen was cultured in petri dishes on PDA agar, incubated at 25 ± 1ºC to induce sporulation as described by Rathour *et al.* (2006). After sporulation, the conidia were harvested by adding 10 ml of sterilized distilled water per petri plate and gently scraped the surface to harvest the spores. Conidial suspensions at a concentration of about 10^4 spores/ml were prepared in sterilized distilled water with 0.1% Tween 20 to increase spore dispersion (Prasad *et al.*, 2009). Conidial densities were counted using a haemocytometer. Established cultures were subsequently maintained as described by Valent *et al.* (1986) and used for further studies as and when required.

4.3.3 Determination of pathogenic variation of *Pyricularia oryzae* by host differentials

The experiment was conducted in the screen house at SUA to assess virulence (aggressiveness) of *P. oryzae* isolates on a set of ten rice blast differential monogenic varieties and to distinguish the pathogen pathotypes (races). These differentials included IRBLk-Ka (*Pik*), IRBLkm-Ts (*Pik-m*), IRBLb-w/co, IRBLkp-K60 (*Pik-p*), IRBLz-Fu (*Piz*), IRBLa-C (*Pia*), IRBLi-F5 (*Pii*), IRBLta2-Pi (*Pita-2*), St and IRBLt-K59 (*Pit*) and were collected from AfricaRice Tanzania office.

Four hundred seeds for each variety as recommended by ISTA (2005) were sown in eight 15 cm diameter plastic pots containing sterilized moist silt loam soil arranged in a Completely Randomised Design (CRD) with three replicates. Fifty seeds were sown in each pot and each pot represented one replication. Inoculum preparation was done as

described by Thinlay *et al.* (2000). Rice seedlings were inoculated with *P. oryzae* at the 4-5 leaf stage (21-day-old seedlings) in the evening using a low-pressure spray bottle with a suspension of conidia 2 x 10^5 spores/ml following procedures described by Zhang *et al.* (2009). Disease reaction was assessed seven to ten days after inoculation based on the IRRI (1996) standard evaluation scale of 0 – 9 where: 0 = no lesions; 1 = small, brown, specks of pinhead size; 3 = small, roundish to slightly elongated, necrotic, gray spots about 1-2 mm in diameter; 5 = typical blast lesions infecting < 10% of the leaf area; 7 = typical blast lesions infecting 26-50% of the leaf area; 9 = typical blast lesions infecting >51% leaf area and many dead leaves (Prabhu *et al.*, 2006; Windarsih *et al.*, 2014). The pathogenic race of each isolate was determined by the reaction of the rice differential varieties used.

4.3.4 Characterization of *Pyricularia oryzae* by PCR

4.3.4.1 DNA extraction

Total DNA was extracted from seven isolates of *P. oryzae*. The pathogen isolates were grown on 10 g of potato dextrose agar supplemented with 2 g of yeast extract per liter in Erlenmeyer flasks for hours without agitation followed by ten days with constant agitation in the darkness at 24ºC. One or two mycelial paper discs were transferred to 250 ml Erlenmeyer flasks containing 150 ml of the culture medium. The harvested mycelia was freeze-dried, lyophilized and macerated in liquid nitrogen. DNA extraction was done following the procedure described by Prabhu *et al* (2002).

About 300 mg of powdered mycelia was suspended in 700 µl of extraction buffer (50 mM Tris-HCl, pH 8.0; 50 mM EDTA; 3% sodium dodecyl sulfate, wt/vol and 1% of

mercaptoethanol) at 65ºC for 1 h. The cellular proteins were precipitated with 30 µl of potassium acetate (3 M and pH 5.2). DNA was precipitated in 200 µl of cold isopropanol, washed with 70% ethanol, dried under vacuum and re-suspended in TE buffer (10 mM Tris-HCl, pH 8.0; 1.0 mM EDTA), containing 10 mg/ml of RNase A and incubated at 37ºC for 30 min. The DNA concentration was estimated by fluorometer and adjusted to 10 ng/µl. The DNA pellets were dried overnight and dissolved in 1 ml of TE buffer (pH 8.0). Quantification of DNA was performed on 0.8% agarose gel and diluted with sterile distilled water to a concentration of 25 ng for PCR analysis. The PCR contained the following reaction mixture (25 µl): 50 ng DNA, 2.5 µl 10 x buffer reaction (200 mM Tris-HCL, Ph 8.4 and 500 mM of KCl) , 2.0 µl 50 mM, $MgCl_2$; 0.5 µl dNTP (10 mM each dATP, dGTP, dCTP and dTTP); 1.25 µl of each primer (100 mM); 5 units of Taq polymerase.

4.3.4.2 Polymerase chain reaction (PCR) amplification

The PCR amplification reaction was carried out in a thermocycler with the following temperature conditions as described by Bussaban *et al.* (2005). An initial PCR cycle was performed at 95ºC for 4 minutes, primer annealing at 55ºC for 30 s, polymerization at 72ºC for 1 minute. This was followed by 30 cycles of 94ºC for 5 minutes, 55ºC for 30 s and 72ºC for 1 minute. Final extension was performed at 72ºC for 7 minutes. The PCR products were separated in agarose gel electrophoresis 2% in TBE 0.5 x with 100 volts for 30 minutes (Plate 4.1) then visualized in UV light after soaking in ethidium bromide. Four primers (Table 4.1) were used to amplify the targeted DNA fragments.

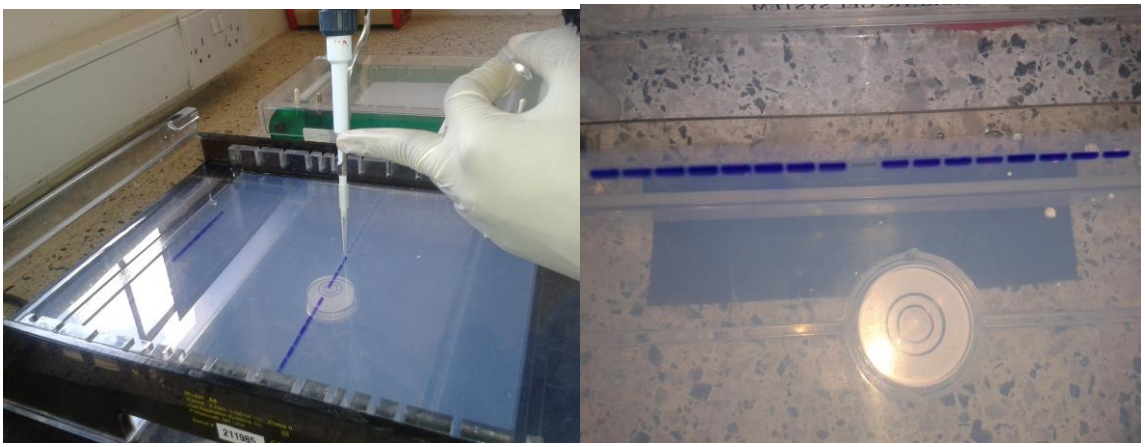


Plate 4.1: Loading of DNA in the agarose jel for amplification

(photos: C. J. Chuwa, MARI, Dar es Salaam).

4.3.4.3 Statistical analysis

Data were analyzed using both GenStat® Executable release 14 Statistical Analysis and Microsoft Excel Software. Data were subjected to analysis of variance (ANOVA) tests. When significant differences were found, means were separated and assessed using Duncan's Multiple Range Test (DMRT). Significant differences between treatment means were tested using the Least Significant Difference (LSD) at 5% level of probability. The dendrogram was constructed with UPGMA cluster analysis of all seven isolates studied using simple similarity coefficients.

4.4 Results and Discussion

4.4.1 Identification and pathogenicity test of *Pyricularia oryzae*

Of the 320 isolates of rice blast disease collected, only seven strains were identified as *Pyricularia oryzae* based on morphology and PCR. The pathogen *P. oryzae* obtained from the blotter method was cultured on PDA medium. The strains from leaf component DAK, KAH, KAP, KIK, MOS, MSU and SUA showed excellent sporulation and growth on PDA medium (Plate 4.2a).

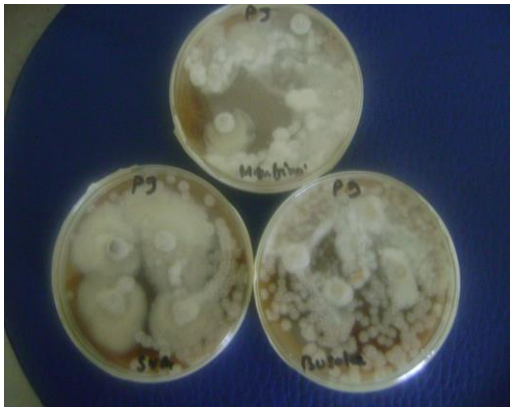
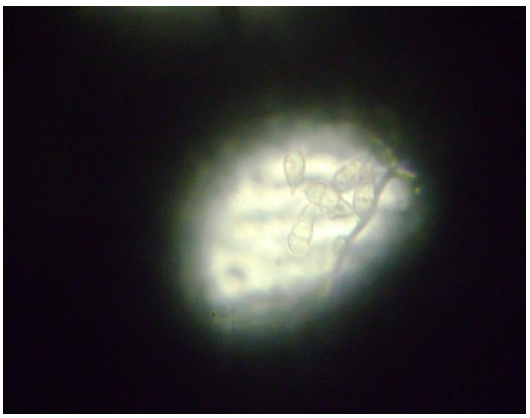
A  B 

Plate 4.2: a) Colony growth and b) conidia morphology of the three isolates *Pyricularia oryzae* (photos: C. J. Chuwa, SUA, Morogoro).

These results correspond with the study of Hosseini-Moghaddam and Soltani (2013) found that PDA containing segments of rice leaf, better favored the growth of *P. oryzae*. Conidia were pyriform, almost hyaline to pale olive, 2-septate and 3-celled (Plate 4.2b).

4.4.2 Pathogenic variation of Pyricularia oryzae using rice differentials

The *P. oryzae* strains pathotyped by inoculation on the international rice differentials showed compatible reactions (Table 4.1). The International rice differentials IRBLk-Ka (*Pik*), IRBLkp-K60 (*Pik-p*), IRBLa-C, IRBLi-F5 (*Pii*), IRBLta2-Pi (*Pita-2*), St and IRBLt-K59 (*Pit*) were resistant to all *P. oryzae* strains. However, differentials IRBLb- w/co, IRBLkm-Ts (*Pik-m*) and IRBLz-Fu (*Piz*) were susceptible.

Table 4.1: Reaction of differential rice varieties to seven strains of *Pyricularia oryzae* collected from Kilimanjaro, Mbeya, Morogoro and Shinyanga regions in Tanzania

	Strains						
Differential	**DAK**	**KAH**	**KAP**	**KIK**	**MOS**	**MSU**	**SUA**
IRBLk-Ka	R	R	R	R	R	R	R
IRBLa-C	R	R	R	R	R	R	R
St	R	R	R	R	R	R	R
IRBLkp-K60	R	R	R	R	R	R	R
IRBLt-K59	R	R	R	R	R	R	R
IRBLta2-Pi	R	R	R	R	R	R	R
IRBLb-w/co	S	S	S	S	R	S	S
IRBLi-F5	S	R	R	R	R	R	R
IRBLkm-Ts	S	S	S	S	S	S	S
IRBLz-Fu	S	S	S	S	S	S	S
Races	**A**	**B**	**B**	**B**	**C**	**B**	**B**

S = Susceptible, R = Resistant

DAK= Dakawa, KAH = Kahama, KAP = Kapwili, KIK = Kikusya, MOS = Moshi and MSU = Msufini.

All *P. oryzae* strains were not pathogenic on IRBLk-Ka, IRBLkp-K60, IRBLa-C, IRBLta2-Pi, St and IRBLt-K59. All strains (KAD, KAH, KAP, MOS, SUA, KIK and MSU) produced susceptible reaction only on rice differentials IRBLKm-Ts and IRBLz-Fu. The strain from Moshi was not able to infect IRBLb-w/co, while the rice differential was susceptible to all other *P. oryze* strains. Dakawa strain was pathogenic on IRBLi-F5 while other strains were not.

However, all seven *P. oryzae* strains were pathogenic on IRBLkm-Ts and IRBLz-Fu (Table 4.1). Similar contradicting results have been reported by Windarsih *et al.* (2014)

when studying molecular marker application for rice blast resistance selection on the double haploid rice population. The virulence of the strain obtained have been reported to be strongly influenced by the varieties from where they were isolated based on nutritional differences between rice varieties (Bonman *et al.*, 1987).

On the basis of the reaction of the ten international differentials, three races (A, B and C) of *P. oryzae* were identified (Table 4.1). Most of the strains belonged to race B (Table 4.1). Race A and C consisted of a single strain each. Race C characterized by resistant reaction of the eight international differentials viz; IRBLk-Ka, IRBLa-C, St, IRBLkp-K60, IRBLt-K59, IRBLta2-Pi, IRBLb-w/co and IRBLi-F5. Race B was characterized by susceptible reaction of three differentials IRBLb-w/co, IRBLkm-Ts and IRBLz-Fu followed by race A which was characterized by susceptible reaction of four differentials. Pathogenic variation has been cited as the principal cause for the frequent breakdown of resistance shortly after varieties are released (Sharma *et al*., 2013). This also agrees with findings reported by Correa-victoria and Zeigler (1993) that pathogenic variation of *P. oryzae* may come from single spore strains originating from single lesion and monoconidial sub-cultures. Gad *et al*. (2013) also found that the strains of *P. oryzae* from the same lesion may differ in pathogenicity, and single-spore sub-cultures may also differ in pathogenicity from the original single-spore cultures. Strains from each rice-growing region were limited; therefore, further collection and evaluation of strains are needed to confirm the findings of this study.

The similarity coefficients of *P. oryzae* strains used in this study varied from 0.3 to 1.0 and clustered broadly into two groups (Figure 4.1). The average similarities within the cluster of strains in group one was significantly smaller than the average similarities among strains from group two. However, on the basis of the reaction types either pathogenic or

non-pathogenic, the seven strains were grouped into three different pathotypes (Figure 4.1).

Pyricularia oryzae strains induced differential reaction on IRBLb-w/co, IRBLi-F5, IRBLkm-Ts and IRBLz-Fu. Rice differentials IRBLb-w/co, IRBLi-F5, IRBLkm-Ts and IRBLz-Fu were susceptible to all strains. This agrees with findings reported by Wang *et al.* (2013) when identifying rice blast disease resistance genes using international rice differential varieties that *P. oryzae* strains can genetically remain stable in the resistant cultivar.

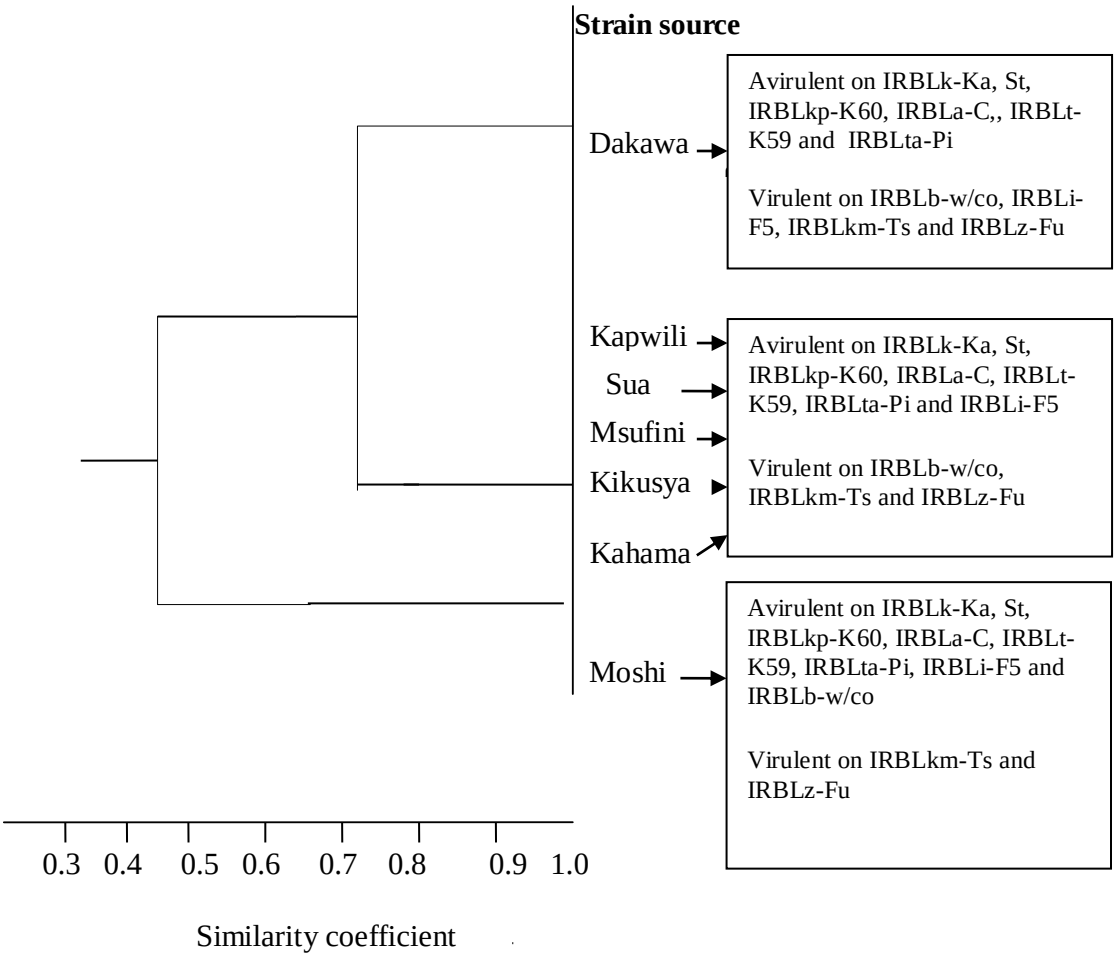


Figure 4.1: Dendrogram showing the relationship between the seven *Pyricularia oryzae* strains

Results showed that both pathogenic and non-pathogenic strains were clustered together in a closely related group. In the present study, the strains were designated into two main groups (Figure 4.1). The first group was designed as slightly virulent strains consisting of MOS strain. Five strains, KAP, SUA, MSU, KIK and KAH were assigned to group two designated as moderately virulent strains with the remaining DAK strains assigned to group three, a severely virulent strains. The present investigation revealed that these three groups of *P. oryzae* strains infected all the international rice differentials. These strains could overcome a great number of resistance genes because each of the differential genotype possessed different resistance gene against rice blast disease. Differences in pathogenicity between individual strains have been used for a long time to determine pathogenic variation of *P. oryzae* (Srivastava *et al.*, 2014).

In pathotype one, only Dakawa strain was included, while a maximum of five strains (Kapwili, Sua, Kikusya, Msufini and Kahama) were included in pathotype 2 (Figure 4.2) and the remaining Moshi strains were grouped in pathotype three. Among the host differentials, IRBLk-Ka, St, IRBLkp-K60, IRBLa-C, IRBLt-K59 and IRBLta-Pi showed resistance to all seven strains and IRBLi-F5 to five strains followed by IRBLb-w/co being resistant to one strain. These results are supported by the study of Karthikeyan *et al.* (2013) on the virulence characteristic analysis and identification of new pathotypes of the rice blast fungus (*Magnaporthe grisea*) in India.

Strains of *P. oryzae* were grouped in different pathotypes based on their reaction on rice varieties known to have sources of resistance. However, the resistance genes *Pik, , Pik-p*, *Pii*, *Pita-2* and *Pit* remained effective against rice blast strains while genes *Pik-m* and *Piz*

were not effective against the disease. Such observations have also been reported by Bonman *et al.* (1987), Correa-victoria and Zeigler, (1993), Dinh *et al*. (1999) and Noguchi *et al*. (2007).

4.4.3 Characterization of Pyricularia oryzae strains using molecular technique

Molecular markers have been used widely to characterize fungal plant pathogen populations, in particular for the characterization of *P. oryzae*. Seven strains of *P. oryzae* were analyzed for genetic variation using five primers which included Bt1a and Bt1b, CAL-228F and CAL-737R, ACT-512F and ACT-783R, ITS1 and ITS4, Pot2-1 and Pot2-2 (Table 4.2). Primers tested were specific to *P. oryzae* from which they were designed.

Table 4.2: Pyricularia oryzae primers for reductase gene amplification used in the current study

Primer	**Primer sequence (5' – 3')**
Forward - ACT-512	ATGTGCAAGGCCGGTTTCGC
Reverse - ACT-783	TACGAGTCCTTCTGGCCCAT
Forward - Bt1a	TTCCCCCGTCTCCACTTCTTCATG
Reverse - Bt1b	GACGAGATCGTTCATGTTGAACTC
Forward - CAL-228	GAGTTCAAGGAGGCCTTCTCCC
Reverse - CAL-737	CATCTTTCTGGCCATCATGG
Forward - ITS1	TCGGTAGGTGAACCTGCGG
Reverse - ITS4	TCCTCCGCTTATTGATATGC
Forward - Pot2-1	CGGAAGCCCTAAAGCTGTTT
Reverse - Pot2-2	CCCTCATTCGTCACACGTTC

Of the ten (10) primers used, Bt1a and Bt1b, Pot2-1 and Pot2-2 did not amplify DNA from all *P. oryzae* strains, suggesting problems with the primers' specificity for the *Pyricularia oryzae* pathogen. However, the primers CAL-228F and CAL-737R amplified genomic DNA of *P. oryzae* strains MSU, DAK, KAP and MOS while SUA, KAH and KIK strains were not amplified (Figure 4.2 and 4.3).

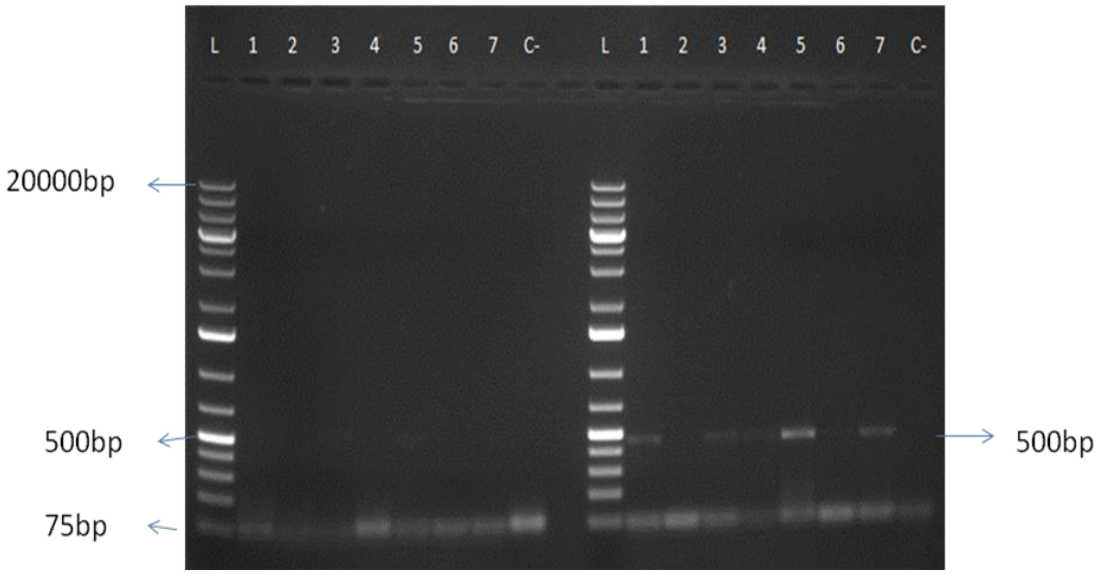


Figure 4.2: Agarose gel eletrophoresis of PCR amplification products of *Pyricularia oryzae* genomic DNA using primers BTta and BTtb (left) and CAL-228F and CAL-737R (right). The *P. oryzae* strains from left to right in the photograph were MSU, SUA, DAK, KAH, KAP, KIK and MOS, represented by numbers 1, 2, 3, 4, 5, 6 and 7, respectively, L = Ladder (1kb+) and C = control. Arrows indicate the two fragments that are characteristic of *P. oryzae*. Molecular sizes are shown in base pairs (bp).

Furthermore, the primer ACT amplified KAP, KIK and MOS strains while MSU, SUA, DAK and KAH were not amplified. The primers ITS amplified *P. oryzae* strains MSU, SUA, KAH, KAP, KIK and MOS. The *P. oryzae* strains SUA and KAH were very faintly amplified. The strain DAK was not amplified by both ACT and ITS primers, suggesting

lack of similarity with the fungal DNA structure (Figures 4.2 and 4.3). Similar results have been reported by Jiaa *et al.* (In Press) in the investigation of an expedited method for isolation of DNA for PCR from *Magnaporthe oryzae* stored on filter paper, who characterized strains from rice and indicated that the strains of *P. oryzae* attacking rice leaves and panicles were genetically distinct from location to location.

The amplification reactions with the three primers generated polymorphic bands. A single 500 bp product was exhibited by all the strains of *P. oryzae* amplified by CAL-228F and CAL-737R (Figure 4.4). Kumar *et al.* (2010) reported similar results when they were identifying blast resistance expression in rice genotypes using molecular markers (RAPD and SCAR). They found that most of the *P. oryzae* strains identified were polymorphic in nature with band sizes between 100 and 500 bp. The primers ACT-512 and ACT-783 amplified *P. oryzae* strains KAP, KIK and MOS collected from Mbeya and Kilimanjaro regions, respectively (Figure 4.5).

Primers ITS 1 and ITS 4 amplified strains MSU, SUA, DAK, KAH, KAP,KIK and MOS of *P. oryzae*, and strains also appeared similar with polymorphic banding pattern of 550 bp. These amplification values were higher than those reported by Kumar *et al* (2010) which ranged from 40 bp to 420 bp. Similar results were reported by Srivastava *et al*. (2014) using REMAP markers and amplification values ranged from 490 to 600 bp.

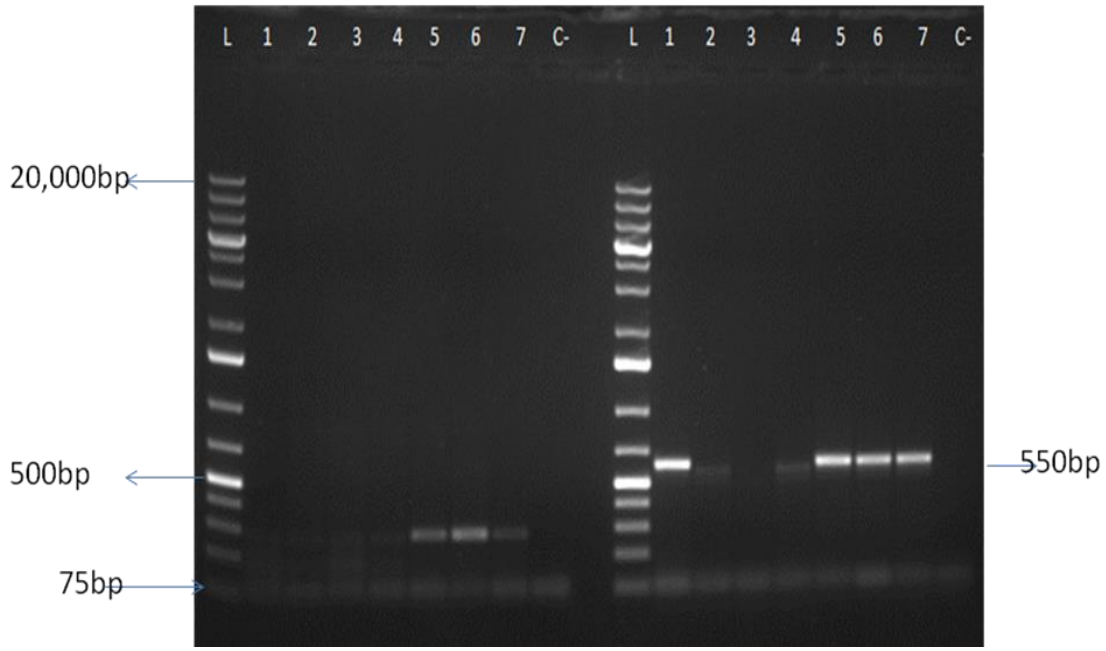


Figure 4.3: Agarose gel eletrophoresis of PCR amplification products of *Pyricularia oryzae* genomic DNA using primers ACT-512F and ACT-783 (left) and ITS1 and ITS4 (right). The *P. oryzae* strains from left to right in the photograph were MSU, SUA, DAK, KAH, KAP, KIK and MOS, represented by numbers 1, 2, 3, 4, 5, 6 and 7, respectively. L = Ladder (1kb+) and C = control. Arrows indicate the two fragments that are characteristic of *P. oryzae*. Molecular sizes are shown in base pairs.

The *Pyricularia oryzae* strains used in the study did not show differences in banding patterns. The banding patterns of seven *P. oryzae* strains from major rice growing areas in Tanzania were all similar (Figure 4.2 and 4.3). This study revealed that the *P. oryzae* strains collected from different major rice growing areas of Tanzania were not significantly different genetically.

PCR products of seven *P. oryzae* strains produced a strong band of 550 bp and very weak bands at ≤500 bp (Figures 4.2 and 4.3). *Pyricularia oryzae* strains MOS, KIK, KAP and MSU were amplified by primers ITS1, ITS4, ACT-512, CAL-228 and CAL-737 and

produced strong bands of 550 bp. This confirmed these isolates to be *P. oryzae* and the PCR with ITS1, ITS4, ACT-512, CAL-228 and CAL-737 primers proved to be a reliable method to differentiate the rice blast pathogen. The number of amplification products obtained was specific to each primer. Overall, the random amplified polymorphic DNA patterns did not show high level of polymorphism. The results indicate that the *P. oryzae* strains genome were genetically stable, but varied in pathogenic (Kang and Lee, 2000; Li *et al.*, 2010; Zhou *et al*., 2007). These results can be used for screening resistance commercial rice varieties for planting in areas where there is high risk of *P. oryzae* infection. This information however, is imperative to develop effective breeding rice varieties resistance to rice blast disease strategies in the country.

4.5 Conclusion

The results of this study indicated that the strains of *P. oryzae* identified greatly differed in pathogenic patterns. Great pathogenic variation was detected in the strains using international rice differential genotypes with different resistance genes. All seven *P. oryzae* strains used were pathogenic on rice differentials *Pik-m*, *Pii* and *Piz*. Based on characterization of *P. oryzae* using international rice differentials, three different races of *P. oryzae* were identified among seven strains. Races A and C comprised of one strain each, whereas, race B comprised of five strains.

The results of molecular analysis showed no differences in banding patterns between strains from major rice growing areas in Tanzania, indicating that all strains analyzed were genetically homogeneous but pathogenically heterogeneous.

In cluster analysis, the strains were grouped into two main groups showing close relationships in pathogenic variation. The highest pathogenic strains; DAK, KAP, SUA, MSU, KIK and KAH were clustered in the same group showing the close correspondence between them. Pathogenic variation among strains plays an important role in rice blast disease dynamic and consequently, in the success of integrated disease control, especially for breeding resistant rice varieties. However, based on the results of the present study, both characterization and pathogenic variation of *P. oryzae* strains should be considered when screening of rice germplasm against *P. oryzae*, the cause of rice blast disease.

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CHAPTER FIVE

5.0 GENERAL CONCLUSION AND RECOMMENDATIONS

5.1 General Conclusion

Blast disease caused by *Pyricularia oryzae* is a major rice production constraint in major rice-growing regions in Tanzania. The present study has confirmed that rice blast is the major disease in the country and causes substantial rice yield losses. The relative high disease incidence and severity of rice blast in the country is influenced by high relative humidity, moderate temperatures and high rainfall. This study has revealed that Mbeya and Morogoro received high rainfall, high relative humidity and have moderate temperature during rice growing seasons (March – June) thus are hot spot areas for rice blast disease. The study has also shown that *Pyricularia oryzae* pathotype composition in Tanzania is complex.

The assessment of yield losses was carried out using statistical and experimental methods. Such methods utilized data of disease pathometric variables to estimate crop losses and yield for analysing yield losses. Both leaf and panicle blast disease caused grain yield losses directly and indirectly. The critical phase for rice blast disease was during tillering and grain filling stage. At these stages, considerable reduction in number of tillers per hill and seeds per panicle were recorded. These results have shown significant effect on grain yield losses caused by rice blast. The study indicated that increased severity of rice blast disease caused by *P. oryzae* on both leaves and panicles was due to increased temperature, significantly high amount of rainfall and high relative humidity. These results revealed that rice variety IR 64 was resistant to leaf and panicle rice blast disease, with Jaribu 220

being the most susceptible to the disease. The findings in this study have provided very useful information for developing strategies for controlling rice blast disease, especially for rice breeding programs.

The study also investigated the variability of *P. oryzae* using differential rice genotypes and molecular techniques. Genetic analyses based on the differential system are useful tools for the identification of resistance genes, although these are complicated and tiresome. A gene-for-gene interaction operated between specific avirulence genes in *P. oryzae* and a specific resistance gene in a plant. However, the results of PCR of DNA amplification showed that isolates DAK, SUA, KAH, KAP, KIK and MOS collected from Morogoro, Shinyanga, Mbeya and Kilimanjaro regions were genetically similar. The isolates were classified using the primers CAL-228F and CAL-737R, ACT-512F and ACT-783R, ITS1 and ITS4. This study helped to identify the effective and diverse germplasm which can be used as sources of resistance to rice blast disease, and consequently help to design appropriate breeding strategies for development of durable rice blast resistant varieties. Therefore, further research is needed to develop and promote rice blast resistant varieties and farmers must be encouraged to use improved rice varieties as part of rice blast disease management in the country.

5.2 Recommendations

i. In the surveys, high rice blast disease incidence and severity was related to epidemiological factors (temperature, RH, and rainfall) and varietal differences as well as nitrogen fertilizers. Therefore, further research is needed to determine the efficacy of the effect of the N-fertilizers on incidence and severity of rice blast

disease and come-up with the correct recommended rates of application in order to minimize disease severity in major rice growing areas in Tanzania.

ii. High incidence and severity of rice blast disease were recorded in rain-fed ecosystem compared with irrigated ecosystem. Therefore, more irrigation schemes should the constructed in major rice growing areas in Tanzania so that farmers can use irrigation schemes to avoid high risk of rice blast disease.

iii. The rice variety IR 64 grown in Kilimanjaro region in irrigation scheme during the field surveys was found to be resistant to rice blast disease. There is therefore, a need to screen it in order to confirm its level of resistance to rice blast disease so that it can be used in breeding programs.

iv. In grain yield loss assessment IR 64 recorded the lowest grain yield losses per ha and the highest on Jaribu 220. Therefore, these two commercial rice varieties can be used as parents in breeding of resistant varieties to rice blast disease in irrigated ecosystem.

v. All isolates of *Pyricularia oryzae* collected from major rice growing areas of Tanzania were found to be identical genetically while pathogenically were different. Therefore, further molecular analysis including sequencing should be conducted to determine if there is a difference within the isolates.

vi. In depth research on diversity of *Pyricularia oryzae* pathotype should be conducted in order to develop rice varieties with long lasting resistance for Tanzania.

vii. Only three primers amplified the genomes of *P. oryzae* isolates. Therefore, a large number of primers should be included in such studies in order to identify potential molecular variations between strains.

viii. Research on the effect of cultural practices for management of rice blast disease under the prevailing climatic conditions should be conducted in order to encourage farmers for improvement of eco-friendly practices in order to avoid using chemicals.