POPULATION STRUCTURE OF *PYRICULARIA GRISEA* AND REACTION OF RICE CULTIVARS IN THE SOUTHERN HIGHLANDS OF TANZANIA

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

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ABSTRACT

The study was designed to investigate the diversity of *Pyricularia grisea* (Coke) Sacc. population structure and reaction of rice cultivars in Southern highlands of Tanzania. Screen house experiments and a field trapping nursery were used to determine prevalent pathogenic races, effective host resistant genes and reaction of rice cultivars against identified pathogenic races. A total of six pathogenic races U11-i2-k141-z13-ta103, U51-i4-k100-z13-ta103, U11-i4-k041-z11-ta103, U11-i5-k000-z03-ta000, U11-i0-k101-z13-ta100 and U11-i1-k120-z11-ta102 were identified on the basis of the infection type of LIJIANGXINTUAN HEIGU (LTH) monogenic lines. Pathogenic race U11-i2-k141-z13-ta103 was the most virulent strain infecting 44.4 % of the tested varieties, followed by U51-i4-k100-z13-ta103; U11-i4-k041-z11-ta103; U11-i1-k120-z11-ta102; U11-i0-k101-z13-ta100 and U11-i5-k000-z03-ta000 infecting 40.7, 37, 37, 33.3 and 14.8 % of the tested varieties, respectively. Host resistance genes *Pi12, Piz-t, Pi11, Pik-m, Pi12, Pia,* and *Pik-p* were found effective against the available pathogen races. All local varieties tested were susceptible to all races identified differing only on the levels of susceptibility. Uyole inbred lines (UR 319 and UR 400) were susceptible to all races except race U11-i5-k000-z03-ta000 and race U11-i4-k041-z11-ta103. The races identified provide an insight of blast population diversity prevalent in Southern highlands that should be considered when breeding for blast resistance. Effective resistant genes identified can be pyramided to Uyole inbred lines and in commonly grown local susceptible rice varieties to improve blast resistant against prevalent races in Southern highlands.

DECLARATION

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

..... 05/05/2014.....

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The above declaration is confirmed

..... 05/05/2014.....

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(Supervisor)

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DEDICATION

This work is dedicated to my wife Lilian-Zabibu Nyambita, my son Reuben-Prince Maganga and my daughter Sara-Princess Maganga.

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LIST OF ABREVIATIONS AND SYMBOLS

%	percent
***	highly significant different (P<0.001)
°C	degree Celsius
ANOVA	Analysis of Variance
ARI	Agricultural Research Institute
AVR gene(s)	Avirulence gene(s)
Bl	Leaf blast
BLB	Bacterial leaf blight
CABI	Commonwealth Agricultural Bureaux International
CERES	Certification of Environmental Standard
cm	centmetre
CMI	Commonwealth Mycological Institute
COSTECH	Commission of Science and Technology
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
et al.	et alei (and others)
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
g	Gram
h	Hour
ha	Hectare
IRBL	International Rice Bred Line
IRRI	International Rice Research Institute

Kg	Kilogram
K1P	Kikusya 1 Pathotype
K2P	Kikusya 2 Pathotype
LTH	LIJIANGXINTUAN HEIGU
Lsd	Least significant difference
MAS	Marker assisted selection
ml	millilitre
µm	micrometre
mm	millimetre
ML(s)	Monogenic line(s)
MP	Mtowisa pathotype
NAFCO	National Agricultural and Food Company
NBS	National Bureau of Statistics
NIL(s)	Near isogenic line(s)
NP	Namatuhi pathotype
NPK	Nitrogen Phosphorous and Potassium
N1P	Ngana 1 pathotype
N2P	Ngana 2 pathotype
Pi	*Pyricularia*
R	Resistant
R gene(s)	Resistant gene(s)
RCBD	Randomized Complete Block Design
RH	relative humidity
RYMV	Rice yellow mottle virus

S	Susceptible
SES	Standard Evaluation System
SH	Southern Highlands
SHT	Southern Highlands of Tanzania
T	Tonne
USA	United States of America
USDA	United States Department of Agriculture
Var	Variety
VF	Virulent frequency

CHAPTER ONE

1.1 Introduction

Rice (*Oryza sativa*) is the second most important food crop in Tanzania consumed by more than 60 % of the population and grown in all regions at varying levels of importance (FAOSTAT, 2013). With an average yield of 2 t/ha, Tanzania ranks the second to Madagascar as a major rice producer in the Eastern, Central and Southern Africa (Luzi-Kihupi *et al.*, 2009). Rice is also second to maize (*Zea mays*) in terms of consumers' preferences attributed by changes in eating habits, population increase and urbanization (NBS, 2012). It is a preferred grain in the sense that as income rises, consumers shift preference from sorghum and maize toward rice and wheat products.

In southern highlands, rice productivity is low despite the on going efforts to improve rice production in the region. Tanzania average rice productivity is 2 t/ha which is low compared to other countries. The average rice productivity in Africa is 2.8 t/ha while in Asia average productivity is 4.6 t/ha (FAOSTAT, 2013). Poor soil fertility, low yielding varieties, diseases and pests are the major production constraints. Diseases being the main constraint with higher contribution to low yield than other mentioned constraints. Leaf blast (Bl), bacterial leaf blight (BLB) and rice yellow mottle virus (RYMV) are the most important diseases of rice in Tanzania.

Rice blast caused by a fungus *Pyricularia grisea* (Cooke) Sacc. [anamorph] is considered as the major disease of rice because of its wide distribution and high incidence under favourable conditions (Babujee and Gnanamanickam, 2000).

It remains the major threat to rice production worldwide despite extensive research efforts to control the disease (Ou, 1985; Skamnioti and Gurr, 2009; Koide *et al.*, 2010). Outbreaks of rice blast disease are a serious and recurrent problem in all rice-growing regions of the world, and the disease is extremely difficult to control because of high pathogen variability (Talbot, 2003). The disease accounts for 10-15 % of annual yield losses of rice worldwide (Shirasawa *et al.*, 2012). In Tanzania, a yield loss of 18 % was reported by Teri and Ali (1983). Rice blast is widespread in all ecosystems since most of the varieties grown are susceptible to the disease.

There are no specific management practices adopted for rice blast control in Tanzania however, in other parts of the tropics management of rice blast has mainly rely on the use of synthetic chemicals and resistant varieties (Mossini *et al.*, 2004). Chemical control is not environmentally user friendly method and it is expensive for small scale rice farmers to afford. Genetic resistance is the most economic, effective and environmentally sound method for blast control (Yang *et al.*, 2009a). Breeding for host plant resistant is therefore, the best disease control method most affordable by small scale rice producers. Improved varieties resistant to biotic stress so far released in Tanzania include Kalalu and Mwangaza which are resistant to Rice yellow mottle virus (RYMV) and not to blast (Luzi-Kihupi *et al.*, 2009).

More than 80 major resistance genes governing blast resistance such as *Pi-a, Pi-b, Pi-i, Pi-k, Pi-k^{h}, Pi-k^{m}, Pi-k^{p}, Pi-t, Pi-ta, Pi-ta^{2}, Pi-z, Pi-z^{t}, Pi-sh*, Pi-*k^{s}, Pi-I* and *Pita-k^{s}* have been identified (Ballin *et al.*, 2008). Some of them have already been incorporated into several rice cultivars and others have been molecularly cloned and

tagged for marker assisted selection (MAS) (Gu *et al.*, 2008; Ballin *et al.*, 2008; Koide *et al.*, 2009; Suh *et al.*, 2009; Jia *et al.*, 2009; Yang *et al.*, 2009b; Odjo *et al.*, 2011, RoyChowdhury *et al.*, 2012a). Several others *Pi-b, Pi-ta, Pi-9, Pi-36, Pi-37,*and *Pikm,* have been isolated using maps based cloning approach (Ashikawa *et al.*, 2008; Hayash and Yoshida, 2009) while a number of others including *Pi20(t), Pi39(t)* and *Pi-ta*^{2} were mapped at the *Pi-ta* region of chromosome 12 (Jia and Martin, 2008; Li *et al.*, 2008). However, even in the presence of resistant varieties breeding for resistance to rice blast has become a continuous process due to the ability of the fungus to quickly overcome resistance within a short time after the release of new cultivar (Chen *et al.*, 2006). In order to develop effective durable blast resistance cultivars, it is necessary to have a clear understanding of blast pathogen population structure and their level of virulence. An understanding of the structure and dynamics of pathogen population diversity and virulence is essential for prudent implementation of strategies for management of the disease through breeding of durable resistant cultivars (Babujee and Gnanamanickam, 2000).

Development of varieties with broad and durable resistance to rice blast in Tanzania is hampered by lack of adequate information on the population structure and diversity of the blast pathogen. This study seeks to investigate the population structure and diversity of *P. grisea* with implication of rice improvement through breeding for resistant varieties to pathogen population prevalent in Southern highlands of Tanzania.

1.2 The Overall Objectve:

Studies of *Pyricularia grisea* population structure, diversity and their implication in rice improvement through breeding of resistant varieties in Southern highlands of Tanzania (Mbeya, Rukwa and Ruvuma)

1.3 Specific Objectives

(i) To assess pathotype composition of *Pyricularia grisea* populations in Southern Highlands based on differential cultivars.

(ii) To determine the reaction of rice cultivars grown in Southern Highlands against identified pathotypes.

(iii) To explore *Pyricularia grisea* population diversity in Southern highlands of Tanzania.

CHAPTER TWO

2.0 Literature Review

2.1 Rice Production in Tanzania

Rice is the staple crop of economic importance in many countries worldwide (Yoon *et al.*, 2011). In Tanzania, rice is grown under three major ecosystems namely rain-fed lowland, upland rice and under irrigation. It is a dependable crop for many people and it is grown by 16 % of Tanzanian farmers for food and cash earning. Virtually all rice (99 %) is grown by smallholders' farmers, although some of them are part of large-scale rice irrigation schemes that were formerly state- managed farms (NBS, 2007). Tanzania per capita consumption of rice is roughly 16 kg, contributing 8 % of the caloric intake; it is the third most important source of calories in Tanzania after maize (33 % of caloric intake) and cassava (15 %). Rice production has increased from about 450 000 t of milled rice in 2000 to 800 000 t of milled rice in 2010 (Minot, 2010).

Rice is commercialized more than any other staple food crop in Tanzania. According to the 2002-2003 National Agricultural Sample Census, 42 % of rice production is marketed, compared to 28 % of maize and 18 % of sorghum although large number of rice growers may account for that bulk of sales. Tanzania is both an importer and an exporter of rice. Tanzania imported 74 877 t of rice on average in 2010 mostly from Asia (FAOSTAT, 2013). This represents about 10 % of apparent domestic consumption. Rice exports in 2010 were about 12 000 t mostly to Kenya, Zambia, and other countries in the region. However, imported rice is considered inferior to local rice by Tanzanian consumers and thus sells at a discount compared

to domestic rice. In Southern highlands rice is grown in Mbeya, Rukwa, Ruvuma and some parts of Iringa region.

2.2 Rice Blast Disease

2.2.1 History of the disease and geographical distribution

Rice blast caused by *Pyricularia grisea* (Cooke) Sacc. is by far the most important disease of many diseases that attack rice. The disease was first recorded in China 1637, later Japan 1704, Italy 1828, USA 1876, and India 1918 (CABI, 2007). In Tanzania, rice blast was first recorded in 1981 (CMI, 1981), Teri and Ali. (1983) studied the yield loss caused by the diseases and it was also reported by European and Mediterranean Plant Protection Organisation in 2006. Rice blast is found everywhere wherever rice is grown (TeBeest *et al.*, 2007). *Pyricularia grisea* is thought to occur in all countries where rice is commercially grown (Africa, North, Central and South America, Asia, Europe and Oceania).

2.2.2 Disease symptoms

The blast pathogen attacks all aerial parts of the rice plant. The disease is most conspicuous, when it attacks the leaf blades, nodes, panicle and neck, but it is also found on the leaf sheaths, rachis, the joints of the culm and even on the glume (Scheuermann *et al.*, 2012). Symptoms on leaves appear as elliptical or spindle-shaped lesions (0.5 - 1 cm wide), with pointed ends and grey or white centres: dark-green to reddish-brown margins, sometimes with a yellow halo (Plate 1).

Under humid conditions, abundant conidia are produced on both sides of the leaf. Under favourable conditions on a susceptible cultivar several grayish spots may

appear, become larger and broader and coalesce, leading to withering of the whole leaf (Agrios, 2005). Severely infected fields have a scorched appearance. On resistant rice cultivars, hypersensitive spots or small, round to elliptical, brown lesions are formed. On leaf collars, rotting may result in premature leaf fall. On lower nodes, rotting may cause 'white heads'. On panicles, all parts of the rachis may be infected. Most often, the basal node of the panicle is infected, resulting in 'neck rot' or 'rotten neck'. Brown to black spots are also seen on the rachis, particularly where it branches and on the glumes. Early infection results in white heads or partially filled heads while late infection after grain filling results in 'broken necks' (Scheuermann *et al.*, 2012).

Plate 1: Typical symptoms of rice blast disease on leaves of the susceptible variety.

2.2.3 Taxonomy of *Pyricularia grisea*

The blast fungi belong to the group of Ascomycete fungus that attack many graminaceous species and in its asexual form is called *Pyricularia grisea* (Coke)

Sacc, and the sexual or perfect stage is known as *Magnaporthe grisea* (Herbert) Barr (Rossman *et al.*, 1990). The taxonomy of both the anamorphic and teleomorphic states of the rice blast fungus has for many years been unclear. Cavara first described *Pyricularia oryzae* on rice and *Dactylaria parasitans* on crabgrass (*Digitaria spp.*) (Rossman *et al.*, 1990). Since then there has been controversy over whether the species can be reliably separated on the basis of morphological features (Suzuki, 1965) or host specificity (Sprague, 1950). Sprague (1950) retained *Pyricularia oryzae* for the species attacking rice and *Pyricularia grisea* for that on monocots other than rice. Controversial results have, however, been obtained by numerous cross-inoculation experiments, and indeed Ou (1985) considered complexity in patterns of host range to be a reflection of the inherent genetic variability in this organism.

The failure to identify the perfect state added to difficulties until when Hebert (1971) produced *Ceratosphaeria grisea* from *Pyricularia grisea* on Sachs' medium. Barr (1977) transferred the genus to *Magnaporthe* producing *Magnaporthe grisea* (Herbert) Bar. It has been therefore viewed that *Pyricularia grisea* (Cooke) Saccardo as the correct classification of the anamorph causing blast disease on rice and grey leaf-spots on other monocots; whereas *Magnaporthe grisea* (Hebert) Barr as the correct name for its teleomorph (Rossman *et al.*, 1990). Couch and Khon (2002) described *Magnaporthe oryzae* as a new species distinct from *M. grisea* which is nomeniclaturally tied to isolates from *Digitaria*, the scientifically correct name for isolates from oryza sativa and other grasses is therefore *M. oryzae*. According to Couch and Khon (2002) gene trees were inferred for *Magnaporthe*

species using portions of three genes: actin, beta-tubulin, and calmodulin. These gene trees were found to be concordant and distinguished two distinct clades within *M. grisea*. One clade is associated with the grass genus *Digitaria* and is therefore nomenclaturally tied to *M. grisea*. The other clade is associated with *Oryza sativa* and other cultivated grasses and is described as a new species, *M. oryzae*. While no morphological characters as yet distinguish them, *M. oryzae* is distinguished from *M. grisea* by several base substitutions in each of three loci as well as results from laboratory matings which that *M. oryzae* and *M. grisea* are not interfertile.

2.2.4 Pathogen morphology

The mycelia of *P. grisea* are septate and the nuclei within the mycelia and spores of this fungus are haploid. Cultures vary in colour from cream to grey, aerial mycelium scanty to thick cottony. Conidia (Plate 2a) vary from pyriform to obclavate (17 - 23 x 8 - 11 μm), mostly 3-septate, with small basal hilum, acrogenous. Conidiophores (Plate 2b) grey, single or in fascicles, usually simple and showing sympodial growth, geniculate towards apex, with swollen base (Ellis, 1971). Perithecia single or in groups, partially or wholly embedded with long beaks protruding from the surface,dark brown to black, 60 - 300 μm diameter at globose base. Asci cylindrical to subclavate, unitunicate,thin-walled, each with eight ascospores. Ascospores hyaline, fusform, 3-septate with little to no constriction at the septa, slightly curved (4 - 7 x 17 - 24 μm) (Herbert, 1971).

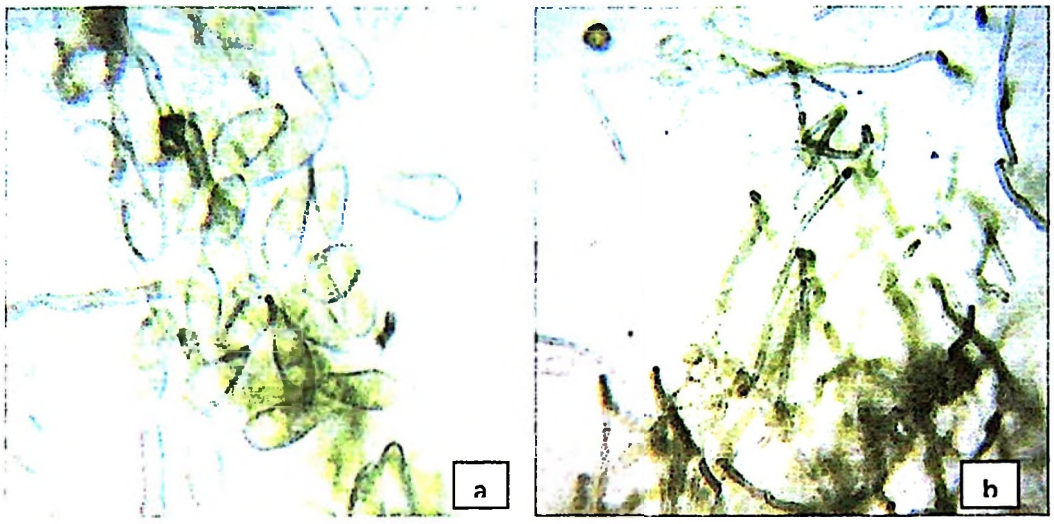

Plate 2a: Conidia of *Pyricularia grisea* as it seen on the view of light microscope (40x)

Plate 2b: Conidia and conidiophore of *Pyricularia grisea* as it seen on the view of light microscope (40x)

2.2.5 Infection process and disease cycle of *Pyricularia grisea*

In the tropics, air borne conidia of *P. grisea* are present all round the year, because of the availability of collateral host grassess such as *Setara intermecia, Digitaria marginata, Panicum repens and Leersia hexandra* (TeBeest *et al.*, 2007). Some of these grasses act as primary sources of inocula although the significance of allo-infection (infection from non-rice hosts) is unclear because recent results indicate its rarity in the field (Borromeo *et al.*, 1993). Seeds are also considered important source of the fungus especially in temperate areas where only one crop is grown per year (Afounda *et al.*, 2009). Infection of rice occurs when conidia are deposited on rice tissues, and germinate by producing a germ tube and an appressorium. The conidia germination and appressoria formation are controlled by environmental stimulus and germ tube is not formed if conidia were maintained in water, avoiding

germination (Lee *et al.*, 2009). After penetration, the primary infection hypha grows rapidly and ramifies within susceptible tissues while growth within tissues of resistant cultivars is often inhibited (TeBeest *et al.*, 2007). Infection and colonization of *P. grisea* can also occur on roots where appressoria are normally not formed and penetration pegs are produced directly from hyphopodium initiating penetration on rhizodermal cells (Sesma and Osburn, 2004). Differentiation of invasive hyphae then occurs and new cells are invaded, but the previously infected cells are not damaged. So, a biotrophic interaction occurs without producing lesions on infected tissues. However, around four weeks after root infection, disease symptom appears on plant stems and leaves, showing the systemic pathogen distribution (Marcel *et al.*, 2010). Plant diseases are often severe during periods of warm temperatures and high moisture (Scheuermann *et al.*, 2012).

Generally, rice blast is favoured by moderate temperatures (24 °C) and periods of high moisture 12 h or longer, conditions readily attainable in flooded rice fields. Rice blast incidence increases with decrease of temperature, while increases with increase in humidity (Shafaullah *et al.*, 20011). Severity is often correlated with the amount of infested material; it is also affected by soil fertility. High levels of organic matter or excessive use of nitrogen can lead to rice blast increase when the cultivar resistance is not specific. However, nitrogen deficiency can predispose plants to disease (Prabhu *et al.*, 1996). Lesions on the young seedlings appear within a few days after infection. Each lesion from a susceptible host can give rise to more than 20 000 conidia over several days (Barksdale and Asai, 1961). These secondary lesions produce more spores and these spores are readily wind disseminated to

nearby healthy leaf tissues. The secondary cycles can be repeated many times during the growing season, with the potential for very high amounts of disease within the crop. Blast disease is polycyclic, with 7 - 8 cycles per year in temperate environments, compared with 10 - 15 cycles per season and 2 - 3 seasons per year in tropical environments. The number of cycles and the number of spores that are produced on each individual lesion can be influenced by many factors, including the temperature, rainfall, the depth of the water in the paddy, the amount of nitrogen used to fertilize the rice, and the level of genetic resistance in the cultivar that is infected (TeBeest *et al.*, 2007).

2.2.6 Epidemiology

Free water is needed for *P. grisea* conidial germination and 92 % RH is needed for infection to occur (Teng *et al.*, 1991). The period of leaf wetness therefore has a large effect on infection by *P. grisea.* At optimal temperatures of 24 - 28 °C, *P. gisea* commences infection immediately after the attachment of conidium to the surface of rice leaf, and subsequently germinated conidia produce appresoria for penetration. Penetration is believed to occur within 24 h depending on strains (Wang and Valent, 2009). The fungus enters the plant either through the cuticle or via stomata. Latent periods require 4 - 6 days at an optimal temperature of 26 - 28 °C (Teng *et al.*, 1991). Conidia are produced from a new lesion about 6 days after inoculation, provided RH is above 93 % (CABI, 2007). A typical lesion releases 2000-6000 conidia per day for a period of 10 - 14 days under laboratory conditions (Ou, 1985). Release of conidia follow a diurnal periodicity, mainly between 2 and 6 h, although in tropics a second peak of spore production in the afternoon following

monsoon rains is possible (Barksdale and Asai, 1961). *Pyricularia grisea* attacks rice plants at all stages of development and can infect leaves, stems, nodes and panicles (Sesma and Osbourn, 2004). It uses a hemibiotrophic infection strategy involving initial proliferation inside living host cells followed by a destructive necrotrophic mode (Park *et al.*, 2009). Certain rice cultivars also suffer from collar rot (Scheuermann *et al.*, 2012), which often results in the premature death of flag leaves. In general, leaf blast is most severe between seedling stages and maximum tillering stages. Disease starts on leaves then apparently declines, largely because leaves become more resistant with age, and leaves on older plants acquire this property faster (CABI, 2007). At heading, disease again increases, this time on nodes and panicles.

2.3. Pathogen Variability

2.3.1 The context of pathogen variability

Pathogens causing fungal diseases are known to be complex and variable (Stakman and Christensen, 1953), *P. grisea* like other fungi shows high degree of variability in the field. New races frequently appear with the ability to infect previously resistant rice cultivars (Suh *et al.*, 2009). Suzuki (1967) described the fungus as "persistently heterokaryotic" after finding great variation in the physiology and pathogenicity. Due to high variability of the fungal population in the field, frequent loss of resistance of newly-released rice cultivars become a major restraint in sustainable rice production (Wang and Valent, 2009). Effective and durable use of blast resistance has been limited because of breakdowns in resistance due to the occurrence of more virulence races (Koizumi, 2008).

2.3.1Mechanism of variability

Heterokaryosis was first considered as the main mechanism of pathogen variability (Suzuki, 1967) whereby the number of nucleus was the basis of this argument. Suzuki (1965, 1967) reported one to thirteen nuclei of various sizes in the conidial and mycelia cells of many isolates of *P. grisea*. Giatgong and Frederiksen (1969) also observed that a few conidial and vegetative cells had more than one nucleus and that anastomosis was found within a monoconidial culture of *P. grisea* isolates. They believed that the pathogenic variability could not be explained on the basis of heterokaryosis. Formerly the number of nuclei was determined by nucleus staining and in all studies where multinucleate cells were reported, it has been suspected that some of the stained bodies in the cells may have been lipids or other bodies rather than nuclei.

With the use of electron microscope Wu and Tsao (1967) made observations and found that the cells of the conidia were uninucleate. This again indicates that heterokaryosis is not the primary mechanism of variation in *P. grisea*. Suzuki (1965, 1967) also reported that the number of chromosomes in the nuclei varied from two to five in the "heterokaryons" or wild types and three, four or five in "homokaryons." Giatgong and Frederiksen (1969) reported six chromosomes, although they occasionally observed three, four, or five chromosomes. Tanaka and Yamasaki (1975) re-examined chromosome numbers and determined the DNA content of nuclei using a micro spectrophotometer. They found that the chromosome number varied from two to12 with higher frequencies at three and six assuming that n equals three and 2n equals six. Tanaka *et al.* (1979) observed that the haploid

number of chromosomes was six. These observations further explained the mechanism for the different number of chromosomes in each nucleus. This appears to be a very significant cytological or genetic explanation for the great pathogenic variability. The frequency and magnitude of variation in chromosome number as shown by Tanaka and Yamasaki (1975) matches that of variability in pathogenicity described above. While other mechanisms of variation may play a part, the differences in chromosome numbers are perhaps the major cause. Success in producing a perfect stage of *P. grisea* by mating isolates from various hosts provides opportunities for further study in mechanisms of variability (Kato and Yamaguchi, 1979). Wu and Tsai (1974) while crossing ultraviolet-induced mutants; also suggested that parasexuality might be the mechanism of variation. Another study at IRRI (unpublished), using chemically induced mutants that differed in nutritional requirements, or in colour, in culture, or both, also suggests that either heterokaryosis or parasexual recombination can occur. Otsuka *et al.* (1965) reported a great difference among isolates of *P. grisea* in utilization of carbon and nitrogen sources, requirement and production of vitamins, as well as enzyme activities. The 47 isolates they studied were classified into 13 groups. These nutritional and biochemical differences also may offer a clue to the basis for variation in pathogenicity.

2.4 Genetics of Host Resistance

In genetic control of plant diseases two modes of resistance can be distinguished; a specific or vertical resistance and a non-specific or horizontal resistance (Sere *et al.*, 2011). The relationships between rice and blast fungus conform principles

governing the host-pathogen relationship as described Flor-gene for gene (Valent, 1990). Both the vertical and the horizontal relationship have been described for the couplet *O. sativa - P.grisea.*

2.4.1 Vertical relationship

Vertical resistance is characterized by the existence of a differential interaction between the pathogen isolates confronted with the host varieties. It is qualitative in the sense that it results in an all-or-nothing reaction that is, a variety is resistant, intermediate, or susceptible based on lesion types (Ou, 1980). Since it is controlled, in general by few major genes, breeders frequently use it to develop resistant materials. Unfortunately, it is not durable, since new pathogen races are able to overcome it in a relatively short time and destroy the efforts of many years of labour (Babujee and Gnanamanickham, 2000, Chen *et al.*, 2006). The existence of a monogenic (or oligogenic) resistance to the blast fungus has been largely confirmed in the couplet *O. sativa - P.grisea* by many studies (Zhou *et al.*, 2004). Such oligogenic system is responsible for a qualitative, complete and non-durable resistance. Genetic studies carried out in Japan by Kiyosawa and Ling (2001) led to identification of 13 dominant genes (*Pi-a, Pi-b, Pi-i, Pi-k, Pi-k^h, Pi-k^m, Pi-k^p, Pi-t, Pi-ta, Pi-ta^2, Pi-z, Pi-z^t, Pi-sh*) against Japanese blast isolates (Kiyosawa and Ling,2001). Many other resistance genes have been described such as *Pi-k^s*, *Pi-I* and *Pita-k^s* (Zhou *et al.*, 2004). When a rice plant lacks vertical resistance against a blast strain, post-infection pathogenic process proceed with the development of lesions and production of new spores. The importance of the diseases at this time depends

on the ability of the variety to slow the epidemic either by reducing the size of the lesions or by reducing the production of new spores.

2.4.2 Horizontal relationship

Horizontal resistance is characterized by the absence of differential interaction between pathogenic races and host plant varieties. Its genetic is determined by a group of genes which act together to produce observable variations. The horizontal resistance has been clearly described in rice under different denominations: field resistance, slow blasting, quantitative resistance, non specific resistance and partial resistance. Scientists agree that this system is stable and durable (Bonman, 1992).

Based on the theory of Van der Plank's (1963), rice blast fall under what is described as the 'compound interest' disease type, as many cycles of the pathogen occur during the same cycle of rice production. For such diseases, control methods that try to reduce the apparent rate of progression of the disease are more efficient than those that seek to reduce or suppress the initial quantity of disease. Therefore, horizontal resistance was described as more efficient than vertical resistance in sustainable control of rice blast (Sere *et al.*, 2011).

2.4.3 Breeding for rice blast resistance

Breeding for disease resistance aims to incorporate genes for durable resistance into improved rice varieties. The use of resistance varieties is the most economic and effective way of controlling rice blast, especially for resource-poor farmers (RoyChowdhury *et al.*, 2012b). However, varieties released as resistant often tend to loose their resistance after some years, and the rice blast prevailed again (Sun *et al.*,

1999). Evidence indicates that the cultivar specificity of *P. grisea* follows gene-for-gene model (Kang, 2000). Numerous *AVR* genes have been genetically identified in *P. grisea* (Chao and Ellingboe, 1997) and correspondingly, numerous *R* genes against *P. grisea* in rice (Valent *et al.*, 1998).The variability of the pathogen and the history of resistance breakdown have led to the development of a number of different plant breeding approaches to achieve durable blast resistance. It includes the use of resistance genes, partial resistance, gene pyramiding and lineage-exclusion hypothesis. The differential system, consisting of monogenic lines and differential blast isolates, has been used for advanced studies on the identification of pathogenicities of blast isolates and estimation of *R* genes in rice varieties (Telebanco-Yanoria *et al.*, 2008; Takehisa *et al.*, 2009). A set of monogenic lines containing 24 major *R* genes (*Pia, Pib, Pii, Pik, Pik-h, Pik-m, Pik-p, Pik-s, Pish, Pit, Pita, Pita-2, Piz, Piz-t, Pi1, Piz-5, Pi3, Pi5(t), Pi7(t), Pi9, Pil2(t), Pi11(t), Pi19* and *Pi20*) in the blast-susceptible recurrent Japonica variety LTH (Telebanco-Yanoria *et al.*, 2008), developed by the International Rice Research Institute, are excellent materials for determining plant-specific resistance in rice breeding (Telebanco-Yanoria *et al.*, 2010; Koide *et al.*, 2011). Another set of near-isogenic lines (NILs) with 27 blast *R* genes were also developed using an indicia variety, CO39 (Telebanco-Yanoria *et al.*, 2011). The resistance spectra of *R* genes against field isolates of *P. grisea* are characterized by international and domestic differential systems, classical genetic analysis, monogenic differentials and near-isogenic differentials (Koide *et al.*, 2011).

2.5 Management of Rice Blast

2.5.1 Use of resistant varieties

Host cultivars that are resistant against leaf and panicle blast have been the most widely used method of disease control (Yang *et al.*, 2009a), but resistance (mainly race-specific resistance) has tended to be ephemeral because of pathogen variability. Forms of 'partial resistance' have been identified but are little understood or used (Roumen, 1994). Cultivars with multiple genes for blast resistance (Marchetti *et al.*, 1996; RoyChowdhury *et al.*, 2012b) and 'durable' forms of resistance in hybrid rice (Chuan *et al.*, 1999) have been described as offering better hopes of sustained activity against the pathogen. Advances in understanding the molecular genetics of the pathogen (Xin *et al.*, 1998), the increasing availability of fertile lines, and advances in mapping resistance genes in the host (Chen *et al.*, 1999) together with knowledge of virulence and avirulence genes in the pathogen (Leong *et al.*, 1994) and pathogen population structures (Zeigler *et al.*, 1994) have improved our understanding of interactions between the rice host and the blast pathogen and should lead to the development of more durable forms of resistance. Marker-aided selection of resistance from recombinant populations have provided new tools for rice breeding and resulted in improved genetic analysis of resistance (Inukai *et al.*, 1996). Host resistance is likely to be more durable when deployed in appropriate rice cultivar mixtures (Mundt, 1994), as a result of the 'mixture effect', consisting of reduced inoculum, barrier and induced resistance (Chin and Wolfe, 1984). Nakajima *et al.* (1996) regarded the same effects as being responsible for effectiveness of multilines for the control of blast. The effectiveness of multilines or mixed varietal plantings has been demonstrated in reducing blast disease in Japan (Ando, 2009)

2.5.2 Cultural practices

Cultural methods are normally unlikely to be implemented in isolation from other agronomic considerations. Avoidance of excessive levels of nitrogenous fertilizer (Prabhu *et al.*, 1996) and moisture stress to crops in order to reduce disease severity has been reported. Field sanitation and synchronized planting reduce carryover and/or spread of disease. The effects of plant density are variable: although closer planting favours the microclimate for disease, plants have less available nitrogen for the fungus. Silicon soil amendments (Thiagalingam and Chin, 1976) are known to increase host resistance.

2.5.3 Chemical control

Fungicidal control is largely practised in temperate or subtropical rice cultivation, mainly in Japan, China, South Korea, Taiwan and, increasingly, Vietnam. Substantial areas under rice cultivation are also treated in Latin America (Colombia and Brazil), in India and in the USA but little are used in other geographical areas either because of cost limitations, or because of lower or inconsistent disease pressure (CABI, 2007). In Tanzania, fungicidal control is hardly used because of cost limitations. However, the major products used in most rice producing areas are mainly systemic fungicides with a residual activity of at least 15 days, although older organophosphorous products such as Edifenphos are still widely used. The modern blasticides include Isoprothiolane, Probenazole, Pyroquilon and Tricyclazole (Filippi and Prabhu, 1997), applied as foliar sprays, as granules into water or seed-box treatments (irrigated lowland rice), or as seed dressings for upland rice. Melanin biosynthesis inhibitors such as Carpropamid (Thieron *et al.*, 1999) or

broad-spectrum fungicides like Azoxystrobin (strobilurin) (Lee and Beaty, 1999) are also widely used products. The application of most products is recommended as protective applications before or shortly after the onset of leaf blast.

2.5.4 Early warning Systems and Integrated pest management

Blast-forecasting systems have successfully been developed in Japan (Uehara *et al.*, 1988) and Korea (Kim *et al.*, 1988), based on meteorological data. In India and Thailand, simple models have been developed but are not widely used. Other models such as integrating CERES-Rice, a rice growth simulation model with BLASTSIM, a blast epidemic simulation model and BLASTAM, a tool for forecasting leaf blast occurrence have been successful in predicting leaf blast at specific locations but require further validation (Luo *et al.*, 1997). In general, reliable forecasting models are not yet available in the tropics. Empirical (Pinnschmidt *et al.*, 1994) and mechanistic (Bastiaans *et al.*, 1994) models relating disease intensity to yield loss are being utilized to construct damage functions to aid timely intervention with control measures (Teng, 1994).

CHAPTER THREE

3.0 Materials and Methods

3.1 Assessment of pathotype composition of *Pyricularia grisea* population in southern highlands using differential cultivars

3.1.1 Plant materials

Rice plant materials used were 34 monogenic lines and varieties; two Uyole inbred lines UR319 and UR400 and four local varieties wahiwahi, mwasungo, zambia and kilombero. Monogenic lines and varieties were provided by Africa rice, Dar es Salaam centre. The names of monogenic lines and varieties and their respective resistance genes are shown in (Appendix 1).

3.1.2 Samples collection

Samples of leaves infected with rice blast were collected randomly from local and improved varieties in farmers' fields at hot spots areas of Mbeya, Rukwa and Ruvuma regions for pathogen isolation in the laboratory. Leaf samples of about 6 inches were collected at the epidemic initiation and early in the morning when high spore production was observed on lesions (Pinnschmidt *et al.*, 1994). Leaf samples were then packed in small (6.5 x 3.625") brown envelopes and transported immediately to the laboratory for isolation. Sample collection and isolation were done regional wise to facilitate immediate transfer of samples to the laboratory for isolation, the longest time taken from the farthest point of collection like villages of Sumbawanga and Songea rural districts to the laboratory was 2 days which is within 3 days recommended for the samples to remain viable before isolation (Koide *et al.*, 2011). Leaf samples taken from each plant were packed in separate envelopes, 30

envelopes containing leaf samples were collected from each village on different farms making a total of 180 envelopes in all of the three regions. In Mbeya region, disease leaf samples were collected from Kyela District at Kikusya and Ngana villages. In Rukwa region disease leaf samples were collected from Sumbawanga rural District at Muze and Mtowisa villages and in Ruvuma disease leaf samples were collected from Songea rural District at Namatuhi and Mpitimbi villages.

3.1.3 Pathogen isolation and differentiation of isolates

Isolation of *P. grisea* was made on water agar medium (20 g Agar Powder, 1 g Streptomycin, 1 g Penicillin and 1000 ml distilled water) (Wagtech), using single spore technique. Diseased leaf tissues were cut into small pices of about 0.5 - 1 cm and then surface sterilized using 10 % chlorox to minimize the chances of other organisms other than fungi of interest to grow. The leaf pieces were then transfered with sterile foceps into water agar and incubated for 24 - 48 h at 28 °C. After 24 h of incubation, using a sterile needle, spores were picked from sporulating lesions and placed gently on the surface of a fresh water agar medium in Petri dishes. The spores were then dispersed on the surface of the medium and single spores selected and demarcated under a stereomicroscope. Demarcations help in identification of selected single spore from unselected ones. After 10 h, the germinating single marked conidia were transferred into starch medium (20 g Agar Powder, 10 g Starch, 2 g Yeast extract , 1 g Streptomycin, 1 g Penicillin in 1000 ml distilled water) medium (Technopharmchem) and then incubated at 28 °C for 10 days to allow sporulation. Cultural characteristics (colony growth rate, colony color, texture and zonation) were studied and used in the differentiation of isolates

3.1.4 Preservation and storage of isolates

A piece of fully grown mycelia from the incubated isolates were taken and put into starch media petri dish and incubated at 28 °C for 4 days. At day four of the incubation process, sterile filter paper disks (1 x 1 cm) were placed inside starch agar plates in 20 mm diameter in front of the actively growing edge colonies. The sterile filter paper disks were left inside the agar media and the petri dishes returned to the incubator for the next six days until when the disks were overgrown and covered by *P. grisea* mycelium and spores. Disks colonized by mycelium of *P. grisea* were then taken from the agar media and put into small envelopes (8 x 10 cm) which were placed in the deciccator containing silica gel for drying. The thoroughly dried envelopes containing filter paper discs were then put into vacuumed (without air) plastic bags and then stored at -20 °C (Valent *et al.*, 1991; Couch and Khon, 2002).

3.1.5 Grouping of identified isolates into respective pathotype using differential cultivars

In the grouping of identified isolates into respective pathotype, 27 monogenic lines and a blast-susceptible Japonica-type variety, Lijiangxintuanheigu (LTH), a total of 28 entries were seeded in a tray (57 × 35 cm) filled with soil mixture of sand and humus in the ratio of 1:3, respectively (Namai and Ehara, 1986). A randomized complete block design (RCBD) with three replications for each isolate was used and rice seeds were pre-germinated in sterile Petri dishes (for 4 days) before transplanted in trays under sterile condition. Each tray was divided into 28 lines to accommodate all the 28 entries each line having 4 plants. NPK and Urea fertilizers at a rate of 30 g

and 6 g per tray respectively were applied at transplanting for NPK and 21 days after transplanting for Urea. Classification of identified isolates into respective pathotypes was made based on reaction of isolates on differential cultivars after inoculation

3.1.5.1 Preparation of inoculum and inoculation of isolates on differential cultivars

Stored isolates cultures were revived on starch agar media and incubated for 10 days to allow sporulation. Fully grown mycelia from the incubated isolates were then scratched out and placed in dark box for 48 h at 28 °C to obtain the most active and effective fungal spores. Inoculum was prepared by suspending the fungal spores in sterile distilled water prior to inoculation and adjusted to 5 x 10^4 spores/ml using haemacytometer. Plants were inoculated by spraying 10 ml of aqueous spore suspension containing 5 x 10^4 spores/ml on leaves using hand sprayer. Plants were inoculated 4 weeks after transplanting. Tween 80 (two drops) was added into the spore suspension prior to inoculation to increase adhesiveness of spores on leaves. After inoculation plants were covered overnight with plastic cardboard on screenhouse benches for three consecutive days to create high humidity approaching 100 % necessary for the infection.

3.1.5.2 Disease assessment of inoculated differential cultivars

Assessment and evaluation of monogenic lines and varieties were done 8 days after inoculation using 0 - 5 assessment scale described by Hayash and Fukuta (2009). Infection type scores were determined by comparing the interval of large vertical vascular bundle with the width of the lesion on cultivar where a mean score mean

score of 0 - 2 = Resistant and 3 - 5 = Susceptible with exception to IRBLsh-S and IRBLta2-Pi, where susceptibility starts with a mean rating of 3.5 and in IRBL5-M a mean score of over 1.5 was considered susceptible.

3.1.5.3 Pathotypes virulent potential and distribution frequency

Virulent potential of identified pathotypes were assessed as percentage disease severity while virulence frequency of the pathotypes was calculated on the basis of number of cultivars infected by particular pathotypes.

3.1.5.4 Pathotypes designation

Designation of pathogenic races was done on the basis of the infection type of LTH monogenic lines using a new system to classify rice blast isolates proposed by Hayash and Fukuta (2009). In this system, blast isolates are characterized by a reverse octal system, based on Gilmour's octal code developed for the assignment of pathogen race names (Gilmour, 1973). This nomenclature method is excellent in regularity and flexibility, and suitable for a differential system composed of many varieties. The selected LTH monogenic lines are divided into groups with 3 lines, and each of the 3 lines is given the code number 1, 2, 4, for group 1, 2 and 3 respectively. The total of the code numbers of the three LTH monogenic lines, which are compatible to blast fungus, show the race number. After assessment and evaluation of the monogenic lines and varieties, the responses were changed into resistance (R) or susceptible (S) according to their mean scores as described in section 3. 1. 4. 3. Monogenic lines were then put into five group in a table describing the Resistance (*R*) gene locus they carry, Target *R* genes they have,

names of monogenic lines, Code number, responses of monogenic lines and the sum of responses according to its respective codes number where 0 will be given for avirulent line and 1, 2, or 4 for virulent based on the genes in each line (Appendix 3). The LTH monogenic lines were categorized into 3 different classes and given codes 1-3. 1st line having code number 1 contains sh-B, LTH, i-F5, ks-F5, km-Ts, k-ka, 9-W, z-FU, ta2-Pi, ta-K1 and 19-A monogenic lines. 2nd line having code number 2 contains b-B, a-A, 3-CP4, 1-CL kp-K60, z5-CA, ta2-Re, ta-CP1 and 20-IR24 monogenic lines. 3rd line having code number 3 contains t-K59, 5-M, khK3, 7-M, zt-T and 12- M monogenic lines (Hayash and Fukuta, 2009). In designating the pathotype name, from each group the responses were summed with 0 for avirulent and 1, 2 or 4 for virulent based on the line they belong. A race number is sum of a code number showing a susceptible reaction. U mentioned before a number shows an international blast pathogenicity race and i, k, z, ta before each number shows locus of the multiple allele.

3.2 Reaction of Selected Cultivars to Identified Strains of *Pyricularia grisea*

In screening cultivars for resistance to identified strains of *P. grisea* on some rice varieties and inbred lines, two Uyole selected inbred lines (UR319 and UR400) and four local varieties (Wahiwahi, Mwasungo, Zambia and Kilombero) were seeded in a tray (57 × 35 cm) filled with soil mixture of sand and humus in the ratio of 1 to 3 respectively (Namai and Ehara, 1986). RCBD with three replications for each isolate was used and rice seeds were pre-germinated in sterile Petri dishes (for 4 days) before transplanted in trays under sterile condition. In a tray the two Uyole lines and four local varieties were transplanted into two rows, each row having 12 plants.

NPK and Urea fertilizers at a rate of 30 g and 6 g per tray respectively were applied at transplanting for NPK and 21 days after transplanting for Urea. Preparation of the inoculum and inoculation of test materials was done as described in section 3. 1. 4. 1. Observations on reaction type for each inbred line were recorded 8 days after inoculation. IRRI (1996) Standard Evaluation System (SES) on a scale 0 - 9 was used where 0 = No typical susceptible lesion observed, 1 = Small brown specks of pin-point size without sporulating centre, 3 = Small roundish to slightly elongated, necrotic grey spots, about 1-2 mm in diameter, with a distinct brown margin, 5 = Typical susceptible blast lesions 3mm or longer, infecting less than 10 % of the leaf area, 7 = Typical susceptible blast lesions infecting 11-50 % of the leaf area and 9 = More than 75 % leaf area affected.

3. 3 Population Diversity of *Pyricularia grisea* in Southern Highlands of Tanzania (Field Study).

3.3.1 Trapping nursery

A trapping nursery was used in a study to determine the *P. grisea* population diversity. The design and layout of the trapping nursery were as shown in Appendix 4. Field experiment was setup in a randomised completely block design with four replications using 35 rice varieties. The 35 rice varieties used for the study includes monogenic lines (MLs), differential varieties and one susceptible local check. Each rice variety was sown in four lines of 50 cm length with 10 x 10 cm spacing between lines and holes. In each rice variety, three seeds per hole were used and plants were thinned to one making a total of 24 plants per variety. In order to increase and homogenize the inoculums, three infecting rows were planted between replications

fifteen days before the entries. After emergence of the seedlings in each of the infector row, infected leaves from diseased plants around the site were collected, chopped into small pieces and disseminated between the rows. The trials were implemented three weeks after the beginning of the rice growing season in the site in order to obtain inoculums from neighbouring farmers' fields to increase the blast disease pressure in the experimental sites.

3.3.2 Fertilizer application and disease evaluation

NPK and Urea fertilizers were applied in the infecting rows at the rate of 82.8 g of NPK/band at sowing and 124.2 g of Urea/band at 14 days after sowing. The same amount of fertilizer was applied in the entries sown 15 days after the infecting rows were sown. Disease evaluation started 21 days after sowing. In each variety, severities of all the 24 plants were assessed separately, one by one using SES on a scale 0-9. The score of each plant were then recorded and the average score were considered as the severity score of that variety. Blast symptoms were scored once a week and five evaluations were done to get the maximum disease severity. Symptoms were recorded on each variety by considering plants without symptoms or with only hypersensitive lesions as resistant to the blast population (Thinlay *et al.*, 2000). Trapping nursery is based on the fact that the relationship between virulence genes of pathogens and resistance genes of rice varieties follows the gene-for-gene theory (Flor, 1971). Then the reaction of each variety with known resistance gene is an indication of the presence or absence of the corresponding avirulence gene without prejudging their association into distinct races (Sere *et al.*, 2004). Therefore, virulence genotypes of pathotypes can be inferred when resistance genotypes are

known for each differential variety and these can distinguish pathotypes (races) based on its reaction pattern or qualitative differences in reactions to different pathogen strains (Kobayashi *et al.*, 2007)

3.4 Data Analysis

Data obtained were subjected to analysis of variance (ANOVA) and mean were separated by Student-Newman-Keuls test (Abdi and Williams, 2010) at P = 0.05 using GENSTAT statistical package. The following statistical model was used:

$Y_{ij} = \mu + t_i + eij$. Where; Yij = Response of variables investigated, μ = the overall mean of the experiment, t_i = the effect of the ith treatment, eij = the random error effect due to inherent variability of the experiment.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Assessment of Pathotype Composition of *Pyricularia grisea* Population in Southern Highlands based on Differentials

4.1.1 Pathogen isolation and cultural differentiation of the isolates

Three-celled, hyaline, pyriform to nearly ellipsoid conidia (Plate 2a) and long, slender, mostly simple conidiophores (Plate 2b) were seen during pathogen isolation. The morphology of conidia and conidiophores were exactly like those described by Ellis (1971). A total of 122 isolates were recovered from the leaf samples collected following single spore isolation, 30 isolates were recovered from Ruvuma, 30 isolates from Rukwa and 62 isolates from Mbeya (Appendix 5).

There was no cultural difference among isolates in-terms of colour as they all appeared white, the noticeable difference were on growth, where isolate 1 (Plate 3a) and isolate 2 (Plate 3b) had fast growth followed by isolate 4 (Plate 3d) while isolate 3 (Plate 3c), isolate 5 (Plate 3e) and isolate 6 (Plate 3f) had the slowest growth respectively.

There were also differences in aerial growth, colony smoothness growth and zonation. Isolate 1 (Plate 3a) and isolate 4 (Plate 3d) showed fluffy growth characteristics while the rest had smooth growth. Isolate 2 (Plate 3b) and isolate 4 (Plate 3d) had zonation while the rest had no zonation. Variations observed in cultural characteristics are important from the point of view of the biology of the pathogen as it occurs in nature; it is closely linked with the question of physiological races of pathogen (Meena, 2005). Physiological races or pathotypes are normally

alike in morphology but differ in certain cultural, physiological, biochemical, pathological and other characters.

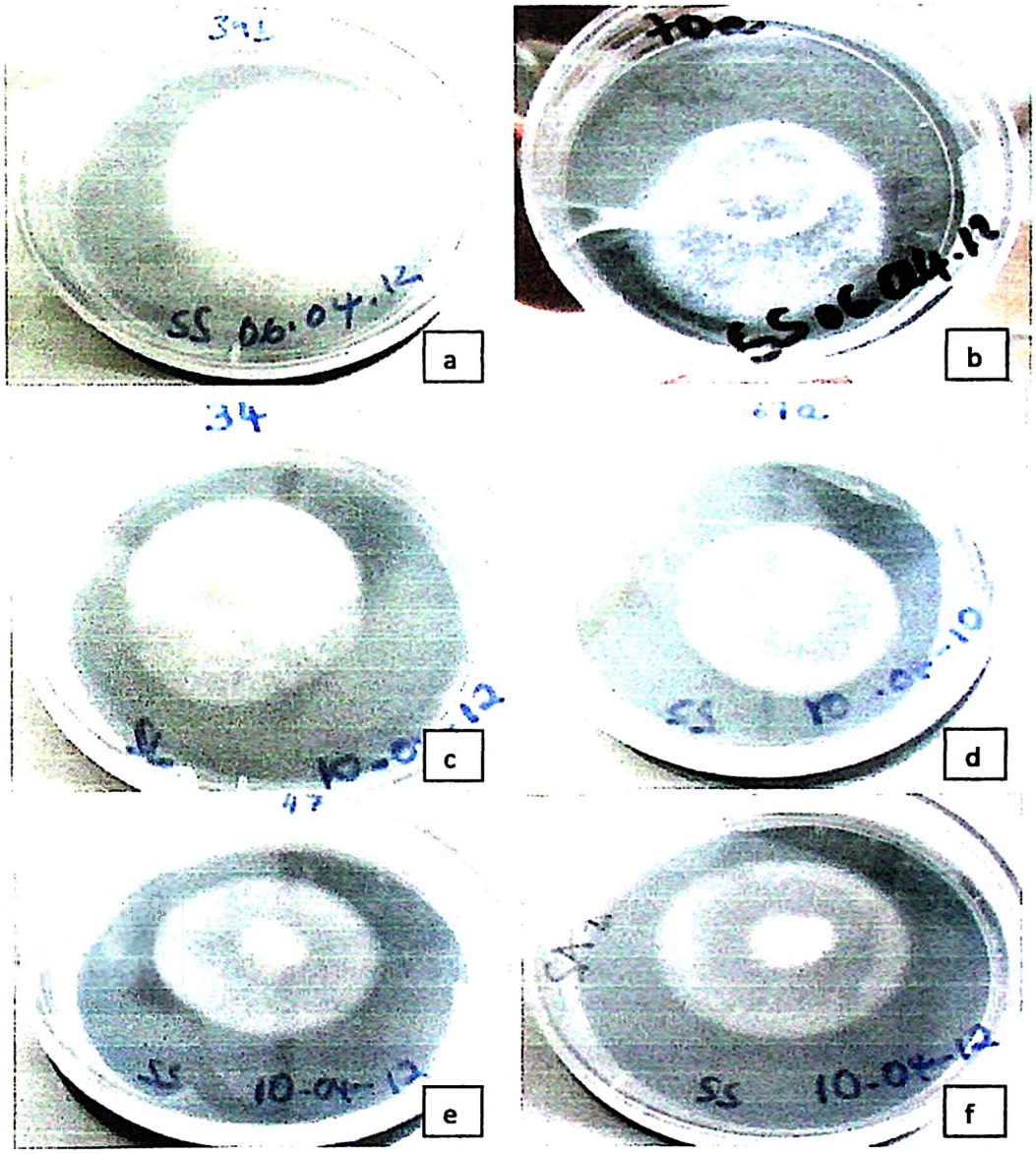

Plate 3: Cultural variation of different *Pyricularia grisea* isolates grown on starch agar medium

Where: a = Isolate 1; b =Isolate 2; c = Isolate 3; d =Isolate 4; e = Isolate 5; f =Isolate 6

4.1.2 Pathotype classification

In pathotype classification, six pathotypes were identified following inoculation of six isolates on differentials. Of the 122 isolates, only six produced enough spores for inoculation while others produced very few spores and some didn't produce spores at all and some died in the process. The six pathotypes identified were Mtowisa (MP), Namatuhi (NP), Kikusya 1 (K1P), Kikusya 2 (K2P), Ngana 1(N1P) and Ngana 2 (N2P) (Table 1a and b). The names represent the place where the pathotype were collected. These pathotypes all together represent the composition of *P. grisea* population available in southern highlands, it might be not exhaustive but it gives the picture of the composition of *P. grisea* population in the area. Results in Table 1a and b showed that monogenic rice lines and varieties have different resistant respond to different *P. grisea* pathotypes identified. This was possible due to the different response the *R* gene and *avr* gene on interaction between the resistance genes in rice lines to *avr* genes in blast races (Utami *et al.*, 2011).

4.1.2 .1 'Mtowisa' pathotype

This pathotype was collected from Rukwa region and had the virulence frequency of 37 %.The pathotype was virulent to 10 monogenic lines that harbours *Pi20, Pi9, Pi5, Pi19, Pif, Pik, Pik-h, Pita-2, Piz* and *Pish* resistant genes and avirulent to 17 monogenic lines that harbours *Pib, Pit, Pia, Pii, Pi3, Pik-s, Pik-m, Pi1, Pik-p, Pi-7(t), Pize-5, Piz-t, Pi12(t), Pita,Pi11,Pi1b,*and *Pi33* resistant genes (Table 1b).

Table 1a: Severity scores of rice monogenic lines (MLs) against the six pathotype of *Pyricularia grisea* identified in Southern Highlands of Tanzania

Var name	*R* gene	Pathotypes					
		K1P	N1P	MP	K2P	N2P	NP
C103 TTP	*Pi1b*	0.0^{a}	3.7^{cm}	0.0^{a}	2.0^{b}	0.0^{a}	2.0^{b}
IRBL 5-M	*Pi5*	0.0^{ab}	2.0^{bd}	2.3^{bh}	2.3^{bc}	0.0^{a}	0.0^{a}
IRBLB-B	*Pib*	0.0^{ac}	2.3^{bj}	2.0^{bc}	2.0^{b}	2.0^{bd}	2.0^{b}
IRBLKM TS	*Pik-m*	1.7^{ad}	2.0^{bg}	2.0^{bf}	2.0^{b}	2.0^{bd}	2.3^{b}
IRBLKP-K60	*Pik-p*	2.0^{dc}	1.0^{ab}	2.7^{ch}	2.3^{bd}	1.7^{b}	2.3^{b}
IRBL 1-CL	*Pi1*	2.0^{dc}	2.3^{bh}	2.0^{b}	2.0^{b}	2.0^{bc}	3.7^{c}
IRBL 7-M	*Pi7*	2.0^{df}	0.0^{a}	0.0^{a}	2.0^{b}	2.0^{bd}	3.7^{c}
St1	*Pif*	2.0^{af}	2.0^{bd}	4.0^{hi}	2.7^{bf}	3.3^{bg}	0.0^{a}
IRBLTA CP 1'	*Pita*	2.0^{ag}	2.3^{bk}	2.3^{bh}	2.7^{bg}	2.7^{bf}	0.0^{a}
IRBL 12-M	*Pi12*	2.3^{dh}	1.3^{ab}	2.7^{ch}	0.0^{a}	0.0^{a}	0.0^{a}
IRBL-A	*Pia*	2.3^{di}	1.7^{b}	2.0^{bd}	0.0^{a}	0.0^{a}	1.7^{b}
IRBL 11-ZH	*Pi11*	2.7^{dj}	2.3^{bi}	0.0^{a}	2.3^{bc}	2.0^{bc}	2.0^{b}
IRBLI-F5	*Pii*	2.7^{dj}	2.0^{bf}	0.0^{a}	4.0^{h}	0.0^{a}	3.7^{c}
IRBLT-K59	*Pit*	2.7^{dj}	4.0^{el}	2.0^{bg}	2.0^{b}	0.0^{a}	0.0^{a}
IRBLZT-T	*Piz-t*	2.7^{dj}	1.7^{b}	2.3^{bh}	2.0^{b}	2.0^{be}	0.0^{a}
IRBLK KA	*Pik*	3.0^{dj}	2.7^{bl}	4.0^{hi}	0.0^{a}	3.0^{bf}	2.0^{b}
IRBLZ FU	*Piz*	3.7^{dj}	4.3^{lm}	4.0^{hi}	3.7^{ch}	3.3^{bg}	4.0^{ef}
LTH		3.7^{dj}	3.7^{bg}	3.7^{bi}	3.7^{ch}	3.7^{cg}	3.7^{cd}
IRBL 19-A	*Pi19*	4.0^{ej}	4.0^{hm}	4.0^{hi}	0.0^{a}	2.0^{bd}	0.0^{a}
IRBL 20-IR 24	*Pi20*	4.0^{ej}	4.0^{hm}	3.7^{bi}	2.7^{bc}	0.0^{a}	3.7^{c}
IRBL 9-W	*Pi9*	4.0^{ej}	4.0^{cm}	3.7^{bi}	0.0^{a}	4.0^{fg}	5.0^{g}
IRBBLKH-K3	*Pik-h*	4.0^{dij}	2.0^{bg}	4.0^{hi}	2.0^{b}	2.0^{bd}	2.0^{b}
IRBLZ5-CA	*Piz-5*	4.0^{dij}	4.0^{cm}	2.3^{bh}	4.0^{h}	4.0^{fg}	2.0^{b}
IR 1529	*Pi33*	4.3^{ej}	0.0^{a}	2.3^{bh}	2.3^{bc}	3.3^{cg}	3.7^{c}
IRBLKS-F5	*Pik-s*	4.3^{ej}	5.0^{m}	0.0^{a}	0.0^{a}	4.0^{fg}	5.0^{eg}
IRBL3-CP 4	*Pi3*	4.7^{j}	2.0^{bc}	2.0^{bc}	2.0^{b}	0.0^{a}	2.0^{b}
IRBLSH-S	*Pish*	4.7^{j}	4.0^{cm}	5.0^{i}	3.3^{ch}	4.7^{g}	4.0^{ee}
IRBLTA 2-Pl	*Pita-2*	4.7^{hj}	4.2^{lm}	4.0^{hi}	3.3^{ch}	4.7^{g}	4.7^{eg}
Means		2.86	2.60	2.46	2.05	2.08	2.39
lsd		1.3	1.13	1.09	0.68	0.97	0.744
F test		***	***	***	***	***	***
cv%		27.2	26.4	27.3	20.3	28.5	19
VF%		44.4	40.7	37	14.8	33.3	37

Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ according to the Student-Newman-Keuls test.

Where: ***= highly significant different (P<0.001); VF = Virulency frequency; KIP = 'Kikusya' 1Pathotype; N1P = 'Ngana' 1 Pathotype; MP = 'Mtowisa' Pathotype; K2P = 'Kikusya' 2 Pathotype; N2P; 'Ngana' 2 Pathotype; NP = 'Namatuhi' Pathotype.

Table 1b: Reaction of rice monogenic lines (MLs) to the six pathotype of *Pyricularia grisea* identified in Southern Highlands of Tanzania

		Pathotypes					
Var name	*R* gene	K1P	N1P	MP	K2P	N2P	NP
C103 TTP	*Pi1b*	HR	MS	HR	R	HR	R
IRBL 5-M	*Pi5*	HR	S	S	S	HR	HR
IRBLB-B	*Pib*	HR	R	R	R	R	R
IRBLKM TS	*Pik-m*	R	R	R	R	R	R
IRBLKP-K60	*Pik-p*	MR	R	MR	R	R	R
IRBL 1-CL	*Pi1*	MR	R	R	R	R	S
IRBL 7-M	*Pi7*	R	HR	HR	R	R	S
St1	*Pif*	R	R	S	MR	MS	HR
IRBLTA CP 1'	*Pita*	R	R	R	MR	MR	HR
IRBL 12-M	*Pi12*	R	R	MR	HR	HR	HR
IRBL-A	*Pia*	R	R	R	HR	HR	R
IRBL 11-ZH	*Pi11*	MR	R	HR	R	R	R
IRBLI-F5	*Pii*	MR	R	HR	S	HR	S
IRBLT-K59	*Pit*	MR	S	R	R	HR	HR
IRBLZT-T	*Piz-t*	MR	R	R	R	R	HR
IRBLK KA	*Pik*	MS	MR	S	HR	MS	R
IRBLZ FU	*Piz*	S	S	S	S	MS	S
LTH		S	S	S	S	S	S
IRBL 19-A	*Pi19*	S	S	S	HR	R	HR
IRBL 20-IR 24	*Pi20*	S	S	S	MR	HR	S
IRBL 9-W	*Pi9*	S	S	S	HR	S	HS
IRBBLKH-K3	*Pik-h*	S	R	S	R	R	R
IRBLZ5-CA	*Piz-5*	S	S	R	S	S	R
IR 1529	*Pi33*	S	HR	R	R	MS	S
IRBLKS-F5	*Pik-s*	S	HS	HR	HR	S	HS
IRBL3-CP 4	*Pi3*	HS	R	R	R	HR	R
IRBLSH-S	*Pish*	HS	S	HS	R	HS	S
IRBLTA 2-PI	*Pita-2*	HS	S	S	R	HS	HS

Where: R = Resistant; MR = Moderately Resistant; HR = Highly Resistant;
S = Susceptible; MS = Moderately Susceptible; HS = Highly Susceptible
KIP = 'Kikusya' 1Pathotype; N1P = 'Ngana' 1 Pathotype; MP = 'Mtowisa' 'Pathotype'; K2P = 'Kikusya' 2 Pathotype; N2P; 'Ngana' 2 Pathotype;
NP = 'Namatuhi' Pathotype.

Variety IRBL 5-M (Table 1a and b) carrying *Pi5 R* gene had a mean score of 2.3 but was susceptible to Mtowisa pathotype while others with the same score were not, suggesting that not only the scores that determine resistant or susceptibility of a variety but the genes it carries (Hayash and Fukuta, 2009). The following *R* genes *Pib, Pit, Pia, Pii, Pi3, Pik-s, Pik-m, Pi1, Pik-p, Pi-7(t), Piz-5, Piz-t, Pi12(t), Pita,Pi11,Pi1b,*and *Pi33* which shown to be effective against Mtowisa pathotype, may be useful in preventing rice blast in Rukwa region.

4.1.2.2 'Namatuhi' pathotype

This pathotype was collected from Ruvuma region and had the virulence frequency of 37 %. The pathotype was virulent to 10 monogenic lines that harbours *Pi1, Pi20, Pi33, Pi7, Pii, Pish, Piz, Pita-2, Pi9* and *Pik-s* resistant genes and avirulent to 17 monogenic lines that harbours *Pib, Pia, Pik-h, Pik, Pi3, Pi5(t), Pik-m, Pik-p, Pi-7(t), Piz-5, Piz-t, Pi12(t), P19(t) , Pif, Pi11, Pi1b* and *Pit* resistant genes (Table 1b). The following *R* genes *Pib, Pia, Pik-h, Pik, Pi3, Pi5 (t), Pik-m, Pik-p, Pi-7(t), Piz-5, Piz-t, Pi12 (t), P19 (t), Pif, Pi11, Pi1b* and *Pit* which shown to be effective against Namatuhi pathotype, may be useful in preventing rice blast in Ruvuma region.

4.1.2.3 'Kikusya' 1 pathotype

This pathotype was collected from Mbeya region and had the virulence frequency of 44.4 %. The pathotype was virulent to 13 monogenic lines that harbours *Pik, Piz, Pi19, Pi20, Pi9, Pik-h, Piz-5, Pi33, Pik-s, Pi3, Pish, and Pita-2* resistant genes and avirulent to 15 monogenic lines that harbours *Pib, Pit, Pia, Pii, Pi5(t), Pik-m, Pi1, Pik-p, Pi7(t), Piz-t, Pita ,Pi1b, Pi12, Pif and Pi11* resistant genes (Table 1b). With the virulence frequency of 46.4% Kikusya 1 pathotype was the most virulent

pathotype of all the pathotypes identified in this study. The following *R* genes *Pib, Pit, Pia, Pii, Pi5(t), Pik-m, Pi1, Pik-p, Pi7(t), Piz-t, Pita, Pi1b, Pi12, Pif* and *Pi11* which shown to be effective against Kikusya 1 pathotype, may be useful in preventing rice blast in Mbeya region when combined with other *R* genes from Kikusya 2, Ngana1 and Ngana 2 Pathotypes.

4.1.2.4 'Kikusya' 2 pathotype

This pathotype was collected from Mbeya region and had the virulence frequency of 14.8 %. The pathotype was virulent to five monogenic lines that harbours *Pish, Pita-2, Piz, Pii* and *Piz-5* resistant genes and avirulent to 21 monogenic lines that harbours *Pib, Pia, Pikh, Pik, Pi3, Pik-s, Pik-m, Pi1, Pik-p, Pi-7(t), Piz-t, Pi12(t), Pita, P19(t), Pi20(t), Pi9, Pi1b, Pit, Pi11, Pi33,* and *Pif* resistant genes (Table 1b). With Virulence frequency of 17.9 %, Kikusya 2 was the least virulent of all the six pathotypes identified in this study. The following *R* genes *Pib, Pia, Pikh, Pik, Pi3, Pik-s, Pik-m, Pi1, Pik-p, Pi-7(t), Piz-t, Pi12(t), Pita, P19(t), Pi20(t), Pi9, Pi1b, Pit, Pi11, Pi33,* and *Pif* which shown to be effective against Kikusya 2 pathotype, may be useful in preventing blast disease in Mbeya region when combined with other *R* genes from Kikusya1, Ngana1 and Ngana 2 Pathotypes.

4.1.2.5 'Ngana'1 pathotype

Ngana1 pathotype from Mbeya region had the virulence frequency of 40.7 %. The pathotype was virulent to 10 monogenic lines that harbours *Pi1b, Pi19, Pi20, Pi9, Pish, Pit, Piz-5, Pita-2, Piz* and *Pik-s* resistant genes and avirulent to 18 monogenic lines that harbours *Pib, Pia, Pii, Pi3, Pik-m, Pi1, Pikh, Pik-p, Pi-7(t), Piz-t, Pi12(t), Pita ,Pi33,Pif, Pi1, Pi11,Pi9* and *Pik* resistant genes (Table 1b). *R* genes *Pik, Pi33*

and *Pik-h* which were susceptible to Kikusya1 pathotype were resistant to Ngana1 pathotype, on the other hand, *Pi1b, Pit* and *Pik-s* which were susceptible to Ngana1 pathotype were resistant to Kikusya1 pathotype suggesting that the variety can be susceptible to one pathotype while resistant to the other. It is important therefore to breed varieties with broad resistance that will be resistant to all prevalent virulent races of the pathogen in the area. The following *R* genes *Pib, Pia, Pii, Pi3, Pik-m, Pi1, Pikh, Pik-p, Pi-7(t), Piz-t, Pi12(t), Pita ,Pi33, Pif, Pi1, Pi11, Pi9* and *Pik* which shown to be effective against Ngana 1 pathotype, may be useful in preventing blast disease in Mbeya region when combined with other *R* genes from Kikusya1, Kikusya 2 and Ngana 2 Pathotypes.

4.1.2.6 'Ngana' 2 pathotype

Ngana 2 pathotype from Mbeya region and had the virulence frequency of 33.3 %. The pathotype was virulent to 9 monogenic lines that harbours *Pik, Pi33, Pif, Piz, Pi9, Pik-s, Piz-5, Pish* and *Pita-2* resistant genes and avirulent to 18 monogenic lines that harbours *Pib, Pia, Pii, Pi5(t), Pikh, Pi3, Pik-m, Pi1, Pik-p, Pi-7(t), Piz, Piz-5, Pi12(t), Pita, P19(t) , Pi20(t) ,Pi1b,Pit,Pi11* and *Pi9* resistant genes (Table 1a). The following *R* genes *Pib, Pia, Pii, Pi5(t), Pikh, Pi3, Pik-m, Pi1, Pik-p, Pi-7(t), Piz, Piz-5, Pi12(t), Pita, P19(t) , Pi20(t) ,Pi1b, Pit, Pi11* and *Pi9* which shown to be effective against Ngana 2 pathotype, may be useful in preventing blast disease in Mbeya region when combined with other *R* genes from Kikusya1, Kikusya 2 and Ngana 1 Pathotypes.

Resistant genes *Pia, Pi11, Pib, Pik-m, Pik-p, Pi12* and *Piz-t* were the most effective *R* genes of all, being able to confer resistance to all the six pathotypes (Table 1 b).

Resistant gene *Pib* was also found to confer high levels of resistance to most blast races in Japan (RoyChowdhury *et al.*, 2012b) and conveys resistance to seven blast races in USA. *Pib* has also been introgressed into various *japonica* cultivars independently from two Indonesian and Malaysian cultivars (Jia *et al.*, 2010). It was also introgressed into rice cultivar Saber from the Chinese *indica* cultivar Teqing in USA (McClung, 2004). Similarly, *Pikh, (Pikm)* and *Piks* have been reported in US cultivars that provide resistance to the blast races, IB54, IB45, IH1 and IG1, and IB54 respectively (Jia and Moldenhauer, 2010).

Piz was the least effective *R* genes of all failing to confer resistance to any of the six pathotypes (Table 1b). *Piz R* gene in rice is well known to confer resistance to a wide range of the rice blast fungus, *P. grisea* (RoyChowdury *et al.*, 2009) but it is not effective in these pathotypes possibly due to pathogen variability (Chen *et al.*, 2006, Suh *et al.*, 2009, Wang et *al.*, 2009) . Likewise *R* genes *Pi1, Piz-5(Pi2), Pi5* and *Pi9* reported to have resistance to a broad spectrum of *P. grisea* isolates (Jeung *et al.*, 2007) were effective to some but not all pathotypes of Southern Highlands. *Pi1* was effective to all pathotypes except Namatuhi, *Piz-5* was effective to only Mtowisa and Namatuhi, *Pi5* was effective to Kikusya 1, Ngana 2 and Namatuhi, *Pi9* was effective to only Kikusya 2 pathoypes (Table 1 b). Although *Pi9* is regarded as a broad-spectrum *R* gene and is thus used in the breeding programme (Cho *et al.*, 2007), it has potential to be damaged whenever the blast isolate is dominant (Koide *et al.*, 2011). This is what seems more likely happened to *Pi9* in this study.

4.1.3 Pathotype designation

Identified pathotypes were designated into respective pathogenic races using a new designation system proposed by Hayashi and Fukuta (2009) described in section 3.1.5.3. The pathogenic races designated included U11-i2-k141-z13-ta103 (Kikusya1), U51-i0-k100-z13-ta103 (Ngana1), U11-i4-k041-z11-ta103 (Mtowisa), U01-i5-k000-z03-ta000 (Kikusya2), U10-i0-k101-z13-ta100 (Ngana2), and U10-i1-k120-z11-ta102 (Namatuhi). In this system, blast isolates were characterized by a reverse octal system, based on Gilmour's octal code developed for the assignment of pathogen race names (Gilmour, 1973). The designation summarizes the classification of each pathotype in a way that it offers sufficient range in regularity, flexibility and extension to take in new resistant genes that will be identified in the future (Koide *et al.*, 2011).

4.1.3.1 Pathogenic race U11-i2-k141-z13-ta103 (Kikusya1 pathotype)

U11-i2-k141-z13-ta303 is an international blast pathogenicity race number given to Kikusya1 pathotype derived from information on Table 2. Pathogenicity race numbers 11, 2, 141, 13 and 103 represent the sum of code numbers of monogenic lines that showed a susceptible reaction to Kikusya1 pathotype in group 1, 2, 3, 4, and 5, respectively. In this case 11, 2, 141, 13 and 103 means kikusya 1 showed susceptible reaction to monogenic lines that harbours the following *R* genes; *Pish* and LTH of group 1, *Pii* and *Pi5(t)* of group 2, *Pik-s* , *Pikh* and *Pik* of group 3, *Pi9(t)*, *Piz* and *Piz-t* of group 4 and *Pita-2*, *Pi19(t)* and *Pi20(t)* of group 5 respectively (Table 2).

Table 2: Designation of *Pyricularia grisea* pathogenic race U11-i2-k141-z13-ta303 based on the reaction of monogenic line with Lijiangxintuan heigu genetic background

	Group of MLs											
	I		II	III			IV		V			
R gene locus	-		Pii	Pik			Piz		Pita			
Target R gene	*Pish*	+	*Pii*	*Pik-s*	*Pik-m*	*Pik*	*Pi9(t)*	*Piz*	*Pita-2*	*Pita*	*Pi19(t)*	
	Pib	*Pia*	*Pi3*	-	*Pi1*	*Pik-p*	-	*Piz-5*	*Pita-2*	*Pita*	*Pi20(t)*	
	Pit	-	*Pi5(t)*	-	*Pikh*	*Pi7(t)*	-	*Piz-t*	*Pi12 (t)*	-	-	
Monogenic line	sh-B	LTH	i-F5	ks-F	km-Ts	k-Ka	9-W	z-Fu	ta2-Pi	ta-K1	19-A	
(IRBL)	b-B	a-A	3-CP4	-	1-CL	kp-K60	-	z5-CA	ta2-Rc	ta-CP1	20-IR24	
	t-k59	-	5-M	-	kh-K3	7-M	-	zt-T	12-M	-	-	
Code number	1	1	1	1	1	1	1	1	1	1	1	
	2	2	2	-	2	2	-	2	2	2	2	
	4	-	4	-	4	4	-	4	4	-	-	
Scores	S	S	R	S	R	S	S	S	S	-	S	
	R	R	S	-	R	R	-	S	-	R	S	
	R	-	R	-	S	R	-	R	R	-	-	
Race number	1	1	2	1	4	1	1	3	1	0	3	

Where: R = Resistant; S = Susceptible; IRBL = International Rice Bred Line;
ML = Monogenic line; LTH = LIJIANGXINTUAN HEIGU

4.1.3.2 Pathogenic race U51-i4-k100-z13-ta103 (Nganal pathotype)

U51-i4-k100-z13-ta103 is an international blast pathogenicity race number given to Nganal pathotype derived from information on Table 3. Pathogenicity race numbers 51, 4, 100, 13 and 103 represent the sum of code numbers of monogenic lines that showed a susceptible reaction to Nganalpathotype in group 1, 2, 3, 4, and 5, respectively. In this case 51, 4, 100, 13, and 103 means Nganal showed susceptible reaction to monogenic lines that harbours the following *R* genes; *Pish, Pit* and LTH of group 1, *Pi5(t)* of group 2, *Pik-s* of group 3, *Pi9(t), Piz* and *Piz-5* of group 4 and *Pita-2, Pi19(t)* and *Pi20(t)* of group 5 respectively (Table 3).

Table 3: Designation of *Pyricularia grisea* pathogenic race U51-i4-k100-z13-ta103 based on the reaction of monogenic line with Lijiangxintuan heigu genetic background

	Group of MLs											
	I		II	III			IV		V			
R gene locus	-		Pii	Pik			Piz		Pita			
Target R gene	*Pish*	+	*Pii*	*Pik-s*	*Pik-m*	*Pik*	*Pi9(t)*	*Piz*	*Pita-2*	*Pita*	*Pi19(t)*	
	Pib	*Pia*	*Pi3*	-	*Pi1*	*Pik-p*	-	*Piz-5*	*Pita-2*	*Pita*	*Pi20(t)*	
	Pit	-	*Pi5(t)*	-	*Pikh*	*Pi7(t)*	-	*Piz-t*	*Pi12 (t)*	-	-	
Monogenic line	sh-B	LTH	i-F5	ks-F5	km-Ts	k-Ka	9-W	z-Fu	ta2-Pi	ta-K1	19-A	
(IRBL)	b-B	a-A	3-CP4	-	1-CL	kp-K60	-	z5-CA	ta2-Re	ta-CP1	20-IR24	
	t-k59	-	5-M	-	kh-K3	7-M	-	zt-T	12-M	-	-	
Code number	1	1	1	1	1	1	1	1	1	1	1	
	2	2	2	-	2	2	-	2	2	2	2	
	4	-	4	-	4	4	-	4	4	-	-	
Scores	S	S	R	S	R	R	S	S	S	-	S	
	R	R	R	-	R	R	-	S	-	R	S	
	S	-	S	-	R	R	-	R	R	-	-	
Race number	5	1	4	1	0	0	1	3	1	0	3	

Where: R = Resistant; S = Susceptible; IRBL = International Rice Bred Line;
ML = Monogenic line; LTH = LIJIANGXINTUAN HEIGU

4.1.3.3 Pathogenic race U11-i4-k041-z11-ta103 (Mtowisa pathotype)

U11-i4-k041-z11-ta103 is an international blast pathogenicity race number given to Mtowisa pathotype derived from information on Table 4. Pathogenicity race numbers 11, 4, 041, 11 and 103 represent the sum of code numbers of monogenic lines that showed a susceptible reaction to Mtowisa pathotype in group 1, 2, 3, 4, and 5, respectively. In this case 11, 4, 041, 11 and 103 means Mtowisa pathotype showed susceptible reaction to monogenic lines that harbours the following *R* genes; *Pish* and LTH of group 1, *Pii* and *Pi5(t)* of group 2, *Pikh* and *Pik*, of group 3, *Pi9(t)* and *Piz* of group 4 and *Pita-2, Pi19(t)* and *Pi20(t)* of group 5 respectively (Table 4).

Table 4: Designation of *Pyricularia grisea* pathogenic race U11-i4-k041-z11-ta103 based on the reaction of monogenic line with Lijiangxintuan heigu genetic background

	Group of MLs											
	I		II	III			IV		V			
R gene locus	-		Pii	Pik			Piz		Pita			
Target R gene	*Pish*	+	*Pii*	*Pik-s*	*Pik-m*	*Pik*	*Pi9(t)*	*Piz*	*Pita-2*	*Pita*	*Pi19(t)*	
	Pib	*Pia*	*Pi3*	-	*Pil*	*Pik-p*	-	*Piz-5*	*Pita-2*	*Pita*	*Pi20(t)*	
	Pit	-	*Pi5(t)*	-	*Pikh*	*Pi7(t)*	-	*Piz-t*	*Pi12(t)*	-	-	
Monogenic line	sh-B	LTH	i-F5	Ks-F5	km-Ts	k-Ka	9-W	z-Fu	ta2-Pi	ta-K1	19-A	
(IRBL)	b-B	a-A	3-CP4	-	1-CL	kp-K60	-	z5-CA	ta2-Re	ta-CP1	20-IR24	
	t-k59	-	5-M	-	kh-K3	7-M	-	zt-T	12-M	-	-	
Code number	1	1	1	1	1	1	1	1	1	1	1	
	2	2	2	-	2	2	2	2	2	2	2	
	4	-	4	-	4	4	4	4	4	-	-	
Scores	S	S	R	R	R	S	S	S	S	-	S	
	R	R	R	-	R	R	-	R	-	R	S	
	R	-	S	-	S	R	-	R	R	-	-	
Race number	1	1	4	0	4	1	1	1	1	0	3	

Where: R = Resistant; S = Susceptible; IRBL = International Rice Bred Line;
ML = Monogenic line; LTH = LIJIANGXINTUAN HEIGU

4.1.3.4 Pathogenic race U11-i5-k000-z03-ta000 (Kikusya2 pathotype)

U11-i5-k000-z03-ta000 is an international blast pathogenicity race number given to Kikusya2 pathotype derived from information on Table 5. Pathogenicity race numbers 11, 5, 000, 03 and 000 represent the sum of code numbers of monogenic lines that showed a susceptible reaction to Kikusya2 pathotype in group 1, 2, 3, 4, and 5, respectively. In this case 11, 5, 000, 03 and 000 means kikusya 2 showed susceptible reaction to monogenic lines that harbours the following *R* genes; *Pish* and LTH of group 1, *Pii* and *Pi5(t)* of group 2, none of group 3, *Piz* and *Piz-t* of group 4 and none of group 5 respectively (Table 5).

Table 5: Designation of *Pyricularia grisea* pathogenic race U11-i5-k000-z03-ta000 based on the reaction of monogenic line with Lijiangxintuan heigu genetic background

	Group of MLs											
	I		II	III			IV		V			
R gene locus	-		Pii	Pik			Piz		Pita			
Target R gene	*Pish*	+	*Pii*	*Pik-s*	*Pik-m*	*Pik*	*Pi9(t)*	*Piz*	*Pita-2*	*Pita*	*Pi19(t)*	
	Pib	*Pia*	*Pi3*	-	*Pi1*	*Pik-p*	-	*Piz-5*	*Pita-2*	*Pita*	*Pi20(t)*	
	Pit	-	*Pi5(t)*	-	*Pikh*	*Pi7(t)*	-	*Piz-t*	*Pi12 (t)*	-	-	
Monogenic line	sh-B	LTH	i-F5	ks-F5	km-Ts	k-Ka	9-W	z-Fu	ta2-Pi	ta-K1	19-A	
(IRBL)	b-B	a-A	3-CP4	-	1-CL	kp-K60	-	z5-CA	ta2-Rc	ta-CP1	20-IR24	
	t-k59	-	5-M	-	kh-K3	7-M	-	zt-T	12-M	-	-	
	1	1	1	1	1	1	1	1	1	1	1	
Code number	2	2	2	-	2	2	-	2	2	2	2	
	4	-	4	-	4	4	-	4	4	-	-	
Scores	S	S	S	R	R	R	R	S	R	-	R	
	R	R	R	-	R	R	-	S	-	R	R	
	R	-	S	-	R	R	-	R	R	-	-	
Race number	1	1	5	0	0	0	0	3	0	0	0	

Where: R = Resistant; S = Susceptible; IRBL = International Rice Bred Line;
ML = Monogenic line; LTH = LIJIANGXINTUAN HEIGU

4.1.3.5 Pathogenic race U11-i0-k101-z13-ta100 (Ngana2 pathotype)

U11-i0-k101-z13-ta100 is an international blast pathogenicity race number given to Ngana 2 pathotype derived from information on Table 6. Pathogenicity race numbers 11, 0, 101, 13 and 100 represent the sum of code numbers of monogenic lines that showed a susceptible reaction to Ngana2 pathotype in group 1, 2, 3, 4, and 5, respectively. In this case 11,0, 101,13 and 100 means Ngana 2 showed susceptible reaction to monogenic lines that harbours the following *R* genes; *Pish* and LTH of group 1, none of group 2, *Pik-s* and *Pik* of group 3, *Pi9(t), Piz* and *Piz-t* of group 4 and *Pita-2, Pi19(t)* and *Pi20(t)* of group 5 respectively (Table 6).

Table 6: Designation of *Pyricularia grisea* pathogenic race U11-i0-k101-z13-ta100 based on the reaction of monogenic line with Lijiangxintuan heigu genetic background

	Group of MLs											
	I		II	III			IV		V			
R gene locus	-		Pii	Pik			Piz		Pita			
Target R gene	*Pish*	+	*Pii*	*Pik-s*	*Pik-m*	*Pik*	*Pi9(t)*	*Piz*	*Pita-2*	*Pita*	*Pi19(t)*	
	Pib	*Pia*	*Pi3*	-	*Pi1*	*Pik-p*	-	*Piz-5*	*Pita-2*	*Pita*	*Pi20(t)*	
	Pit	-	*Pi5(t)*	-	*Pikh*	*Pi7(t)*	-	*Piz-t*	*Pi12 (t)*	-	-	
Monogenic line	sh-B	LTH	i-F5	ks-F5	km-Ts	k-Ka	9-W	z-Fu	ta2-Pi	ta-K1	19-A	
(IRBL)	b-B	a-A	3-CP4	-	1-CL	kp-K60	-	z5-CA	ta2-Re	ta-CP1	20-IR24	
	t-k59	-	5-M	-	kh-K3	7-M	-	zt-T	12-M	-	-	
	1	1	1	1	1	1	1	1	1	1	1	
Code number	2	2	2	-	2	2	-	2	2	2	2	
	4	-	4	-	4	4	-	4	4	-	-	
Scores	S	S	S	R	R	R	R	S	R	-	R	
	R	R	R	-	R	R	-	S	-	R	R	
	R	-	S	-	R	R	-	R	R	-	-	
Race number	1	1	5	0	0	0	0	3	0	0	0	

Where: R = Resistant; S = Susceptible; IRBL = International Rice Bred Line;
ML = Monogenic line; LTH = LIJIANGXINTUAN HEIGU

4.1.3.6 Pathogenic race U11-i1-k124-z11-ta102 (Namatuhi pathotype)

U11-i1-k124-z11-ta102 is an international blast pathogenicity race number given to Namatuhi pathotype derived from information on Table 7. Pathogenicity race numbers 11, 1, 121, 11 and 102 represent the sum of code numbers of monogenic lines that showed a susceptible reaction to Namatuhi pathotype in group 1, 2, 3, 4, and 5, respectively. In this case 11, 1, 121, 111 and 102 means Namatuhi pathotype showed susceptible reaction to the following *R* genes; *Pish* and LTH of group 1, *Pii* of group 2, *Pik-s*, *Pi1*and *Pi7(t)* of group 3, *Pi9(t)* and *Piz* of group 4 and *Pita-2* and *Pi20(t)* of group 5 respectively (Table 7).

Table 7: Designation of *Pyricularia grisea* pathogenic race U11-i1-k124-z11-ta102 based on the reaction of monogenic line with Lijiangxintuan heigu genetic background

	Group of MLs											
	I		II	III			IV		V			
R gene locus	-		Pii	Pik			Piz		Pita			
Target R gene	*Pish*	+	*Pii*	*Pik-s*	*Pik-m*	*Pik*	*Pi9(t)*	*Piz*	*Pita-2*	*Pita*	*Pi19(t)*	
	Pib	*Pia*	*Pi3*	-	*Pi1*	*Pik-p*	-	*Piz-5*	*Pita-2*	*Pita*	*Pi20(t)*	
	Pit	-	*Pi5(t)*	-	*Pikh*	*Pi7(t)*	-	*Piz-t*	*Pi12 (t)*	-	-	
Monogenic line	sh-B	LTH	i-F5	ks-F5	km-Ts	k-Ka	9-W	z-Fu	ta2-Pi	ta-K1	19-A	
(IRBL)	b-B	a-A	3-CP4	-	1-CL	kp-K60	-	z5-CA	ta2-Re	ta-CP1	20-IR24	
	t-k59	-	5-M	-	kh-K3	7-M	-	zt-T	12-M	-	-	
	1	1	1	1	1	1	1	1	1	1	1	
Code number	2	2	2	-	2	2	-	2	2	2	2	
	4	-	4	-	4	4	-	4	4	-	-	
Scores	S	S	S	S	R	R	S	S	S	-	R	
	R	R	R	-	S	R	-	R	-	R	S	
	R	-	R	-	R	S	-	R	R	-	-	
Race number	1	1	1	1	2	4	1	1	1	0	2	

Where: R = Resistant; S = Susceptible; IRBL = International Rice Bred Line;
ML = Monogenic line; LTH = LIJIANGXINTUAN HEIGU

The six pathogenic races identified reveal the nature and structure of blast population available in Southern Highlands of Tanzania. With this information fighting against rice blast disease through the use of resistant varieties could probably be much easier as appropriate resistant genes to be incorporated into current cultivar have been identified in this study. Identification of functional blast resistance genes for a particular region is a prerequisite for their meaningful deployment (Sridhar *et al.*, 1999). Normally, a poor pre-release resistant variety when challenged by an adequate and unknown pathogen population may lead to breakdown of resiatance (Zeigler *et al.*, 1995).

The approach to monitor pathogen populations has tremendously been valuable in the development and deployment of host resistance (Andrivon and De Vallavieille-

Pope, 1993), and has provided important insights into the evolution of pathogen populations in response to selection by host resistance genes (Wolfe and McDermott, 1994). Information on pathogen virulence potential is essential in developing strategies to increase the durability of resistance through adequate characterization of suitable screening sites and appropriate deployment of resistant cultivars (Xia *et al.*, 2000). Most of the races identified were widely distributed in the study area covering more than 80 %, suggesting that the identified races were the main cause of increase in rice blast incidence and low yields. The information obtained from pathotype designation will assist in to build up the durable protection system for blast disease in southern highlands, through enhancement of communication among pathologists and breeders at the global level.

4.2 Resistance of Rice Cultivars to Blast

All selected local varieties were susceptible to the pathogenic races identified differing only on the levels of susceptibility (Table 8 a and b). Uyole inbred lines on the other hand, were susceptible to all pathogenic races except pathogenic race U11-i5-k000-z03-ta000 to which they were resistant and to pathogenic race Pathogenic race U11-i4-k041-z11-ta103 in which UR 400 was resistant. In terms of levels of cultivar resistance Uyole inbred lines perform better than local varieties. Despite being susceptible to at least one pathogenic race Uyole inbred lines had the lowest disease severity compared to the local varieties. This shows some resistance potential in these inbred lines.

Table 8a: Severity scores of selected local rice varieties and Uyole rice inbred lines against the six pathotypes of *Pyricularia grisea* identified in Southern Highlands of Tanzania.

Variety	Pathotypes					
	K1P	N1P	MP	K2P	N2P	NP
UR400	6.7^{a}	6.3^{a}	4.3^{a}	3.0^{a}	5.3^{a}	5.7^{a}
UR319	7.0^{a}	6.7^{a}	5.7^{ab}	4.3^{ab}	6.3^{a}	6.3^{a}
wahiwahi	7.3^{a}	7.3^{a}	7.0^{ab}	5.3^{ab}	7.0^{a}	6.7^{ab}
mwasungo	7.7^{a}	7.3^{a}	7.3^{ab}	5.7^{ab}	7.3^{a}	7.0^{ab}
zambia	7.7^{a}	7.7^{a}	7.7^{ab}	6.3^{b}	7.7^{a}	8.3^{bc}
kilombero	8.3^{a}	8.3^{a}	8.7^{b}	6.7^{b}	8.3^{a}	9.0^{c}
Mean	7.44	7.28	6.78	5.22	7.00	7.17
lsd	1.32	1.81	2.34	2.25	2.25	1.45
F test	***	***	***	***	***	***
Cv%	9.7	13.7	19	23.6	17.7	11.1

Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ according to the Student-Newman-Keuls test.

Where ***= highly significant different (P<0.001); KIP = Kikusya 1Pathotype; N1P = Ngana 1 Pathotype; MP = Mtowisa Pathotype; K2P = Kikusya 2 Pathotype; N2P; Ngana 2 Pathotype; NP = Namatuhi Pathotype.

Table 8b: Reaction of selected local rice varieties and Uyole rice inbred lines against the six pathotypes of *Pyricularia grisea* identified in Southern Highlands of Tanzania.

Variety	Pathotypes					
	K1P	N1P	MP	K2P	N2P	NP
UR400	S	S	MR	R	MS	MS
UR319	S	S	MS	MR	S	S
wahiwahi	S	S	S	MS	S	S
mwasungo	S	S	S	MS	S	S
zambia	S	S	S	S	S	HS
kilombero	HS	HS	HS	S	HS	HS

Where: R = Resistant; MR = Moderately Resistant; HR = Highly Resistant, S = Susceptible; MS = Moderately Susceptible; HS = Highly Susceptible

Among the selected local varieties 'Wahiwahi' had the lowest disease severity followed by 'Mwasungo' and 'Zambia'. 'Kilombero' on the other hand recorded the

highest disease severity of all the local varieties selected. Information of cultivar resistance and effective resistant genes obtained in this study will be useful in development and improvement of durable blast resistant varieties in Southern highlands.

The resistant genes identified can be incorporated into Uyole inbred lines and commonly grown local susceptible rice varieties to develop and come up with more durable resistant varieties for Southern highland rice farmers. In Colombia the combination of the blast resistance genes (*Pi-1, Pi-2, Pi-33*) has proven to confer stable blast resistance to local cultivars after several years of testing under high blast pressure in the field and greenhouse inoculations (Victoria and Martinez, 2009). Indeed, pyramided lines carrying the major *R* gene combinations *Pi1+Piz-5* and *Pi1+Piz-5+Pi-ta* were found to broaden the resistance spectrum of each individual gene in both India and the Philippines (Hittalmani, 2000). Similarly, the rice breeding line 'Jefferson', which carries *Pik* and *Piz* genes, has retained its resistance since its first use in 1997 (Fjellstrom, 2004). In short molecular markers tightly linked to resistance genes have facilitated to most extent their pyramiding into single elite cultivars, with the possibility of creating more durable or broad spectrum resistance (Saghai-Maroof *et al.*, 2008).

4.3 *Pyricularia grisea* Population Diversity in Southern Highlands of Tanzania

Blast resistance testing in the field measures the level of tested *R* gene in the face of the blast pathogen genetic diversity that develops in the testing field (Utami *et al.*, 2011). Results in Table 9 shows that 15 monogenic lines, four varieties (Tetep, Aichi a-Asahi, LTH and Nippon Bare) and a susceptible check SARO 5 were succumbed by races of *P. grisea* when exposed to the natural inoculums in the study area. On the other hand 14 monogenic lines and one variety (moroberekan) were resistant. The difference between resistance and susceptible varieties were clearly evident within 3 months (Plates 4).

Plate 4: Severity differences between a highly rice blast susceptible variety (left hand side) and a highly resistant variety (right hand side) in field trapping nursery.

Some of the susceptible varieties were completely wiped out indicating presence of very strong virulent races in the study area. This is similar to what have been reported by Scheuermann *et al.* (2012), where in the presence of favourable conditions, the lesions may enlarge and coalesce to kill the entire leaf and sometimes under severe conditions, the plant can be killed.

Table 9: Severity scores and reaction of monogenic lines and varieties to different *Pyricularia grisea* races in the field trapping nursery.

NILs/Varieties	*R* genes	Severity	Reaction Pattern
75-1-127	*Pi9*	0.16[a]	R
IRBL 19-A	*Pi19*	1.35[b]	R
IRBLZT-T	*Piz-t*	1.71[c]	R
IRBLT-K59	*Pit*	1.92[c]	R
IRBLTA CP 1'	*Pi9*	2.19[d]	R
IRBL 11-ZH	*Pi11*	2.33[e]	R
IRBLKP-K60	*Pik-p*	2.46[f]	R
IRBLKM TS	*Pik-m*	2.52[f]	R
Moroberekan	*Pi5(t),Pi7*	2.63[f]	R
IRBL 12-M	*Pi12*	3.00[g]	R
IRBL 7-M	*Pi7*	3.29[g]	R
IRBL-A	*Pia*	3.25[g]	R
C039	*Pia*	3.54[h]	R
IR 1529	*Pi33*	4.23[i]	R
IRBL 9-W	*Pi9*	4.35[j]	R
SARO 5		4.54[j]	S
C103 TTP	*Pi1b*	4.58[j]	S
St1	*Pif*	4.77[k]	S
IRBLKS-F5	*Pik-s*	4.92[k]	S
IRBLK KA	*Pik*	5.25[l]	S
IRBL 20-IR 24	*Pi20*	5.31[l]	S
Tetep	*Pikh+Pi-1+Pita+Pitp?*	5.79[m]	S
IRBL3-CP 4	*Pi3*	5.81[m]	S
IRBLI-F5	*Pii*	5.94[n]	S
Aichi a-Asahi	*Pia+Pi19*	6.00[n]	S
IRBBLKH-K3	*Pik-h*	6.04[o]	S
IRBLZ FU	*Piz*	6.08[o]	S
IRBLZ5-CA	*Piz-5*	6.10[o]	S
IRBLB-B	*Pib*	6.38[p]	S
LTH		6.59[p]	S
IRBL 1-CL	*Pi1*	7.00[q]	S
IRBLTA 2-PI	*Pita-2*	7.63[r]	S
NipponBare	*Pia+Pish*	7.94[s]	S
IRBL 5-M	*Pi5*	8.00[s]	S
IRBLSH-S	*Pish*	8.35[t]	S
Mean		4.63	
lsd		2.006	
F test		***	
CV%		30.9	

Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ according to the Student-Newman-Keuls test.

Where: ***= highly significant different (P<0.001); R = Resistant; S = Susceptible

The resistance of rice variety to blast disease is influenced by the genetic diversity of the pathogen population in the field (Utami *et al.*, 2011). The pathogen attack ability is heavily influenced by the virulence factors possessed by a certain race of the blast pathogen (McDonald and Linde, 2002) governed by interaction between the blast resistance gene (*R* gene) in a rice line with avirulence gene that is owned by the blast pathogen. Interaction of *O. sativa* and *P. grisea* which follows the classical gene-for-gene interaction where an *R* gene named as *Pyricularia (Pi)* is effective in preventing *P. grisea* races that contain the corresponding avirulence gene (Flor, 1971).

Sere *et al.* (2004) reported that the reaction of each variety with known resistance gene is an indication of the presence or absence of the corresponding avirulence gene without prejudging their association into distinct races. Results in Table 9 shows that, there is presence of 13 *P. grisea* avirulence genes and absence of the other 15 *P. grisea* avirulence genes which implies that 13 strains of *P. grisea* can be controlled by effective *R* genes while 15 strains still need to be monitored. Pathogen virulence monitoring is still used extensively in many pathosystems today and continues to provide timely information about the structure of pathogen populations that is relevant to breeding programs and resistance deployment (Odjo *et al.*, 2011).

Information on population diversity is therefore essential for developing strategies to increase the durability of resistance in the breeding process as explained by Xial *et al.* (2000). Effective *R* genes may be useful in preventing blast disease in the Southern Highland of Tanzania because the use of host resistance had already

proven to be the most effective and economical method for controlling rice blast (Wang *et al.*, 2010; RoyChowdhury *et al.*, 2012a). This can be achieved through pyramiding these *R* genes into susceptible rice varieties commonly cultivated by poor resource farmers in the Southern Highlands as demonstrated by Zeigler *et al.* (1995). With DNA markers closely linked to some of these major blast *R* genes being available (Ballini *et al.*, 2008; Lee *et al.*, 2009; RoyChowdhury *et al.*, 2012a), their incorporation into superior local rice cultivars can now be accelerated using marker-assisted selection.

CHAPTER FIVE

5.0 Conclusions and Recommendations

5.1 Conclusions

The current study to determine population structure of *pyricularia grisea* (cooke) sacc. and reaction of rice cultivars carried out in the Southern Highlands of Tanzania identified six pathogenic races (U11-i2-k141-z13-ta103, U51-i4-k100-z13-ta103, U11-i4-k041-z11-ta103, U11-i5-k000-z03-ta000, U11-i0-k101-z13-ta100, and U11-i1-k124-z11-ta102). Pathogenic race U11-i2-k141-z13-ta303 was the most virulent race infecting 44.4 % of the varieties tested, followed by U11-i4-k041-z11-ta103; U11-i1-k120-z11-ta102; U50-i4-k100-z13-ta103; U11-i0-k101-z13-ta100 and U11-i5-k000-z03-ta000 infecting 40.7 %, 37 %, 37 %, 33.3 % and 14.8 % of the tested varieties respectively. The pathogenic races identified provide the insight of blast population available in the Southern Highlands that breeders need to consider while breeding for resistant varieties.

In the study seven resistance genes *Pi12, Piz-t, Pi11, Pik-m, Pi12, Pia*, and *Pik-p* were found effective and can be used to protect rice against virulent prevalent races identified. Out of 27 *R genes* used in the study, 20 *R* genes *Pi1b, Pif, Pik-s, Pik, Pi20, Pi3, Pii, Pik-h, Piz, Pi19, Piz-5, Pib, Pit, Pi1, Pi7, Pita-2, Pi5, Pi9, Pi33* and *Pish* were not effective against the prevalent races in Southern Highlands. This shows that there is big pressure of rice blast disease in the study area that lack corresponding resistant gene.

The study also shows that all selected local rice varieties were susceptible to all pathogenic races identified differing only on the levels of susceptibility. Uyole inbred lines on the other hand, were susceptible to all pathogenic races except pathogenic race IJ11-i5-k000-z03-ta000 to which they were resistant and to pathogenic race Pathogenic race IJ11-i4-k041-z11-ta103 in which UR 400 was resistant.

With information of prevalent *P. grisea* races identified, cultivar reaction and effective resistant genes now on hand, development of improved durable rice blast resistant varieties in Southern Highlands is now possible. The effective resistant genes identified can now be incorporated into Uyole inbred lines and commonly grown local susceptible rice varieties in order to develop and come up with more durable resistant varieties for Southern Highland rice farmers.

5.2 Recommendations

(i) Breeding for rice blast resistance using identified effective *R* genes *Pi12, Piz-t, Pi11, Pik-m, Pi12, Pia,* and *Pik-p* is recommended for sustainable rice blast disease management in the Southern Highlands of Tanzania.

(ii) The study tested few selected local rice varieties and Uyole inbred rice lines, there is a need of screening for blast resistance of more local rice germplasm available in the Southern Highlands to find out their resistance status against rice blast disease in order to provide wide range of cultivars to be used in breeding programs.

(iii) The study have revealed effective *R* genes and prevalent pathogenic races in Southern Highlands using differential cultivars. another study using molecular tools is recommended. The use of molecular techniques is rapid and more efficient that involve many samples in a short period of time.

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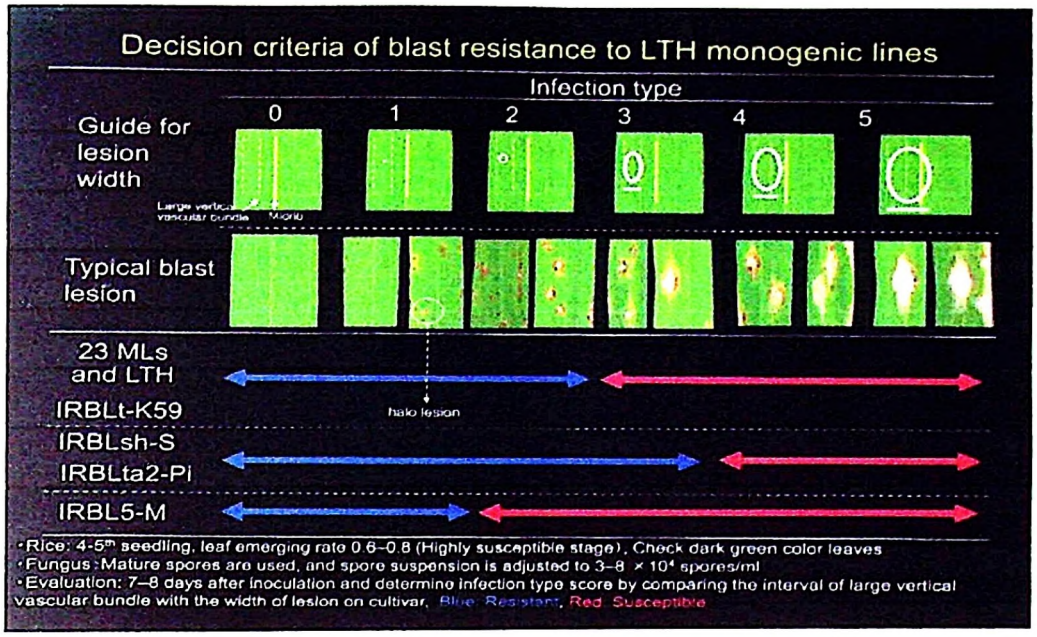
APPENDICES

Appendix 1: Identity of rice monogenic lines (MLs) and their corresponding resistant genes used in this study.

Var Number	Var Name	Resistance genes
V01	IRBL 1-CL	*Pi1*
V02	IRBL 11-ZH	*Pi11*
V03	IRBL 12-M	*Pi12*
V04	IRBL 19-A	*Pi19*
V05	C103 TTP	*Pi1b*
V06	IRBL 20-IR 24	*Pi20*
V07	IRBL3-CP 4	*Pi3*
V08	IR 1529	*Pi33*
V09	IRBL 5-M	*Pi5*
V10	Moroberekan	*Pi5(t),Pi7*
V11	IRBL 7-M	*Pi7*
V12	75-1-127	*Pi9*
V13	IRBL 9-W	*Pi9*
V14	C039	*Pia*
V15	IRBL-A	*Pia*
V16	Aichi a-Asahi	*Pia+Pi19*
V17	NipponBare	*Pia+Pish*
V18	IRBLB-B	*Pib*
V19	St1	*Pif*
V20	IRBLI-F5	*Pii*
V21	IRBLK KA	*Pik*
V22	IRBBLKH-K3	*Pik-h*
V23	Tetep	*Pikh+Pi-1+Pita+Pitp?*
V24	IRBLKM TS	*Pik-m*
V25	IRBLKP-K60	*Pik-p*
V26	IRBLKS-F5	*Pik-s*
V27	IRBLSH-s	*Pish*
V28	IRBLT-K59	*Pit*
V29	IRBLTA CP 1'	*Pita*
V30	IRBLTA 2-PI	*Pita-2*
V31	IRBLZ FU	*Piz*
V32	IRBLZT-T	*Piz-5*
V33	IRBLZT-T	*Piz-t*
V34	LIJIANG XINTUAN HEIGU	

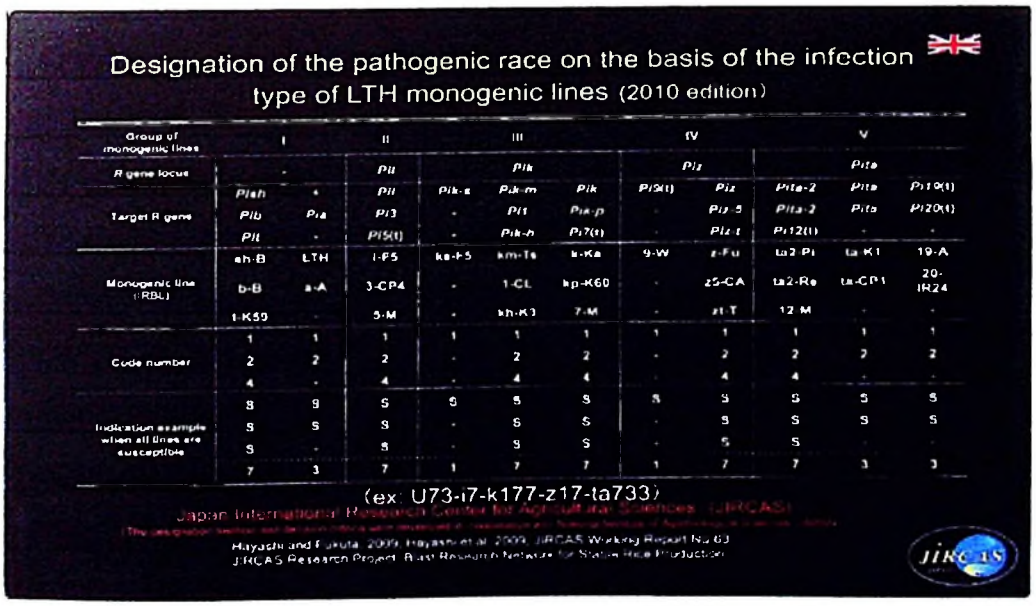
Source: Telebanco-Yanoria *et al.* (2010)

Appendix 2: Guidelines for disease assessment using decision criteria of blast resistance to Lijiangxintuanheigu monogenic lines



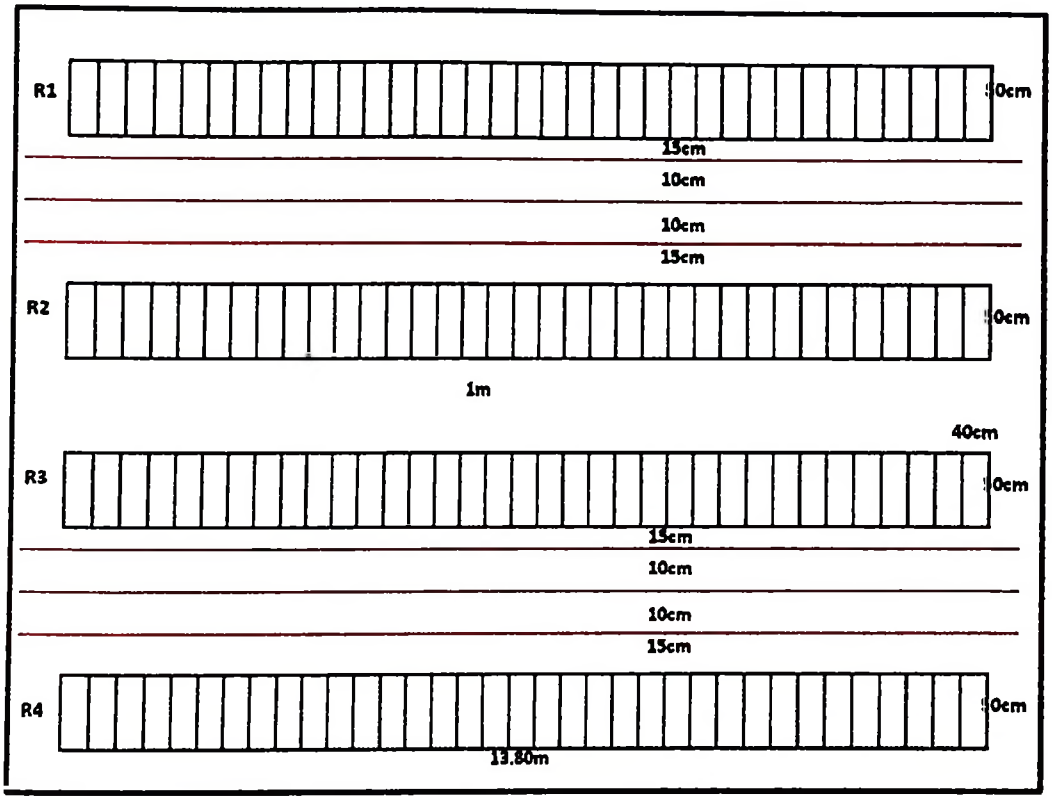
Source:Hayash and Fukuta (2009)

Appendix 3: Guideline for designation of the pathogenic race on the basis of infection type of LijiangXintuanheigu monogenic lines (2010 Edition)



Source: Havash and Fukuta (2009)

Appendix 4: Trapping nursery field layout



Appendix 5: Number of *Pyricularia grisea* isolates obtained from different regions of Southern Highlands.

Region	No. of Districts	No. of villages	No. of Isolates
Ruvuma	1	2	30
Rukwa	1	2	30
Mbeya	1	2	62
Total	**3**	**6**	**122**