OCCURRENCE OF RICE BLAST IN TWO AGRO-ECOLOGIES OF BURUNDI AND ASSESSMENT OF VARIETAL RESISTANCE AND DIVERSITY OF *Pyricularia oryzae* STRAINS

ESTELLA NIYONKURU

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

In Burundi, rice is a very important staple food, generating income for smallholder farmers and the business community. Unfortunally constrained by rice blast disease cause by fungus *Pyricularia oryzae*, which can cause up to 100% loss if not managed. The present study was conducted in middle and high altitudes agro-ecologies of Burundi with an overall objective to increase rice productivity by identifying resistant rice cultivars for management of the disease. Occurrence of blast disease on rice cultivars was caary out in a triplicated split plot experiment in a Randomized Complete Block Design (RCBD) in middle and high altitudes zones. Rice blast disease incidence and severity were significant different between locations (P =0.000). The high altitude agro ecological zone had the higher disease incidence (68.68%) and severity (77.53%), than the disease incidence (3.42%) and severity (20.74%) recorded in Middle altitude agro-ecological zone. When compared between rice growth stages, the disease occurrence and severity were significantly different ($p < 0.05$). In high altitude, rice blast incidence (74.12 - 90.89%) and severity (48.15 - 100%) were recorded at the booting stage and dough stage. In middle altitude, incidence of disease was recorded at tillering (2.49 - 9.67%), booting (3.07 - 9.83%) and dough (0.49 - 1.68%) stages and blast severity was observed at tillering (11.11 - 33.33%) and booting stage (11.11- 48.155%). The Area Under the Disease Progress Curve (AUDPC) showed that the disease progress in all cultivars increased exponentially from tillering to the dough stage, but at this dough stage the disease progress plateaued in some cultivars.

Another study was conducted in the both laboratories in IRRI-Burundi and Plant Molecular Biology at Sokoine University of Agriculture to establish genetic relatedness of rice blast (*Pyricularia oryzae*) isolates from two agro ecologies of Burundi. For the

thirty five (35) Pyricularia oryzae isolates for two AEZ, a set of five primers were used for molecular markers targeting the International Transcribed Spacer (ITS) and Translation Elongation Factor (EF) regions by using polymerase chain reaction (PCR) followed with Sanger sequencing. The results showed differences in banding patterns between isolates: ITS1 and 2R, ITS3F and 4R, ITS1F and 4R, ITS4F and 5R and EF1-983F and EF1-2218R showed bands size of 220bp, 350bp, 390bp, 550bp and 1235bp respectively. Phylogenetic analysis confirmed the narrow genetic diversity between *Pyricularia oryzae* isolates collected in high and middle altitudes regions of Burundi. Phylogenetic analysis showed that the isolates used from Burundi belong to *Pyricularia oryzae* and that the isolates vary considerably depending on the specific hosts. Some isolates were from hosts other than rice, *Ryegrass*, millet, wheat and *Graminis-tritici* for different locations.

A screen house experiment in a Complete Randomized Design (CRD) with three repetitions was conducted in IRRI-Burundi to evaluate 10 cultivars for resistance to a particular artificially inoculated *Pyricularia oryzae* isolates. The results indicated a significant difference between isolates and cultivars in incidence and severity. Of the ten rice cultivars, only three were resistant (R) (Mugwiza, Rufutamadeni and V18) to all isolates of *Pyricularia oryzae*.

Rice blast disease is distributed in middle and high altitudes agroecologies of Burundi. The study recommends more studies in different season to evaluate the incidence and severity of rice blast in relation to paddy yield and the genetic diversity of isolates for other locations in Burundi, not yet covered by the current study.

DECLARATION

I, **Estella Niyonkuru** do here by declare to the senate of the 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.

..…..… …….

Estella Niyonkuru Date

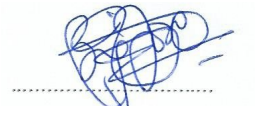
(MSc. Crop Science Candidate)

The above declaration is confirmed by;

..…..… …….

Dr. Madege Raphael Richard Date

(Supervisor)



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Dr Joseph Bigirimana Date

(Supervisor)

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DEDICATION

This dissertation is dedicated firstly to God, my beloved husband Jerome AHISHAKIYE; my children Geoffrey Aime AHISHAKIYE and Davina Olivia AHISHAKIYE and Egide NIYONKURU family.

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LIST OF ABREVIATIONS AND SYMBOLS

µl	Micro-litre
AEZ	Agro-ecological zone
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of variance

AUDPC	Area Under Disease Progress curve
bp	Base pair
CRD	Complete Randomized Design
CTAB	cetyltrimethylammonium bromide
DMRT	Duncan's multiple range test
DNA	Deoxyribonucleic Acid
EF	Elongation factor
FAO	Food and Agriculture Organization
HA	High Altitude
IBM- SPSS	Statistical Package for the Social Sciences
IGEBU	Institut Geographique du Burundi (Geographical Institute of Burundi)
IRRI	International Rice Research Institute
ISSR	Inter Simple Sequence Repeat
ITS	Internal transcribe spacer
MEGA	Molecular Evolutionary Genetics Analysis
MINEAGRIE	Ministère de l'Environnement, de l'Agriculture et de l'Elevage (Ministry of Environment, Agriculture and Livestock)
NA	Not applicable
PCR	Polymerase Chain Reaction
POKG	*Pyricularia oryzae* isolates from HA
PORG	*Pyricularia oryzae* isolates from MA
RAPD	Randomized Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design

RFLP	Restriction Fragment Lenght Polymorphism
SCAR	Sequence Characterized Amplified Region
SSA	Sub-Saharan Africa
SSR	Single Sequence Repeat
SUA	Sokoine University of Agriculture

CHAPTER ONE

1.0 INTRODUCTION, JUSTIFICATION AND OBJECTIVES

Cultivated rice belongs to *Poaceae* family and the genus *Oryzae* (Bajaj and Mohanty, 2005). There are many rice species of which two cultivated such as *Oryza sativa* L. of Asian origin and *Oryza glaberrima* Steud of Sgrican origin (Mgonja, 2016). Rice (O*ryza sativa* L.) is a cereal crop with its fruit rich in starch and the second most produced food grain in the world after wheat (Phadikar *et al*., 2012), with an estimated world production of 769 million tons of paddy (FAO, 2017). The role of rice on human life is obvious because more than the half of the world's population depends on it (Nalley *et al*., 2016); and provides 21% of energy and 15 % of proteins (Zibaee, 2013).

In Burundi, rice has been cultivated for the first time in lowland areas of Imbo plain to be distributed in the whole country (Nsanzineza, 2021). Nowdays, rice is a very popular and important staple food in Burundi generinting income for smallholder's farmers (Baramburiye *et al*., 2010). In Burundi, as elsewhere in Africa rice cultivation generates employment and household income especially the resource poor families (Dar *et al*., 2010, Ehiakpor *et al.*, 2017). Despite the importance of the crop, yet the production is still very low. For example, Ndayiragije *et al*. (2017) reported 2 t/ha of yield during the cropping seasons 2011/12 whereas it fells to 1.91t/ha in 2012/13. The low rice yield can be ascribed to a number of biotic (insects, Weeds, bacteria, fungi and viruses, etc.) and abiotic (salt stress, cold, etc.) constraints. However, diseases remaining a major factor causing yield loss and low profit in rice production despite the use of different pesticides and preventive agricultural practices (MINEAGRIE, 2016).

Among rice diseases, rice blast incited by *Pyriculariae oryzae* (Carava) is the most important due to its contribution in the reduction of yield. The disease is present in about 85 rice growing countries worldwide (TeBeest *et al*., 2012). It can cause yield loss ranging from 11 to 30% per year representing a loss of about 157 million tons of rice which can feed 60 million people worldwide (Nalley *et al.*, 2016). In Africa, rice blast is one of the major constraints of rice production (Talbot, 2003), causing yield loss of up to 100% (Séré *et al*., 2011). Rice blast disease development depends on the presence of the pathogen, the host and favorable conditions for its development (Cartwright *et al*., 2013). According to Shahriar *et al*. (2020), high relative humidity (above 80 %), low temperature (15ºC- 26ºC) are factors favorite to rice blast development. It can infect seeds and all the aerial tissues of rice plants (leaves, neck, nodes and panicles) at any growth stage of rice development resulting in improper filling of rice grains (Asibi *et al*., 2019).

In Burundi, blast is known to be a common rice disease in all agro-ecological zones (Nizigiyimana, 1986) and can cause yield losses of more than 10% (MINEAGRIE, 2016). To control rice blast disease, different methods are used such as timely sowing, proper use of nitrogen fertilizers, tillage, weed control and crop rotation. However, the use of resistant varieties is still the most effective approach for managing rice blast. In Burundi (Imbo plain), a recent study done on the evaluation of rice blast disease severity, indicated high variation in variety resistance depending on the locations and cropping seasons (Nsanzineza, 2021).

The resistance variability observed among resistant varieties is partly associed with variations within *P.oryzae* strains (Correa-Victoria and Zeigler, 1993) which, consequently lead to resistance degradation of rice varieties (Ballini *et al*., 2008). For

that matter, the genetic diversity of rice has great potential to identify rice genotypes with resistance traits to the disease (Asibi *et al*., 2019). In Burundi, apart from Imbo plain region having the information on rice blast disease, in other regions, updated information on rice blast disease occurrence, *P. oryzae* strains variability and resistance levels of commonly cultivated rice cultivars are still limited. Hence, this study aimed to bridge those gaps.

1.1 Justification

Burundi's economy is mainly based on agriculture with 90% of the active population and provides 95% of the food supply (Gahiro, 2013). The majority of farmers depend on subsistence farming (MINEAGRIE, 2018). The rice production under optimal conditions leads to a turnover of more than 10 t /ha whereas in under suboptimal conditions, the yield is normally less than 1 t /ha (Sattari *et al*., 2014). The national average of rice yield in Burundi is low (2.5 to 3 t / ha) compared to the potential rice yield of 5.5 to 8 t /ha (MINEAGRIE, 2016). The low yield is mainly associated to rice blast disease caused by a fungus, *Pyricularia oryza*e. The pathogen attacks rice crop from seedling stage to maturity (Nalley *et al*., 2016) and can cause up to 100% yield loss (Sharma *et al*., 2012).

In Burundi, blast disease caused yield loss of more than 10% (MINAGRIE, 2016). To control the disease, rice farmers in Burundi use different methods such as fungicides and agricultural practices. Unfortunately, due to environmental variability and continued applications of the pesticides, the pathogen can develop resistance especially when used at suboptimal dosage (MINAGRIE, 2018). Therefore, in an integrated pest management scheme, the use of resistant varieties is believed the most affordable and environmentally friendly approach in controlling diseases. Burundi is endowed with

diverse types of improved and local varieties of rice. Depending on environmental conditions and applied agricultural practices, the population of rice blast fungus may be viewed as an aggregate of clones or strains with particular spectrum of virulence on different rice cultivars. Evidence related assays to identify strains of rice blast pathogen in Burundi is currently limited and therefore remains potential research gap. In order to reverse the current rice blast disease problem in Burundi, evaluation of the susceptibility of various rice genotypes to the disease, will help in identifying rice cultivars with resistance to effectively control the blast disease. It is therefore justifiable that this research was proposed to bridge the identified research gaps.

1.2 Objectives

1.2.1 Overall objective

Increase rice productivity by identifying resistant rice cultivars for management of rice blast diseases in two agro-ecologies of Burundi.

1.2.2 Specific objectives

i. To determine rice blast disease occurrence on rice cultivars grown in high and middle altitudes agro ecologies of Burundi.
ii. To establish genetic relatedness of rice blast fungus (*Pyricularia oryzae*) isolates from middle and high altitudes agro ecologies of Burundi.
iii. To evaluate the pathogenicity of *Pyricularia oryzae* isolates obtained from cultivars grown in Middle and High altitudes zones of Burundi.

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CHAPTER TWO

Rice blast in East Africa; what do we know?

Estella, Niyonkuru[1]; Madege, Richard Raphael[1]; Joseph, Bigirimana[2,] and Georges, Habarugira[2]*

[1]Sokoine University of Agriculture, College of Agriculture, Department of Crop Science and Horticulture

[2]International Rice Research Institute-Burundi (IRRI-Burundi)

**Correspondent author, E-mail: estelleniyonkuru@yahoo.fr Tel: +257 61388208*

2.0 Introduction

Globally, rice is a crop of great importance, because it is the third most abundantly produced cereal after wheat and maize (Barthelemy *et al*., 2016). It provides 21% of the world's human energy per capita and 15% of protein (Abed-Ashtiani *et al*., 2012; Zibaee, 2013). Two species are recognized and domesticated: *Oryzae sativa* L. the most cultivated and the most productive of the world originating in South and East Asia and *Oryzae glaberrina* Steud. , Originating in West Africa (Lu, 1999; Fuller and Castillo, 2013). Rice can be grown in diverse climatic conditions, both in dry and wetland environments at high and low altitudes (Fahad *et al*., 2019). In both ecosystems, rice production is constrained by various biotic and abiotic stresses (MINEAGRIE, 2016). Rice blast caused by a fungus *Pyricularia oryzae* (Law *et al*., 2017), is the major constraints responsible for reduced rice yield globally, but especially in low income countries including those in the East African block. This article is a systematic review to elucidate what the research communities in East Africa have done in relation to rice blast.

2.1 Rice Blast Disease Development

Pyricuralia oryzae is a microscopic fungus, belonging to the class of Ascomycetes (Rossman *et al*., 1990) and rice blast is spread by asexual spores (conidia) (Asibii *et al*., 2019). Its vegetative apparatus consists of a thallus with partitioned hyphae (Chauhan *et al.*, 2017). The fungus infects any organ of the rice plant such as the leaf, neck, panicle rachis, stem node and seed (Correa-Victoria, 2001). Pathogen infection is initiated when the conidia are deposited on leaves of young seedlings, afterwards, these spores produce initial infections on young plants when they settle on the leaves, germinate and invade leaf tissue (Asibii *et al*., 2019). Infected seeds are a primary source of inoculum and dead infected seeds may serve as the primary inoculum when placed in the field during seedling development (Hubert *et al*., 2015). The pathogen spends seasons as mycelium and conidia on infected rice straw and seeds and possibly on weed hosts. The fungus produces and releases conidia during periods of high relative humidity (12hours or more) and temperature (24^{o}C) (Kato, 2001; TeBeest *et al*., 2012; Miah *et al*., 2017). When rice leaves or stem surfaces are wet, the conidia germinate and the germ tube produces an appressorium through which the fungus enters plant surfaces or penetrates through stomata (Wilson and Talbot, 2009; Miah *et al*., 2017). Conidia, dispersed by wind, can produce symptoms within 4-5 days of infection (Chauhan *et al*., 2017; Kato, 2001). A single leaf lesion can generate 20 000 conidia and an infected rice spikelet can produce up to 60 000 conidia overnight (Kato, 2001; Wilson and Talbot, 2009). Each reproductive cycle, from infection by conidia to the production of new conidia by the lesion formed lasts between 7 days (Figure 2.1) (Fetene, 2019).

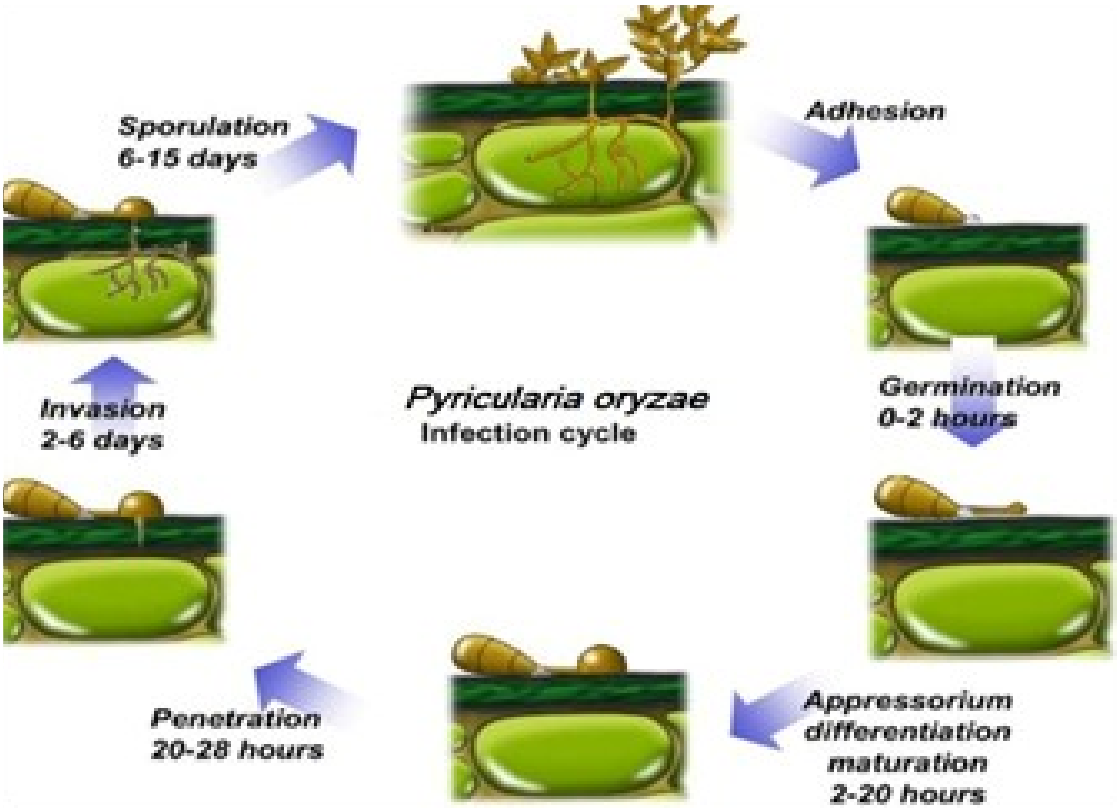

Figure 2.1: Life cycle of rice blast fungus (Roy-Barman and Chattoo, 2005)

2.2 Rice Blast Disease Symptoms

The blast fungus damages all above ground parts at different growth stages of rice. The pathogen produces lesions on leaves (leaf blast), leaf collars (collar blast) neck node and panicle (panicle Blast) (Singh *et al*., 2019). Initial symptoms appear as white to gray-green lesions or spots, with dark green borders, then few days later the lesions appear. On leaves, symptoms appear as grey-green and water-soaked with a darker green border on susceptible cultivars and older lesions often turn light beige in color with necrotic borders. On resistant cultivars, lesions often remain small (1-2 mm) and brown to dark brown in color (IRRI, 2014). On necks and panicles, lesions can be found on panicle branches, spikes and spikelets, which are often grey-brown discolorations of panicle branches and over time the branches may break off at of the lesion. On seeds, symptoms consist of brown spots and sometimes with the classic diamond-shaped lesion often seen on leaves (Tebeest *et al*., 2012) (Figure 2.2). Rice blast symptoms vary according to the

color and shape depending on varietal resistance, environmental conditions and growth stage of plants (Asibi *et al*., 2019).

Figure 2.2: Symptoms of blast disease on rice leaves (a), collar and neck (b), panicle (c) and grain (d) (Miah *et al*., 2017)

2.3 Origin and Distribution of Rice Blast Disease

The disease was first reported as "rice fever" in China by Soong Ying-shin in 1637 and later reported in Japan by Imochi-byo in 1704 and was first recorded in 1913 and as the first devastating epidemic disease in 1919, in the Tanjore delta of the former state of Madras in India (Singh, 1968). It is currently found in about 85 countries across the world (TeBeest *et al*., 2012).

In Africa, rice blast disease was first reported in Uganda in 1922 (Small, 1922), in Burundi appeared in 1986 on Yunnan 3 variety in fields of high elevation (Buyenzi region) (Niyonkuru, 2004), Kenya in 1924 and RDC in 1932 (Asumaya, 1965). In Tanazania, rice blast has been reported in all rice-growing areas according to Teri and Ali (1983), but the first report on entering of rice blast disease in Tanzania in unknown (Hashim, 2020). Rice blast caused by fungus *Magnoporthe oryzae* is one of the most important diseases leading to severe yield losses (Abed-Ashtiani *et al*., 2012), because of its yield loss potential leading up to 100% under favorable conditions (Babujee and Gnanamanickam, 2000; Piotti *et al*., 2005; Fetene, 2019).

2.3.1 Magnitude of rice blast disease in East Africa

Rice blast, caused by the fungus *Pyricularia oryzae*, is an important and serious disease of rice (*Oryza sativa* L.) worldwide and in Africa, which can cause yield losses of up to 100% (Séré *et al*., 2011). The disease has been reported to cause yield losses of up to 40% in Tanzania (Hubert *et al*., 2015), 10% in Burundi (MINEAGRIE, 2016) 48% in Kenya (Kiroho *et al*., 2013) and can be up to 100% in Uganda (Zewdu *et al*., 2017).

In Burundi, the total destruction on rice fields was observed in 1990 (Niyonkuru, 2004). In 1991, the blast destroyed Malgaches variety in Bugesera region (Nzeyimana, 2015). In 1988, the rice blast disease caused 5% of field incidence (Nizigiyimana, 1993), which can lead to a yield loss of more than 10% (MINEAGRIE, 2016). In addition, recent studies indicated a high variation in the severity score (7 - 9) of the disease on the most cultivated varieties in the Imbo plain (Nsanzineza, 2021).

In Tanzania, according the recent study conducted in 2012 and 2014 on the occurrence of rice blast, the results indicated a higher incidence (74.38%) and severity (87.62%) of

the disease in the rainfed ecosystem of Mbeya and Morogoro. While the lower disease incidence (19.38%) and severity (41.5%) was recorded in the irrigated ecosystem of the Kilimanjaro region (Chuwa, 2016). In addition, the same author reported equally that most of the ten rice varieties used in his study were susceptible to the disease and can cause grain yield losses ranging from 11.9 and 37.8% per hectare, which are close to the results found by Hubert *et al.* (2015). Another study conducted in the 2017and 2019 by Hashim (2020), indicated the higher blast disease severity of 100% and 98.8% in the respective districts Mvomero and Karogwe, than in Morogoro rural (88.1%) and Muheza (87.3%) districts.

In Uganda, the highest incidence of blast rice was recorded in rainfed lowland rice (72.18%), followed by irrigated lowland rice (59.53%) and upland rice. rainfall (47.27%), which is also the first report on the prevalence of blast in smallholder rice fields (Amayo *et al*., 2020). In Kenya, the rice blast incidence was ranked as the most destructive disease at 98%, according to farmers, with the main cause being excessive use of nitrogen fertilizer occupying 58% compared to other inputs (Kiroho *et al*., 2013).

2.3.2 Favourable conditions of rice blast disease development

In rice cultivation, high humidity ranging from 85-89 % and temperatures ranging from 12-32^{0}C have been reported as favorable conditions for rice blast disease development (Greer and Webster, 2001, Bundo and Coca, 2016; Nalley *et al*., 2016). In addition, cloudy weather, longer duration of dew, infected rice residues (Bundo and Coca, 2016; Raveloson *et al*., 2018) and infested seeds left on the soil surface for more than several weeks after planting, well after seedling emergence (Teebest *et al*., 2012) can trigger an outbreak of the blast disease.

The results reported by Chuwa (2016) in some areas in Tanzania during 2012/2014, showed that the climatic conditions have a strong influence on the development of blast because it varied according to the ecological zones (irrigated and rainy region): Case Mbeya region which had a maximum temperature of 26.7ºC, a minimum temperature of 15ºC, a relative humidity of 90.5% and rainfall of 93.2mm during the two seasons. Another study made by Hashim (2020), showed that the high disease severity was influenced by genotypes, rice growing season and changes in weather condition. During the study, the rainfall in Morogoro and Mvomero was varied from 532.9 mm to 486.5 mm and Muheza 738.0 mm to 578.9 mm; high relative humidity in Morogoro and Mvomero between 88% to 83% and Muheza 83% to 80%. In Kenya, results reported by Kiroho *et al*. (2013) showed that farmers attributed the blast disease to various causes, including climate change.

A study carried out in the high and middle altitude regions of Burundi indicated that climatic conditions have an influence on the development of the blast disease. The results showed a higher occurrence of the disease in the high altitude region compared to the middle altitude region. Those regions had a minimum temperature of 23.6°C and a relative humidity of 75.1% and a minimum temperature of 28.4^0C and a relative humidity of 73.2% respectively during the study.

2.4 Rice Blast Disease Management

The methods used to manage rice blast disease are cultural, chemical, forecasting and biological methods (Gladieux *et al*., 2018, Hashim, 2020) in the context of an integrated disease management scheme.

2.4.1 Chemical method

Chemicals are used to directly control blast. Fungicides such as Carbendazim, edifenphos, triadimefon and tricyclazole are recommended for control of blast disease (Tuli *et al*., 2017). Soil amendment with silicon significantly reduces the severity and incidence of blast (Seebold *et al*., 2004). Foliar spraying of isoprothiolane at 1.5 ml / l, followed by carpropamid and carbendazim (Varma and Santhakumari, 2012). Disease incidence and severity decreased by 78.3% and 89.7% for isoprothiolane, followed by carpropamide by 67.5 and 80.5% and carbendazim by 56.9 and 73.1% disease incidence and intensity (Varma and Santhakumari, 2012).

In most cases, the use of pesticides leads to varying degrees of residues in crops and foods and sometimes affects human health and the environment. Carbendazim is classified as a hazardous chemical by the World Health Organization and on the priority list of endocrine disrupting chemicals (Li *et al*., 2020). Research has shown that carbendazim is hardly degradable and its use with excess or overdose leads to residues of this product in agriculture (Dong *et al*., 2018), with its negative impacts on the environment and health such as developmental disorders and reproduction, toxicity and mutagenicity (Da costa *et al*., 2019).

Little information has been found on carpropamid, but it is a poorly water-soluble, volatile, and non-mobile fungicide. It is not very toxic to mammals, but shows a moderate level of toxicity to birds, fish and earthworms. To mitigate some negative environmental impacts of synthetic pesticides, a trend to use natural plant extracts to protect rice against pests and diseases and has been suggested (Mohammed *et al.*, 2019).

2.4.2 Cultural methods

The cultural method involves the combination of different practices such as (1) Crop rotation which is a technique that provides a mechanism that separates viable spores in crop residue from emerging seedlings. (2) Proper fertilizer application, since the overuse of nitrogen fertilizer increases the amount of blast in the fields (TeBeest *et al*., 2012). (3) Use of high quality, disease-free seed, as infested seed left on the soil surface provide an inoculum from which outbreaks develop. (4) Genetic resistance, which many rice cultivars contain genes that confer resistance to one or more of the individual races fungus islands found in the region (Ahn and Mukelar, 1986; Yan *et al*., 2017).

2.4.3 Biological methods

Trichoderma spp. and Bacillus isolated from rice phylloplane have been used as an antagonist biological control agent for many plant fungal diseases (Nascimento *et al*., 2016; Hashim, 2020). *Trichoderma sp.* is a saprophytic fungus (Ali and Nadarajah, 2014), producing substrate-degrading lysing enzymes and high resistance to microbial inhibitors (Strange, 1993). *Bacillus spp.* possesses antagonistic mechanisms of antifungal production and can suppress fungal diseases (Araujo *et al*., 2005). *In vivo* experiments done by Hashim (2020) indicated that, the incidence and severity of rice blast was reduced by 70% and 35, 6%, respectively, in plants treated with *T. asperellum*. While in plants treated with *B. subtilis*, a decrease in the incidence (51, 5%) and severity (29, 1%) of blast was recorded. *In vitro,* the results showed an inhibition of more than 75% of the radial growth of *P.oryzae* by treating the rice plants with *Trichoderma asperellum* and *Bacillus subtilis* (Hashim, 2020).

2.4.4 Forecasting method

The forecasting system can helps to predict the likely epidemic or disease intensity and its management by reducing the use of chemical practice and to provide an accurate forecast before crop losses **(**Rijal and Devkota, 2020).

2.5 Genetic Diversity of *Pyricularia oryzae* Strains in East Africa

The use of resistant cultivars is the most effective and economical approach of managing the disease (Zhou *et al*., 2007; Fukuta *et al.*, 2014 and Khan *et al*., 2016). The knowledge of the mechanisms behind outbreak of rice blast diseases and its association with the diversity of the blast pathogen are important (Khan *et al*., 2016) for developing durable disease management system. *Pyricularia oryzae* isolates collected from Morogoro, Shinyanga, Mbeya and Kilimanjaro in Tanzania showed genetic similarity (Chuwa, 2016). A study done on the genetic diversity of *Pyricularia oryzae* populations from Sub Saharan Africa (SSA) indicated that 42 strains from Burundi, Kenya, Rwanda, Tanzania, Uganda, Benin, Burkina Faso, Ghana, Mali, Nigeria and Togo are more diverse and harbor all the genetic groups reported in Asia, but had significant differences in substructure (Onanga *et al*., 2020).

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CHAPTER THREE

Rice blast disease occurrence on rice cultivars grown in high and middle altitudes agro ecologies of Burundi

Estella, Niyonkuru[1]; Madege, Richard Raphael[1]; Joseph, Bigirimana[2], and Georges, Habarugira[2]*

[1]Sokoine University of Agriculture, College of Agriculture, Department of Crop Science and Horticulture

[2]International Rice Research Institute-Burundi (IRRI-Burundi)

**Correspondent author, E-mail: estelleniyonkuru@yahoo.fr Tel: +257 61388208*

Abstract

Rice blast caused by *Pyricularia oryzae* is the major damaging disease in nearly all rice-growing nations and causes low rice yield. In a triplicated Randomized Complete Block Design (RCBD) experiment implemented in high altitude (HA) and middle altitude (MA) agro-ecologies, where rice blast disease incidence and severity were recorded and analyzed. High significant difference between agro-ecologies ($p = 0.000$) were observed in rice blast disease incidence and severity. High altitude region had the higher disease incidence (68.68%) and severity (77.53%), than the disease incidence (3.42%) and severity (20.74%) recorded in Middle region. No significant differences were recorded for effect cultivars.

Similarly, no significant differences were observed between the location and cultivated varietiy of some variables, except for leaf blast incidence ($p = 0.024$) and severity ($p = 0.030$) at the booting stage. Rice blast disease incidence and severity on cultivars were statistically significantly different ($p < 0.05$) at different growth stages, except for

the severity at the dough stage in the Middle region. The incidence varied from 2.49 to 9.67% and the severity from 11.11 to 33.33% at the tillering stage; varied from 3.07 to 9.83% for incidence and severity from 11.11 to 48.155% at the booting stage and incidence varied from 0.49 to 1.68% the dough stage. In high altitude, rice blast incidence (74.12 - 90.89%) and severity (48.15-100%) were statistically significant respectively at the booting stage and dough stage. The Area under the Disease Progress Curve (AUDPC) showed that the disease progress in all cultivars increased exponentially from tillering to the booting stage, but at the dough stage the disease progress in some cultivars plateaued.

3.1 Introduction

Rice (*Oryza sativa* L.) is the main staple food for more than a half of the world's population (Koide *et al*., 2009). Rice crop provides 20 % of world's dietary energy supply and considered as a source of income for millions of small-holder farmers (Onanga *et al*., 2020). Asian countries produce most of the world's supply whereas in Africa continent, rice demand exceeds its production (Nasrin *et al*., 2015). In sub-Saharan Africa (SSA), the average rice yield remains relatively low (2.2 t / ha) compared to 3.4 t / ha global average (Norman and Kebe, 2006 cited in Onanga *et al*., 2020). This low yield is mainly justified by a series of biotic and abiotic diseases impacting rice production. Among those diseases, rice blast, caused by an ascomycete fungus *Pyricularia oryzae* (telomorph *Magnaporthe oryzae*; synonym *Magnaporthe grisea*) is the most devastating disease (Khan *et al*., 2016).

Pyricularia oryzae is a pathogen originated from Southeast Asia (Saleh *et al*., 2014) and has been distributed in the entire world mainly through the introduction of contaminated new genotypes and seed exchange (Ballini *et al*., 2008; McDonald and Linde, 2002). In

West Africa and the largest area of African production, this pathogen is key constraint for production because of yield losses varying from 3 to 77% (Shahriar *et al*., 2020). Rice blast disease is a seedborne disease (Hubert *et al*., 2015) infecting rice crop from seedling to maturity and huge losses can be observed if it attacks seedling in seedbed and early neck blast (Fetene, 2019).

Rice blast disease symptoms are identified as lesions in the infected area in a circular or diamond shaped form or spindle-shaped dark spots with grey or white centers and brown (IRRI, 2014). The disease attacks all above ground parts of rice (leaves, neck, nodes and panicles) and damages caused by the disease differs depending on the infected parts (Zhu *et al*., 2005 and Asibi *et al*., 2019). For that reason, depending of the infected parts, the disease can be called: leaf, blast, node blast and panicle blast (TeBeest *et al*., 2012). The pathogen inflicts serious damage in case of leaf blast and panicle blast (Seebold *et al*., 2004, Gandalera *et al*., 2013). However, panicle blast is directly linked to rice production by reducing the quality and results in partially filled or unfilled grains (Grill *et al*., 1982’ Simkhada and Thapa, 2021).

Pyricularia oryzae fungus is characterized by high genetic mutation allowing it to develop adaptive traits that improve their propagation in new environments (McDonald and Linde, 2002). Despite efforts by rice farmers to use good cultural practices, rice blast is still one of the most devastating fungal diseases of rice fields (Miah *et al*., 2013; Asibi *et al*., 2019), losses caused by rice blast disease can lead 70 to 80 % of annual rice yield (Nasruddin and Amin, 2013; Rijal and Devkota, 2020). In Burundi, rice blast disease was first reported in 1986 on the variety Yunnan 3 in Gisha Marshland in Ngozi Province (Ndikuryayo, 2015). In 1988 the disease destroyed malagashes rice cultivars at Kobero in Muyinga Province (Nzeyimana, 2015).

Rice farmers know the disease and use all the various control methods at their disposal, but no added value. In addition, there is no updated information on the incidence and severity of rice blast disease under field conditions in some areas of Burundi including high (Buyenzi region) and middle (Mosso region) altitudes zones. That's why the present study is proposed for determining the incidence and severity on rice cultivars commonly grown by rice cultivars in the Buyenzi and Mosso regions. The results will allow researchers to make decisions on appropriate approaches for management of rice blast disease.

3.2 Materials and Methods

3.2.1 Study area

The study was carried out in two agro-ecologies: High altitude (HA) in Buyenzi and Middle altitude (MA) in Mosso regions. Buyenzi region is located at an altitude of 1824m over the sea level, annual mean temperature ranging from 16 to 20°C and annual mean rainfall varying from 1200 -1500 mm. Mosso regions is located at altitude of 1260m. Over the sea level, annual temperature is between 17-25°C and average annual rainfall is 1100 -1500 mm. For each site, two seasons can be distinguished in terms of rice cultivation such as dry season from June to November and wet season from December to May. Table 3.1 show temperature, precipitation and relative humidity when the current experiment was conducted.

Table 3.1: Monthly temperature, precipitation and relative humidity at High (Buyenzi region) and Middle (Mosso region) altitudes zones

Month	Middle altitude					High altitude				
	Temperature		Mean	Precipitation (mm)	Relative humidity (%)	Temperature		Mean	Precipitation (mm)	Relative humidity (%)
	Min.	Max.				Min.	Max.			
January	28	16.3	22.1	231.6	77.7	24.8	14.5	19.7	189.5	79.6
February	28.3	16.6	22.4	164.7	77.7	25.5	14.6	20	135.5	79.3
March	28.8	15.7	22.2	184.6	74.5	25.2	14	19.6	152.4	77.8
April	28.1	16.3	22.2	159.1	77.8	24.4	14.1	19.2	244.8	82.2
May	28.2	15.3	21.8	82	75.7	14.5	14.5	14.5	132.6	79.2
June	29	13.2	21.1	0	66.5	25.7	13.2	19.5	1.7	68.5
July	28.5	12	20.2	0	62.7	25.3	12.8	19.1	0	59.4
Average	**28.4**	**15.1**	**21.7**	**117.4**	**73.2**	**23.6**	**14.0**	**18.8**	**122.4**	**75.1**

Source: IGEBU (2021)

3.2.2 Experimental materials

Rice cultivars used were composed of 10 rice cultivars described in below Table 3.2.

Table 3.2: Description of rice cultivars used for evaluation of rice blast disease in field

S/ No	Cultivar	Local name	Growing region	Date of release
1	IR77713-30-1-1-3	Vuninzara	MA	20111
2	IR79511-47-2-6-5	Gwizumwimbu	MA	2011
3	IR91028-115-2-2-2-1	Mugwiza	MA	2016
4	V46	Kigori	HA	1997
5	Landrace	Watt	HA	NA
6	V564-2-7	Kabuye (Rubabi)	HA	2002
7	V18	Umuzambiya	HA	NA
8	Landrace	Karundi	HA	NA
9	Landrace	Buname	HA	NA
10	Landrace	Rufutamadeni	HA	NA

NA: Not Applicable, HA: High Altitude, MA: Middle Altitude

Those cultivars have been chosen in the two agro-ecological zones because they are commonly grown by majority smallholder farmers. Cultivars like Mugwiza, Gwizumwimbu and Vuninzara have been chosen in the middle altitude whereas Buname, Karundi, Watt, Kigori, Rufutamadeni, V18 and Kabuye have been chosen in the high altitude.

3.2.3 Experimental design

The experiment was carried out at HA and MA regions using 10 cultivars often used by rice farmers (described in Table 3.1). The experiment was laid in a Randomized Complete Block Design (RCBD). Each variety was planted in a plot of 11.2 m^2 and the spacing between plants was 20 cm x 20 cm. The distance between plots was 1m and 2m

between plots. Each block was 25, m long and 5.6m wide. The total experimental area was 142,8m^2 for each block. Nurseries were established in January 2021 and transplanting was done in February 2021, three weeks after seeding.

3.2.4 Assessment of rice blast disease incidence and severity

Rice blast disease incidence and severity were determined from experimental trials set in HA and MA agro-ecological zones. For each location, rice blast disease incidence was recorded on leaves and panicles using the formula described by Hajano *et al*. (2011) whereas disease severity was done referring to the standard evaluation system (SES) for rice as described by IRRI (2014). Thereafter, disease severity was calculated using the formula described by Ghazanfar *et al*. (2009).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants X 100}}{\text{Total number of plants assessed}} \quad \text{…… (Equation 3.1)}$$

$$\text{Disease severity (\%)} = \frac{\text{Average of disease score}}{\text{Maximum numerical value scale}} x100 \quad \text{…. (Equation 3.2)}$$

Leaf blast was recorded twice at tilling stage and at booting stage so that panicle blast was assessed when plants were at dough stage.

$$\text{Disease Severity Index (\%)} \quad \varepsilon \frac{nxv}{N \times V} 100 \quad \text{(Salimah } \textit{et al}\text{. (2019)…. (Equation 3.3)}$$

Where, n: Number of leaves / panicles with disease symptoms

v: Score of affected leaves / panicles

N: Total number of plants observed

V: Maximum numerical value of symptoms

The Area Under Disease Progress Curve (AUDPC) was calculated as described by Mohapatra *et al*. (2008) and Pasha *et al*. (2013).

AUDPC= $[(0.5)\ (Y_{i+1}+Yi)\ (T_{i+1}-Ti)]$……...……. (Equation 3.4)

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

Table 3.3: Standard Evaluation System of rice blast disease on leaf

Code	Description of types of lesion
0	No lesions observed
1	Small, brown, specks of pinpoint size or larger brown specks without sporulation center
3	Small, roundish to slightly elongated, necrotic, sporulation spots, about 1-2 mm in diameter with a distinct brown margin or yellow halo;
5	Narrow or slightly elliptical lesions,1-2mm in breadth, more than 3mm long with a brown margin
7	Broad spindle-shaped lesion with yellow, brown or purple margin;
9	Rapidly coalescing small, whitish, grayish, or bluish lesions without distinct margins

Source: IRRI (2014)

Table 3.4: Standard Evaluation System of rice blast disease on panicle

Code	Description of types of lesion
0	No visible lesions or observed lesions on only a few pedicels
1	Lesions on several pedicels or secondary branches
3	Lesions on few primary branches or the middle part of panicle axis
5	Lesions partially around the base(node) or the uppermost internode or the lower part of panicle axis near the base
7	Lesions completely around panicle base or uppermost internode or panicle axis near base with more than 30% of filled grains
9	Lesions completely around panicle base or uppermost internode or the panicle axis near the base with less than 30% of filled grains

Source: IRRI (2014).

3.3 Data Analysis

Data analysis on rice blast disease incidence and severity were subjected to the analysis of variance (ANOVA), using SPSS (Statistical package for social sciences) (IBM-SPSS version 21). Statistical model: $Yijk = \mu + \alpha i + \eta k\ i + \beta j + \alpha\beta\ ij + \varepsilon k$ Where, μ: Grand mean, αi: mean effect of location, $\eta k\ i$: Error plot, βj: Mean effect of cultivars, $\alpha\beta\ ij$: Interaction between Location and cultivars, $\varepsilon k\ i$: Error split plot. Comparison of means for rice blast disease incidence and severity were performed using Duncan's Multiple Range Test (DMRT) at 5% confidence level. Rice blast disease incidence and severity were the dependent variables and location and Cultivars the independent variables.

3.4 Results

3.4.1 Rice blast disease incidence and severity

Rice blast disease incidence and severity are presented in Table 3.5. Table 3.5 shows that difference between location was statistically significant (p = 0.000). Differences due to variety were not significant (p > 0.05). Similarly, difference due to interaction between location and variety were not significant except for leaf blast incidence (p = 0.024) and severity (p = 0.030) at booting stage.

Table 3. 5: ANOVA for incidence and severity of rice blast disease at different growth stage in two agro-ecologies of Burundi

Growth Stage	Source	Incidence		Severity	
		Computed F	p.Value	Computed F	p.Value
Tillering					
	Location	106.963	0.000	106.963	0.000
	Cultivar	0.239	0.986	0.239	0.986
	Location* Cultivar	1.037	0.429	10.037	0.429

Booting	Location	2673.630	0.000	160.026	0.000
	Cultivar	0.914	0.523	0.538	0.838
	Location*Cultivar	2.470	0.024	20.362	0.030
Dough	Location	50.948	0.000	102.273	0.000
	Cultivar	0.708	0.698	1.295	0.270
	Location*Cultivar	0.725	0.683	1.182	0.333

The results indicated that severe leaves and panicles rice blast disease was recorded in high altitude while it was low in middle altitude (Figure 3.1).

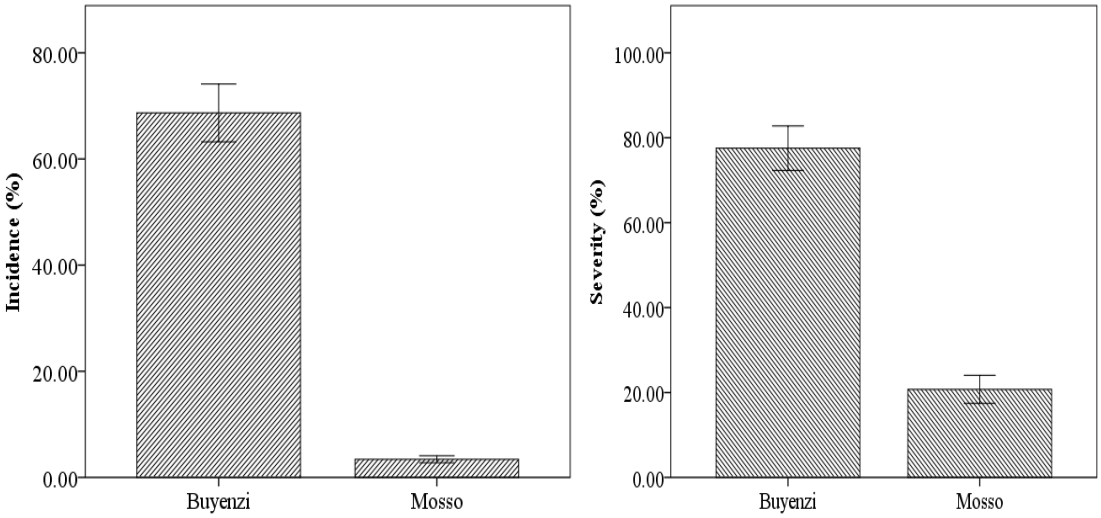


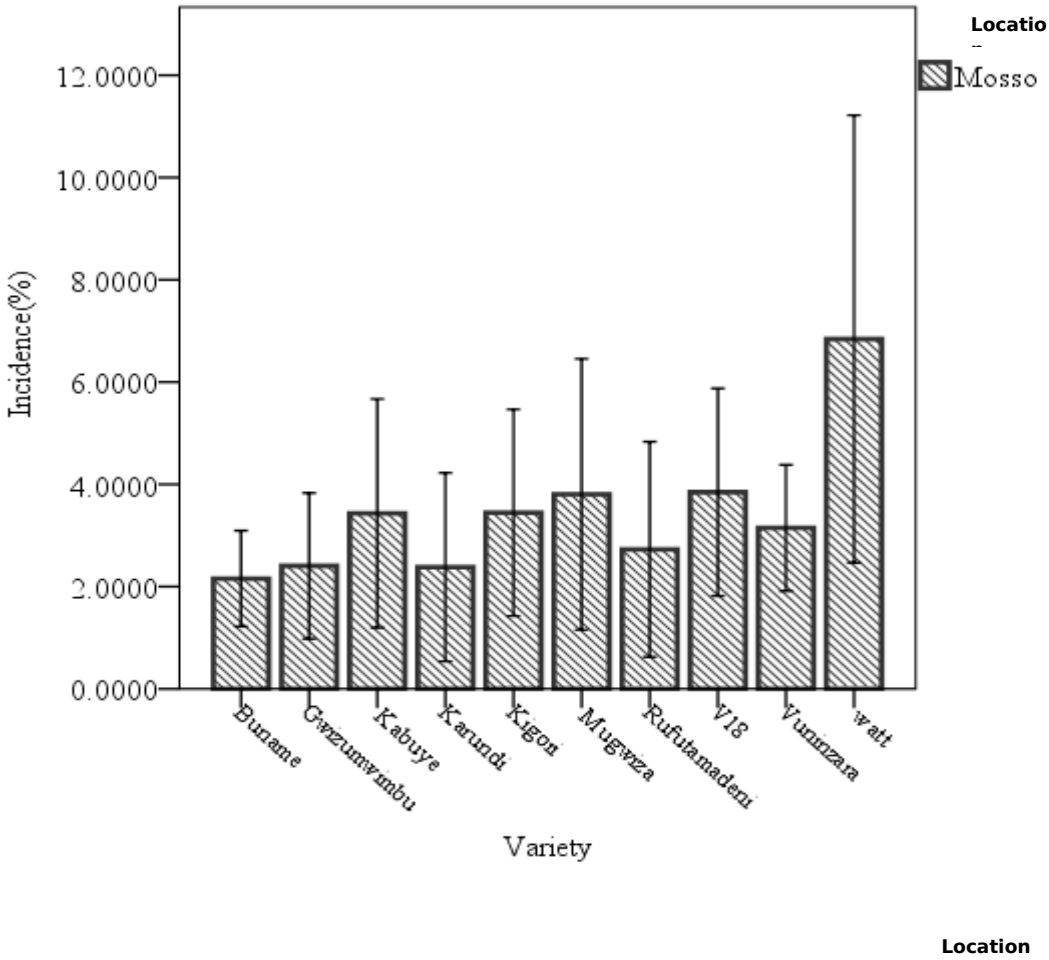
Figure 3.1: Incidence and severity of rice blast disease in two agroecologies of Burundi (high altitude =Buyenzi region and middle altitude = Mosso region)

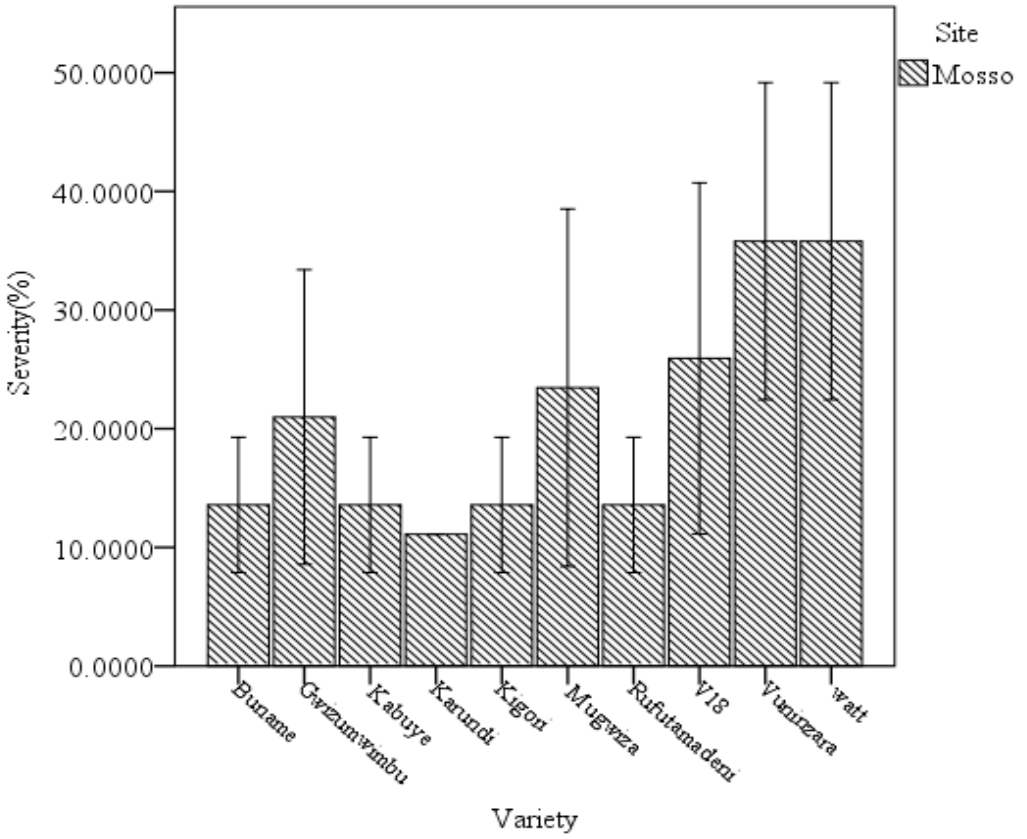
The highest rice blast incidence (68.68%) was observed in HA, while the lowest incidence (3.42%) was registered. Highest rice blast disease severity (77.53%) was recorded in HA AEZ, while it was low (20.74%) in MA.

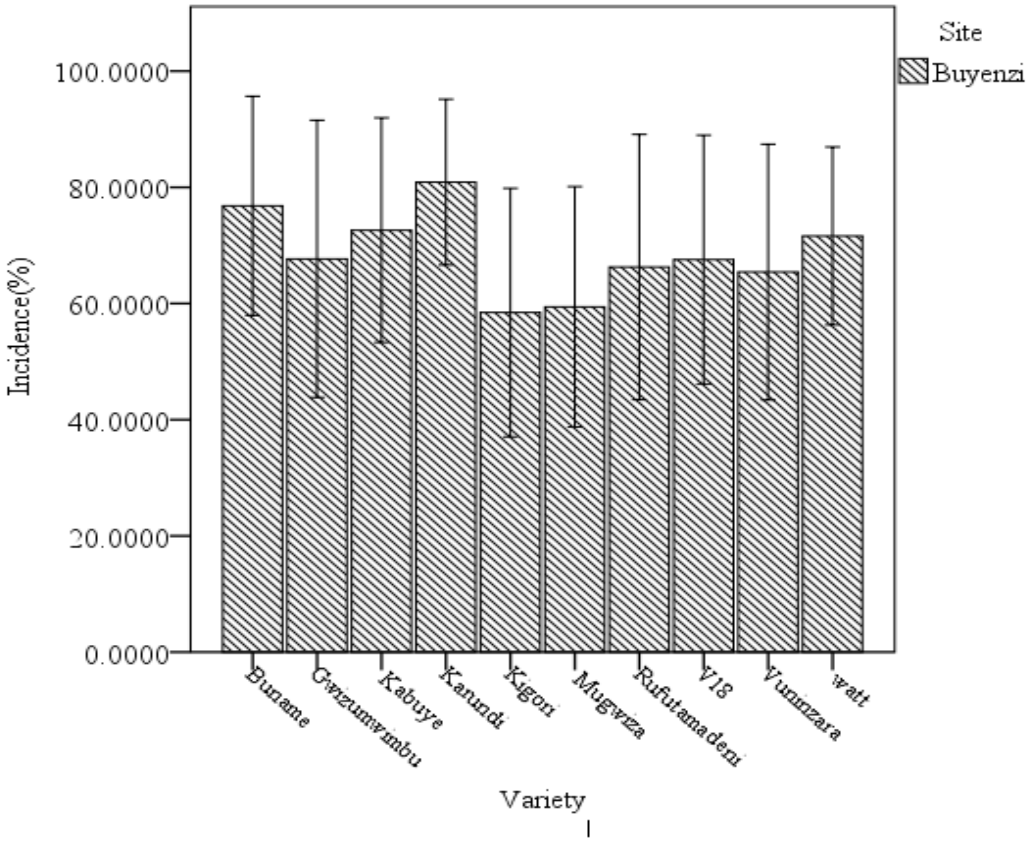
3.4.2 Rice blast disease incidence and severity of ten rice cultivars

A high incidence of rice blast was observed on the Karundi variety (80.98%) in HA, while a low incidence of rice blast disease was observed on the Watt variety (6.84%) in

MA (Figure 3.2). Similarly, a high rice blast disease severity was recorded on Kabuye variety (92.59%) in HA. The cultivars Watt (34.1%) and Vuninzara (34.5%) had low rice blast disease severity in MA (Figure 3.2).


Locatio
Mosso
12.0000
10.0000
8.0000
6.0000
4.0000
2.0000
0.0000
Incidence(%)
Bunane
Gwizumwimbu
Kabuye
Karundi
Kigon
Mugwiza
Rufutamadem
V18
Vumuzara
Watt
Variety
Location


Site
Mosso
50.0000
40.0000
30.0000
20.0000
10.0000
0.0000
Severity(%)
Buname
Gwizumwimbu
Kabuye
Karundi
Kigoi
Mugwiza
Rufutamaderi
V18
Vumizara
watt
Variety


Site
Buyenzi
100.0000
80.0000
60.0000
40.0000
20.0000
0.0000
Incidence(%)
Buname
Gwizumwimbu
Kabuye
Karundi
Kigoi
Mugwiza
Rufutamaderi
V18
Vumizara
watt
Variety

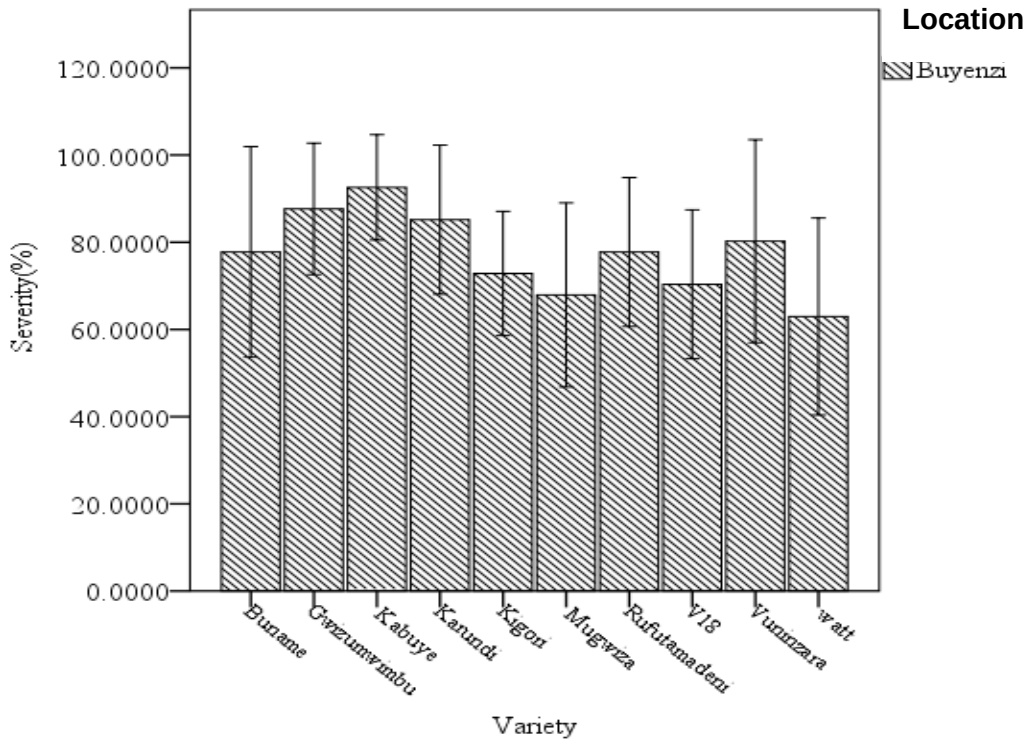


Figure 3.2: Incidence and severity of rice blast disease among the ten cultivars for each location; Mosso and Buyenzi represent MA and HA respectively

3.4.3 Rice blast disease incidence and severity in rice cultivars grown in MA and HA agro ecologies

The incidence and severity of rice blast disease on 10 cultivars at tillering, booting and dough stages in two agro ecologies of Burundi are present in Table 3.6.

Table 3.6: Incidence and severity of rice cultivars at different growth stage in MA and HA agro-ecologies

Varieties	Middle altitude (Mosso region)						High altitude (Buyenzi region)					
	Tillering stage		Booting stage		Dough stage		Tillering stage		Booting stage		Dough stage	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	severity
Vuninzara	3.48a	33.33ab	4.27a	48.15b	1.68b	25.93a	75.48a	77.78a	87.65ab	85.19a	33.09a	77.78ab
Gwizumwimbu	2.49a	18.52ab	3.35a	25.93ab	1.36ab	18.52a	75.23a	77.78a	90.82b	85.19a	37.00a	100.00b
Mugwiza	4.88a	25.93ab	6.86ab	33.33ab	0.66ab	11.11a	72.22a	77.78a	78.12ab	77.78a	27.95a	48.15a
Kigori	4.58a	11.11a	5.08ab	18.52ab	0.66ab	11.11a	70.57a	70.37a	76.46ab	77.78a	28.33a	70.37ab
watt	9.67b	30.74b	9.83b	48.15b	1.01ab	18.52a	71.11a	62.96a	74.12a	62.96a	69.65a	62.96ab
Kabuye	4.48a	11.11a	5.14ab	18.52ab	0.66ab	11.11a	81.02a	85.19a	88.81ab	92.59a	48.01a	100.00b
V18	4.65a	33.33ab	6.22ab	33.33ab	0.66ab	11.11a	77.69a	70.37a	82.63ab	85.19a	42.2a	55.56a
Karundi	2.77a	11.11a	3.88a	11.11a	0.49a	11.11a	79.92a	85.19a	86.33ab	92.95a	76.44a	77.78ab
Buname	2.54a	11.11a	3.07a	11.11a	0.85ab	18.52a	79.48a	85.19a	90.05b	100.00a	60.92a	48.15a
Rufutamadeni	3.09a	11.11a	4.18a	11.11a	0.90ab	18.52a	75.23a	77.78a	82.35ab	85.19a	41.42a	100.0 b
Mean	**4.17**	**20.74**	**5.09**	**25.93**	**0.9**	**15.56**	**75.8**	**77.04**	**83.73**	**84.44**	**46.5**	**71.11**
CV	**60.6**	**71.1**	**54.7**	**60.8**	**65.00**	**55.7**	**10.4**	**35.3**	**9.5**	**23.9**	**63.3**	**28.7**
p-value	**0.010**	**0.012**	**0.023**	**0.047**	**0.034**	**0.427**	**0.723**	**0.987**	**0.017**	**0.655**	**0.567**	**0.046**

Mean in the same column followed by the same letter are not significantly different at $p<0.05$

Incidence of rice blast disease in MA was significant different at tillering stage ($p = 0.010$), booting stage ($p = 0.023$) and dough stage ($p = 0.034$) between cultivars. In the HA zone, the cultivars showed a significant difference in the incidence of the disease at the booting stage ($p = 0.017$). In MA (Mosso region) the highest incidence of the disease was observed on Watt cultivar at tillering (9.67%) and booting (9.83%) stages. While the lowest incidence ranging from 2.49 to 2.77% was recorded on Karundi, Buname and Gwizumwimbu cultivars at tillering stage. At booting stage, the incidence ranged from 3.07 to 3.88% on the same cultivars. At dough stage, rice blast incidence ranged from 0.49 to 1.68%. In HA (Buyenzi region), the high incidence of rice blast was observed on the cultivars Gwizumwimbu and Buname from 90.05 to 90.82%, and the lowest incidence of disease was recorded on Watt variety (74.12%) at booting stage.

Severity of rice blast disease was significant difference at tillering stage ($p= 0.012$) and booting stage ($p = 0.047$) between cultivars in MA. At tillering stage, the high disease severity was observed on Vuninzara and V18 cultivars (33.33%) at the tillering stage, while the low severity (11.11%) was observed on Kigori, Kabuye, Karundi, Buname and Rufutamadeni cultivars. At booting stage, the highest blast severity was recorded on Watt and Vuninzara cultivars (48.15%), while on Karundi, Buname and Rufutamadeni cultivars (11.11%); rice blast disease severity was recorded lowest. In HA, rice blast disease severity was significant different at dough stage ($p = 0.046$) only between cultivars. The high blast severity was observed on Gwizumwimbu, Kabuye and Rufutamadeni cultivars (100%) and the lowest on Mugwiza and Buname cultivars (48.15%) at the dough stage.

3.4.4 Disease Severity Index (DSI) on different rice cultivars in two agroecologies

The results of Table 3.7 showed that the Disease Severity Index (DSI) on the leaves and panicles was not significantly different ($p > 0.05$) between cultivars at different growth

stages in HA region. Significant difference between the cultivars was recorded on severity index at the tillering (p = 0.001), booting (p = 0.009) and dough stage (p = 0.001) in MA (Mosso region).

Table 3.7: Rice blast Disease Severity Index at different growth stage

Varieties	Disease Severity index (%)					
	At tillering stage		At Booting stage		At dough stage	
	Mosso	**Buyenzi**	**Mosso**	**Buyenzi**	**Mosso**	**Buyenzi**
Mugwiza	1.74a	28.87a	2.46a	29.43a	0.08a	18.51a
Buname	0.93a	28.8a	0.533a	31.74a	0.38a	22.55a
Watt	6.42b	31.71a	8.00b	32.12a	0.06ab	24.37a
V18	1.79a	27.51a	3.16a	32.71a	0.08a	25.2a
Kigori	0.67a	18.51a	1.99a	25.4a	0.08a	28.77a
Rufutamadeni	0.52a	24.5a	1.08a	25.48a	0.4a	29.54a
Karundi	0.47a	28.37a	0.86a	28.45a	0.05a	29.87a
Gwizumwimbu	0.81a	24.23a	2.81a	25.88a	0.95b	31.7a
Vuninzara	1.36a	24.5a	3.95a	26.45a	0.92c	32.22a
Kabuye	0.69a	31.55a	1.22a	31.58a	0.08a	32.35a
Mean	**1.53**	**26.8**	**2.61**	**28.92**	**0.63**	**27.5**
CV	**90**	**41**	**77.7**	**36.8**	*****	**47.2**
P.value	**0.001**	**0.921**	**0.009**	**0.985**	**0.001**	**0.928**

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

C.V%=Percent of coefficient of Variation

At tillering stage, Watt variety was recorded with high severity index (6.02%), V18 (1.96%) and Mugwiza (1.74%), while the low severity index was observed on Rufutamadeni variety (0.52%). At booting stage, the high severity index was recorded on Watt variety (8.00%), which was only one different with other cultivars followed by Vuninzara (3.95%), V18 (3.16%), Gwizumwimbu (2.81%) and Mugwiza (2.46%), the

low severity index was observed on Buname variety (0.53%). At dough stage, a high severity index was observed on Gwizumwumbu variety (0.95 %). The low severity index was recorded on Karundi variety (0.05%), followed by Watt variety (0.06%) and respectively to cultivars Mugwiza, V18, Kigori and Kabuye with severity index of 0.08%.

3.4.5 Area under disease progress curve for two agrocologies of Burundi

The Area under Disease Progress Curve (AUDPC) is a useful quantitative summary of disease intensity over time, compare levels of resistance to diseases among varieties of plants. The disease progress was different between rice cultivars, although in all cultivars the disease started slowly and gradually increased exponentially while it decreases timed in others. Leaf and panicle blast disease progress curves for 10 cultivars under field condition are shown in Figure 3.3.

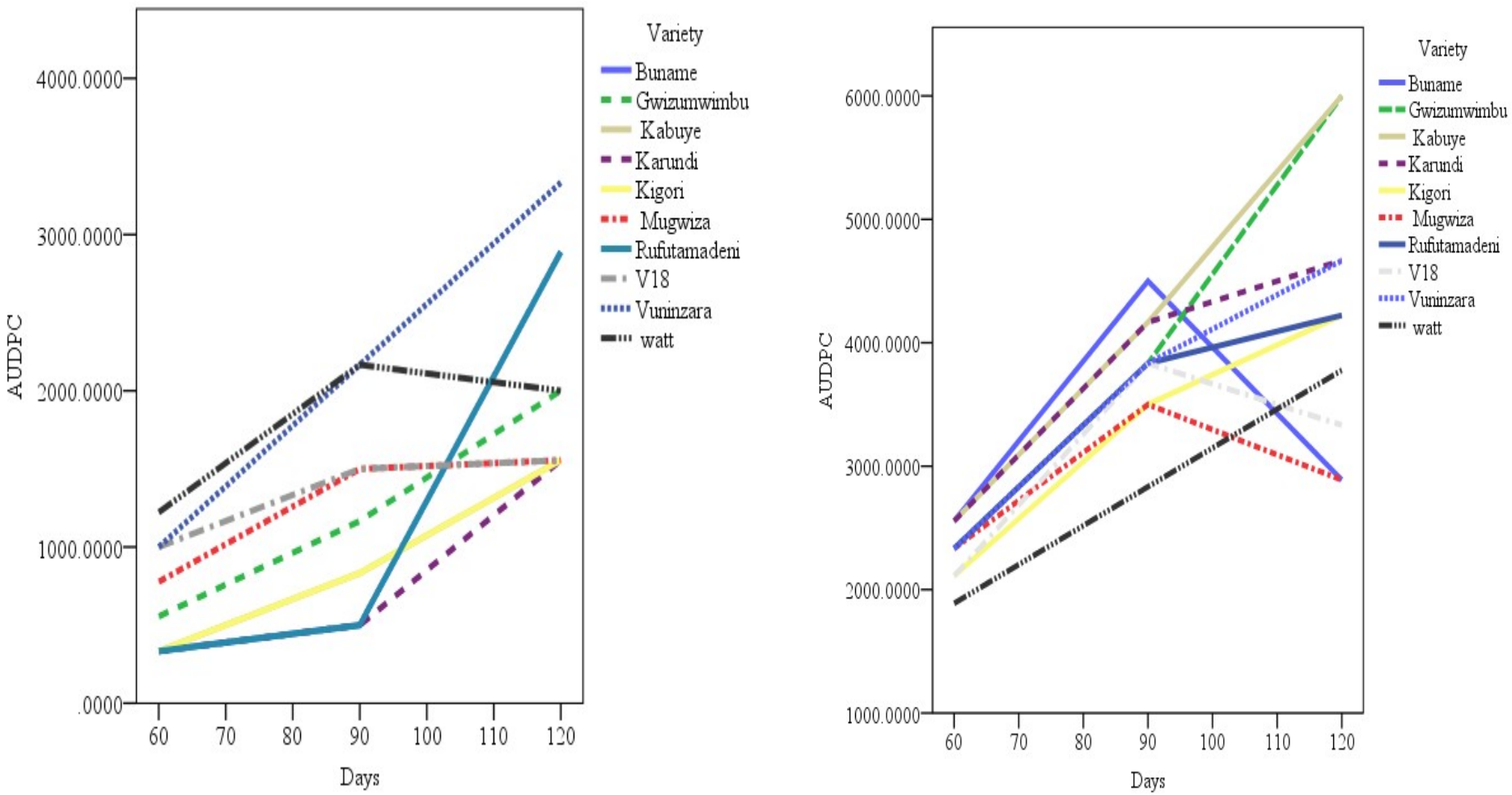


Figure 3.3: Area Under Disease Progress Curve (AUDPC) at Mosso (MA) and Buyenzi (HA) region in two agro ecologies of Burundi

The highest AUDPC values were obtained from Gwizumwimbu and Kabuye (6000.00), Karundi and Vuninzara (4666.67), Kigori and Rufutamadeni (4222.22), Watt (3777.78), V18 (3333.33) and Buname and Mugwiza (2888.89) in the region of Buyenzi. However, the trends of blast disease AUDPC were similar for certain Cultivars such as: (1) Gwizumwimbu and Kabuye; (2) Mugwiza, V18 and Buname; (3) Watt; (4) Kigori and Vuninzara.

In MA region, there were high AUDPC values recorded in Vuninzara (3333.3), Buname and Rufutamadeni (2888.89), Gwizumwimbu and Watt (2000.00); Kabuye Karundi, Kigori, Mugwiza and V18 (1555.56). The cultivars composed by (1) Mugwiza, Watt and V18; (2) Vuninzara, Kigori and Gwizumwimbu (3) Rufutamadeni and Kigori, Cultivars showed similar trends of disease progress.

In HA region, the highest AUDPC values on the rice cultivars Gwizumwimbu and Kabuye show a high level of susceptibility to rice blast because the curve has progressed upwards. Kigori and Vuninzara cultivars showed moderate susceptibility to rice blast disease during growth. High resistance to rice blast disease was observed on Bunane variety, followed by V18 and Mugwiza because trends of progress of disease decreased downwards.

In the MA region, Rufutamadeni variety have been highly susceptible to rice blast disease followed by the Karundi variety beacuse the disease progression curves continued to rise upwards. Vuninzara, Gwizumwimbu, and Kigori Cultivars showed moderate susceptibility to the disease. V18, Watt, and Mugwiza Cultivars showed a high resistance to the disease, the progression curves decreased downwards.

Watt and Mugwiza Cultivars exhibited the same blast resistance behavior traits in both regions, the disease progression curve decreasing downwards. Similarly, Gwizumwimbu variety showed the trend increased of progress disease in both regions. Watt variety showed appropriate disease progression trend for each region, same as Kigori and Vununzara Cultivars. Following these results obtained, the V18 and Mugwiza cultivars can be grown at the same time in these two regions; Buname variety can be planted in the Buyenzi region and Watt variety in the Mosso region.

3.5 Discussion

The rice crop growing in MA agro-ecological zone (AEZ) is less vulnerable to rice blast disease than rice grown in HA agro-ecological zone (AEZ) according this study. The findings reported by Nizigiyimana (1986), releaved that the rice blast disease caused by *Pyricularia oryzae* is known to be a common rice disease in all agro-ecological zones in Burundi. Similarly, results were found by Bhat *et al*. (2013), Chuwa (2016) and Shahriar *et al*. (2020), who reported that incidence and severity of rice blast disease vary based on the locations and environments conditions. The results in this study can be ascribed to differences in ecological condition of the two study locations. For instance, higher incidence and severity of the disease were observed in Buyenzi located in HA AEZ which had minimum temperature of 23.6°C and relative humidity of 75.1 %. These conditions are similar to those reported by Ahmad *et al*. (2011) in Kashmir valley, that the high rice blast disease incidence and severity is favored by minimum temperature of 20-24°C in night and a high humidity above 90%. Unlike Buyenzi, low rice blast incidence and severity were observed in Mosso region located in MA which had a minimum temperature of 28.4°C and relative humidity of 73.2%. Explanation to this can be the same as in Greer and Webster (2001) who reported that temperature above 28°C promote resistance to blast disease in plants rice.

The low incidence observed in middle altitude zone (Mosso) on different cultivars can be justified by the adaptation of the rice cultivars to the climate close to their zone of introduction in Imbo plain. However, the high rice blast disease found in HA (Buyenzi region) can be justified by climatic conditions found; another reason may be that some varieties are introduced by farmers' rice (the illegal distribution of seeds between producers). The difference in disease incidence and severity are also supported by Asfaha *et al*. (2015) who observed that the disease incidence or severity vary from low to high on the rice fields depending on the agro-ecological and cultivars differences.

Within and between location differences in cultivar susceptility to the rice blast at different growth stages were observed in the current study suggesting that age of the plant and environment condition at particular stage could be dictating amount of active inoculum, pathogen virulence and plant resistance. These explanations are in line with Groth and Bond (2007) reported in their finding that incidence and severity of disease depends on inoculum amount, crop growth stage, environment conditions, varietal resistance and cultural practices. Puri *et al*. (2006) in their survey on the effect of blast disease over 45 regions of India found high incidence of 30.45% at dough stage in lower land of rice growing areas compared to high altitude zone.

The Area under Disease Progression Curve (AUDPC) is a parameter that can provide a more prescriptive and practical classification of the disease progression in each variety. This study established 1. Difference cultivars grown in the same AEZ had different rice blast disease progress and 2. Same cultivar grown in different locations had different disease progression. Generally, the progression of the disease tends upwards from the tillering stage to the booting stage, but at dough stage the progression of the disease (AUDPC values) for some cultivars tends upwards and others down. Similar results

were supported by Zewdu *et al*. (2017) in Uganda, that the genotypes were significantly different for final leaf blast severity, panicle blast severity and AUDPC values under field and greenhouse conditions. The results of this study showed that the high value of AUDPC of the blast disease has been recorded in the high altitude region (Buyenzi region). This can be attributed to environmental conditions of regions such as temperature and relative humidity and precipitation. Another reason for the increased severity of rice blast could be other sources of primary inocula and abundance which may come from other hosts.

According to a study carried out by Cui (1995), in certain mountainous regions of the tropical and subtropical zone, the temperature decreases with altitude and, in most of these regions, precipitation increases with altitude; the duration of sunshine and solar radiation is much shorter than in plain areas and relative humidity and dew are highest at night. It has been reported that the low night temperature, which is a characteristic of high altitude areas, led to partial strength degradation (Manibhushanrao and Day, 1972), shorter solar radiation, frequent thick fog and prolonged dew are conducive to rice blast (Changjia *et al*., 2016). The results of another study done in Fuling and Chongqing (Southwest of China) on the distribution of rice blast showed that the severity of rice blast was strongly dependent on altitude (Peng *et al*., 1995). Meteorological factors, including temperature and relative humidity (RH), play an important role in the development of blast severity**.** In Buyenzi region, the average temperature (16.8^{o}C), precipitation (188.7mm) and relative humidity (80.7%) during assessment of disease severity at booting stage (April and May 2021) were high. Similar in Mosso region, the average temperature (22.2^{o}C), precipitation (120.5mm) and relative humidity (76.7%) were high.

Based on the results found, the Buyenzi region (High altitude) can be considered as a hotspot for rice blast disease while Middle altitude can be considered as a zone of low blast disease because of the observed low incidence and severity of rice blast disease. However, low disease such status in MA might change with pathogen adaptability fitness. Previous studies have demonstrated that pathogen could acquire additional fitness through sexual and parasexual recombination mechanisms under field conditions (Hayashi *et al*., 1997). Since in this study, the explained hot spot status in HA is based on damage symptoms, it might also change following climatic changes because level of damage is strongly influenced by environmental factors (Liu *et al*., 2021).

3.6 Conclusion and Recommendations

The rice blast disease incidence and severity were significantly different among regions. No significant difference was recorded for the effect interaction location and cultivar. However significant difference of rice blast disease incidence and severity was recorded between cultivars at different growth stages (Tillering, Booting and Dough stage) in two agro-ecologies of Burundi. V18, Watt and Mugwiza cultivars showed tolerance to the blast disease and can be used in the programs of managing of rice blast disease in both agro ecologies zones. High altitude agroecological zone (HA AEZ) showed a high incidence and severity of disease than the Middle Altitude Ageroecological zone (MA AEZ). The variations of incidence and severity of rice blast disease in two agroecologies zones associated with environmental conditions factors, but also to the illegal introduction of rice varieties by farmers' rice. These results come from one season data hence, disease may be different in other seaseon. It is, therefore, important that any program to develop cultivars for resistant to rice blast disease should consider environmental conditions of different locations, different seasons and cultural practices. More studies are needed for locations not covered by the current study.

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CHAPTER FOUR

Genetic relatedness of rice blast fungus (*Pyricularia oryzae*) isolates from two agro-ecologies of Burundi

Estella, Niyonkuru[1]; Madege, Richard Raphael[1]; Joseph, Bigirimana[2]; Georges, Habarugira[2] and Massawe, Deogracious Protas[3]*

1Sokoine University of Agriculture, College of Agriculture, Department of Crop Science and Horticulture

2International Rice Research Institute-Burundi (IRRI-Burundi)

3 Plant Molecular Biology laboratory at Sokoine University of Agriculture (SUA).

**Correspondent author, E-mail: estelleniyonkuru@yahoo.fr Tel: +257 61388208*

Abstract

Rice blast disease caused by fungus *Pyricularia oryzae* is one of the most destructive diseases in rice-producing areas of Burundi. Studies on characterization and genetic diversity of *P. oryzae* isolates from the high altitude and middle altitude region of Burundi were cunductucted in the both laboratory in IRRI Burundi and Plant Molecular Biology laboratory at Sokoine University of Agriculture *(SUA)*. Molecular analysis using five primers were used for amplification of thirth five *P.oryzae* strains. To understand species diversity of *P.oryzae* isolated, molecular markers targeting the Internal Transcribed Spacer (ITS) and Translation Elongation Factor (TEF) regions followed with Sanger sequencing were used. Thirty-five isolates of *P. oryzae* were amplified in Polymerase Chain Reaction (PCR) using primers TS1F and 2R, ITS3F and 4R, ITS1F and 4R, ITS4F and 5R and EF1-983F and EF1-2218R. The positive PCR amplicons for ITS1F and ITS4R and TEF1-983 and EF1-2218F were Sanger sequenced. The PCR results showed difference in banding patterns between isolates ranging from

220-1235bp. The isolates amplified by TS1F and 2R, ITS3F and 4R, ITS1F and 4R, ITS4F and 5R and EF1-983F and EF1-2218R showed bands size of 220bp, 350bp, 390bp, 550bp and 1235bp respectively. The Sanger sequence products released that all the isolates were *Pyricularia oryzae* from rice host with limited variations in the analyzed genes. Phylogenetic analysis showed narrow genetic diversity between *P.oryzae* collected in high and middle altitudes regions of Burundi. The single nucleotides polymorphisms observed among the isolates in both ITS and EF regions may indicate the level of virulence or pathogenicity among the Burundi Isolates may differs, hence calling for further studies. Therefore, these findings call for plants breeders to initiate/proceed with breeding strategies targeting to overcome the rapid evolving *Pyricularia oryzae* strains by breeding resistant rice cultivars to rice blast disease in the country.

4.1 Introduction

Rice is the staple food for more than half of the world's population (Khush, 2013). Nowadays, rice is a very popular and important staple food in Burundi, generating income for smallholder farmers as well as rural traders (Baramburiye, 2010). Rice is infected with several pathogenic species and diseases, among which rice blast pathogen is the most important and devastating pathogen (Devi *et al*., 2015, Miah *et al*., 2013,). Rice blast disease is caused by *Pyricularia oryzae /Magnoporthe grisea* (Urayama *et al*., 2010; Hosseini Moghaddam and Soltani, 2013), is an important fungal disease known in all rice-growing regions of the world (Ou, 1985). The pathogen infects and damages rice plants at all stages of growth, causing blast symptoms that appear on aerial organs such as leaf, collar, neck, node and seed (Zhu *et al*., 2005). Depending on location and environmental conditions, the incidence and severity of blast varies each year**.** Indeed, the use of resistant varieties incorporating good agricultural practices, which was the most effective and economical way to fight against the disease, is now confronted with

the phenomena of circumvention of the resistance of varieties (Fukuta, 2014). In Philippines, *P.oryzae* exhibits high genetic diversity in host range and varietal specificity (Leung *et al*., 1988). Pathogenicity of *P. oryzae* isolates differs between rice varieties in Burkina Faso et du Togo (Bonman *et al*., 1987, Kassankogno *et al*., 2016), which is the main reason for the failure to obtain cultivars with complete resistance (Thon *et al*., 2006, Kassankogno *et al*., 2016). In addition, the diversity of virulence of blast pathogens makes selection for resistance difficult (Marangoni *et al*., 2013). According to certains pathogens, characteristic of the sexuality and mitosis which allow them to adapt very quickly to changes in their environment (McDonald and Linde, 2002). Similarly, parasexual recombination has been identified as one of the means of variation in *P.oryzae* (Liu *et al*., 2004).

It is better to follow the management practices to control and need to continually monitor the genotypic and pathotype diversity of the pathogen in a given region. Therefore, knowledge of the genetic structure of the pathogen population is necessary in the development of an effective and sustainable management method against the disease. Different molecular approaches are used to characterize strains of blast pathogens (Sharma *et al*., 2002). Molecular techniques are currently an effective for distinguishing and characterization between closely related strains of *P. oryzae*. This study aimed to determine the genetic diversity of *P. oryzae* isolates to establish their phylogenetic relation in the two agro-ecological zones of Burundi (high and middle altitude).

4.2 Materials and Methods

4.2.1 Sampling

Sixty (60) household farms were randomly selected from each of the two agro ecological zones (high and middle altitude) (Figure 4.1), using a Stratified Random Sampling procedure (Boschetti et *al*., 2006). Sampling plant materials was done by collecting

plants with symptoms of blast: blast 60 leaves, 60 nodes and 60 panicles from both Buyenzi and Mosso regions. Samples were kept in labeled envelops gathered in transparent bags at low temperature (cool box) and later in refrigerator maintained at 3-5^{o}C for a systematic isolation of *Pyricularia oryzae* at IRRI Burundi laboratory. Leaves, panicles and nodes were randomly selected during isolation and remained samples were stored again in the refrigerator for future usages.

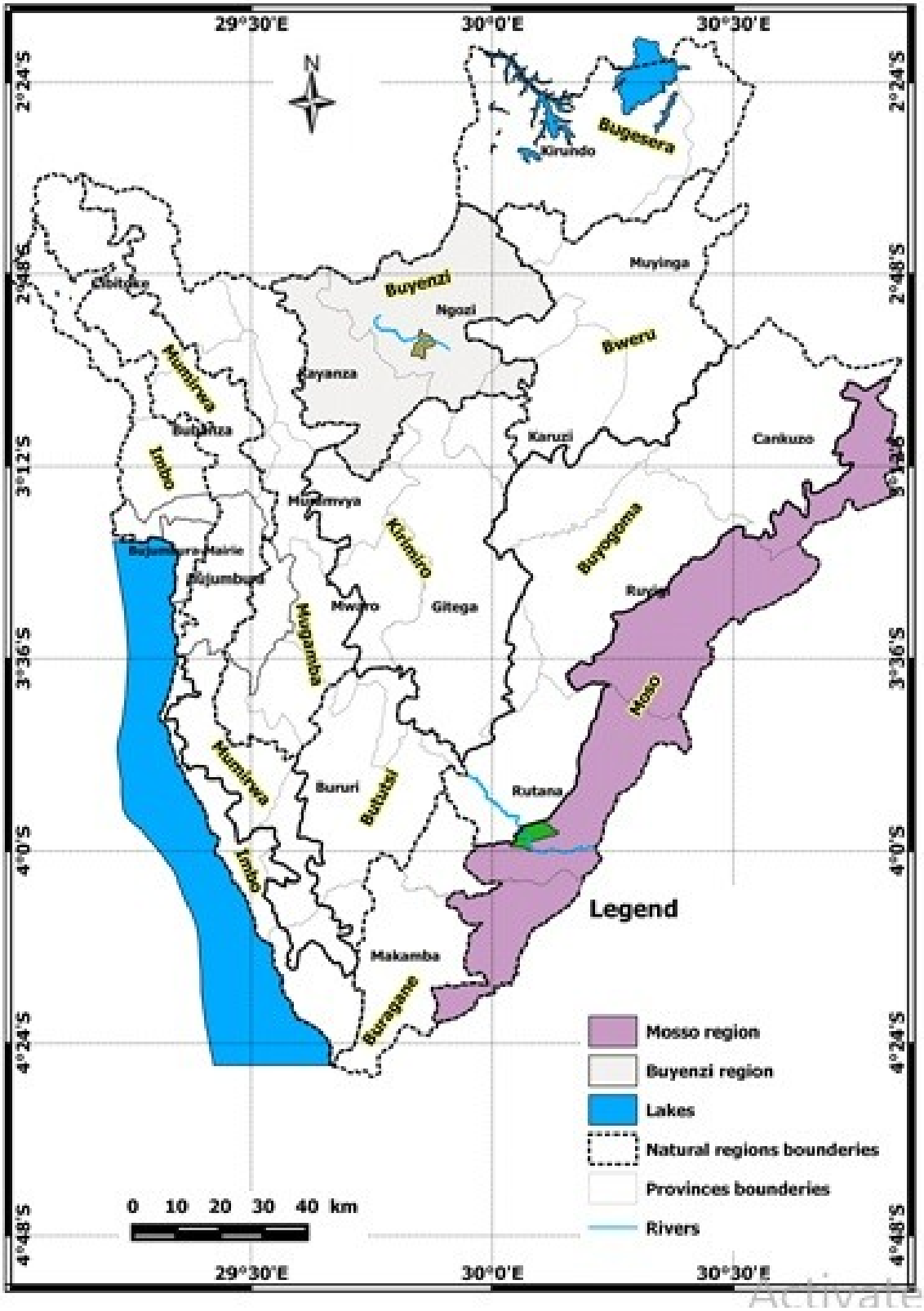


Figure 4.1: Map of Burundi showing the study locations (Buyenzi and Mosso regions)

4.2.2 Isolation of *Pyricularia oryzae*

Isolation of *P. oryzae* was done by cutting infected leaves, nodes, and panicles into small pieces, followed by sterilization done by putting them into Hydrogen peroxide (H_2O_2) for 3 minutes to eliminate saprophytes. Then the samples were rinsed three times with sterile distilled water for 1 minute. Pieces of tissues were placed in sterilized Petri dishes lined with moist filter papers in which incubation was done at room temperature (25-27°C) for 24 hours. After 24hours, fungal spores were harvested, with a glass needle, plated on Water Agar Medium and incubated for 24h at room temperature 25°C). This was followed by microscoping observation to identify germinating spores after which a monosporic culture was established in Potato-Dextrose-Agar (PDA) medium (IRRI, 2013).

4.2.3 Molecular characterization of *Pyricularia oryzae*

4.2.3.1 DNA extraction

Total DNA was extracted from 35 isolates of *P.oryzae* (Table 4.1) according to the procedure described by Murray and Thompson (1980). The harvested mycelium was homogenized after drying them in liquid nitrogen followed by crushing them into powder and put it into the 2 ml Eppendortf tube (Figure 4.2.a). Mortar and pestle were used to macerate of the mycelial. Cethyl Trimethyl Ammonium Bromide (CTAB) solution was added into the eppendortf tube containing the samples and incubated for 15 min at 65°C. After incubation, an equal volume of phenol (450 µl) and Chloroform: isoamyl- alcool (49:1) 450 µl) were added and centrifuged at 1300xg for 5min at 25°C. The upper supernatant was transferred into the new ependorf of 2ml followed by addition of 400 µl chloroform: isoamyl alcool and centrifuged at 1300xg for 2 min at 25°C. The upper supernatant was again transferred to a new Eppendorf of 1.5 µl. The cellular protein was precipitated by adding 0.7 volumes of isopropanol and centrifuged

at 1300xg for 20 min at 25ºC. The DNA pellets were washed with 500 µl of ethanol and centrifuged at 1300xg for 3 min at 25ºC (the process was repeated 3 times) (Figure 4.2.b). The pellet was dried under vacuum and the DNA was dissolved using the hotplate at 65ºC for 30 min by adding 50 µl Tris (10 µl).

a **b**

Figure 4.2: DNA extraction of *Pyricularia Oryzae* (a=crushing the mycelia with nitrogen liquid and b=centrifugation step) at IRRI-Burundi laboratory

Table 4.1: Geographical distribution of isolates used in the molecular Characterization and sequencing

Sample ID	Isolates name	Diseased plant parts	Origin of isolates
1	POKGP2018	Diseased panicle	Buyenzi region
2	POKGL308	Diseased leaf	Buyenzi region
3	POKGP2011	Diseased panicle	Buyenzi region
4	PORGN501	Diseased node	Buyenzi region
5	PORGN502	Diseased node	Buyenzi region
6	POKGL304	Diseased leaf	Buyenzi region
7	POKGP2014	Diseased panicle	Buyenzi region
8	POKGL301	Diseased leaf	Buyenzi region
9	PORGP603	Dideased panicle	Mosso region
10	POKGP208	Diseased panicle	Buyenzi region
11	POKGN106	Diseased node	Buyenzi region
12	PORGP601	Dideased panicle	Mosso region
13	POKGP2010	Diseased panicle	Buyenzi region
14	POKGP206	Diseased panicle	Buyenzi region
15	POKGL307	Diseased leaf	Buyenzi region
16	POKGN103	Diseased node	Buyenzi region
17	PORGP605	Dideased panicle	Mosso region
18	POKGP209	Diseased panicle	Buyenzi region
19	POKGP2016	Diseased panicle	Buyenzi region
20	POKGP203	Diseased panicle	Buyenzi region
21	PORGP608	Dideased panicle	Mosso region
22	POKGP205	Diseased panicle	Buyenzi region
23	POKGP2013	Diseased panicle	Buyenzi region
24	POKGP2012	Diseased panicle	Buyenzi region
25	POKGN101	Diseased node	Buyenzi region
26	POKGP202	Diseased panicle	Buyenzi region
27	POKGP201	Diseased panicle	Buyenzi region
28	POKGP207	Diseased panicle	Buyenzi region
29	POKGP2017	Diseased panicle	Buyenzi region
30	POKGN107	Diseased node	Buyenzi region
31	POKGP204	Diseased panicle	Buyenzi region
32	PORGP606	Dideased panicle	Mosso region
33	PORGP604	Dideased panicle	Mosso region
34	PORGP607	Dideased panicle	Mosso region
35	PORGP602	Dideased panicle	Mosso region

4.2.3.2 Polymerase Chain Reaction (PCR) amplification and sequencing

Five sets of primers namely: EF1-983F and EF1-2218R (Rehner and Buckley 2005; Liu *et al*., 1999), ITS1F and 2R (Gadens and Bruns 1993), ITS3F and 4R (White *et al*., 1990), ITS1F and 4R (White *et al*., 1990) and ITS4F and ITS5R (White *et al*., 1990) were used to amplify the DNA of *P. oryzae* by the Polymerase Chain Reaction (PCR) technique (Table 4.2). A total of 35 independent PCR amplifications were performed for each primer pair. PCR was performed in 25 µl reaction volumes using the Taq 2x Master Mix from New England BioLabs Inc (M0486S). The PCR mix consisted of 12.5 µl of 2x Master Mix, 1.0 µl of forward primers, 1.0 µl of reverse primers, 1.0 µl of DNA template and 5.5 µl of distilled water. PCR amplification was performed using thermal cycling: initiation denaturation at 94°C for 5 min, followed by 32 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 min and final extension at 72°C for 5 min (Figure 4.3.a). The PCR products were separated in a 1.2% agarose gel at 120 volts for 1 hour 30min (Muni *et al*., 2014), stained with ethidium bromide and results scored in a gel documentation system (Figure 4.3.b). The positive PCR amplicons for primer ITS1F and 4R and ITS EF1-983F and EF1-2218R were further amplified in a 50 µl reaction volume, validated by gel electrophoresis and submitted for Sanger sequencing.

Table 4.2: PCR amplification primers used in this study

No	Primer	Primer sequence (5'-3')
1	EF1-983F	GCY CCY GGH CAY CGT GAY TTY
	EF1-2218R	CCC ATR GCT TGY TTR CCC AT
2	ITS1F	AGAGGAAGTAAAAGTCGTAACAAG
	ITS2R	ATATGCTTAAATTCAGGGGG
3	ITS3F	GCA TCG ATG AAG AAC GCA GC
	ITS4F	TCC TCC GCT TAT TGA TAT GC
4	ITS1F	TCC GTA GGT GAA CCT CGC
	ITS4R	TCC TCC GCT TAT TGA TAT GC
5	ITS4F	TCC TCC GCT TAT TGA TAT GC
	ITS5R	GGA AGT AAA AGT GGT AAC AAG G

Figure 4.3: Master Mix (a) and loading of DNA (b) in agarose gel for amplification at Plant Molecular Biology laboratory at Sokoine University of Agriculture (SUA).

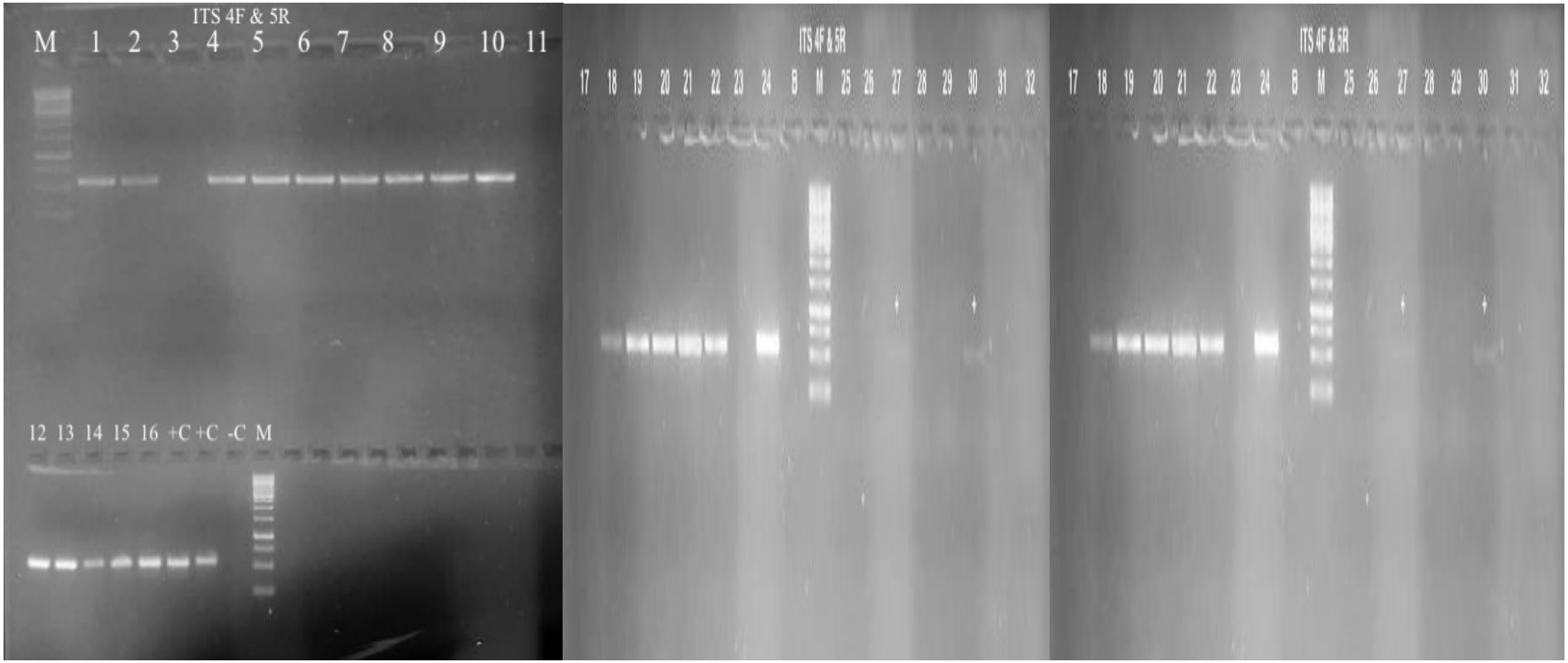
4.3 Data Analysis

The positive PCR products were scored in a binary form, 1 for the presence and 0 for the absence of amplicons. The Sanger sequenced reads were analyzed using different bioinformatics software. The CLC Genomics workbench Software from Qiagen Company Vers. 20.1 (Redwood, CA) was used to trim the ends and validate the base called in a chromatography for each sequenced read, the MacVector software Vers. 18.2 (Apex, NC**)** was used in the alignment of clean reads using the ClustalW option and calculating the similarity matrix among the isolates, while the MEGA 7 software (Kumar *et al.,*2016) constructed the phylogenetic tree to illustrate the evolutionary inference of isolates with other isolates obtained from the GenBank by using Maximum Likelihood with 1000 bootstrapping values.

4.4 Results

4.4.1 Characterization of *Pyricularia oryzae* isolates

A number of 35 isoaltes were used and the results showed that primer EF1-983F and EF1-2218R amplified 16 isolates, 12 isolates are from HA and 4 isolates from MA. ITS1F and 2R amplified 25 isolates, 15 isolates are from HA and 10 isolates from MA. ITS3F and 4R amplified 25 isolates: composed of 15 isolates from HA and 10 isolates from MA. ITS1F and 4R amplified 25 isolates: 13 isolates from HA and 7 isolates from MA. ITS4F and 5R amplified 23 isolates: 16 isolate from HA isolates and 7 isolates from MA (Figure 4.4, Figure 4.5, Figure 4.6 and Figure 4.7).



(1) (2) (3)

Figure 4.4: Amplification using primer ITS 4F and 5R (350bp) with (1) Sample #1 - #16 Negative control as –C; and positive controls as +C, (2). Sample #17 - #32 Negative control as B; and (3) Sample #33 - #35 Negative control as B and positive controls as F1-F5

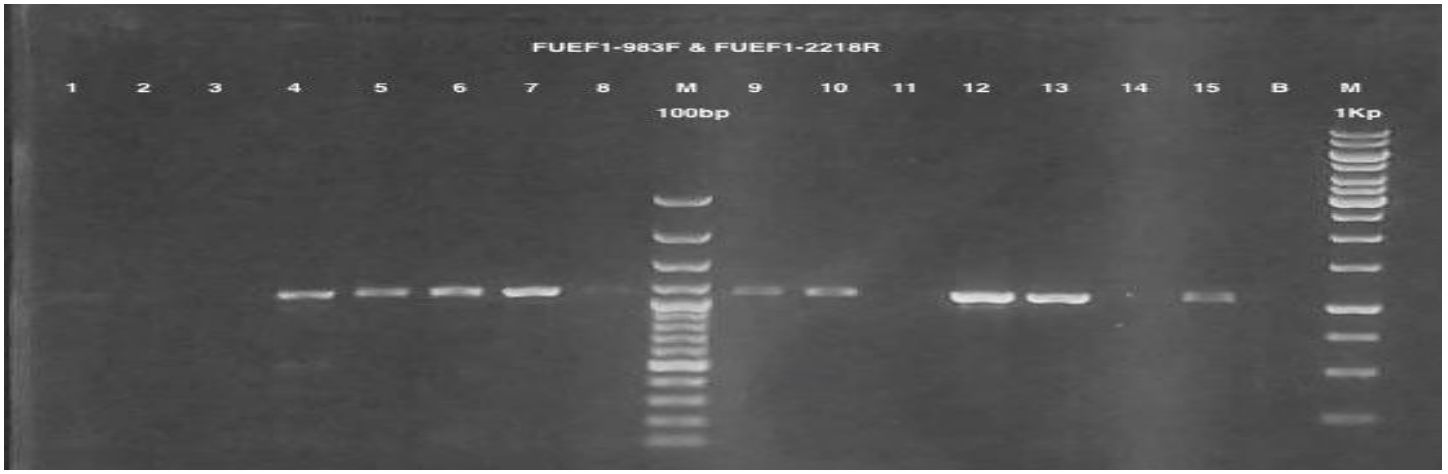


Figure 4.5: Amplification using primer EF1-983F and EF1-2218R (1235) with Sample #1 - #15 Negative control as B

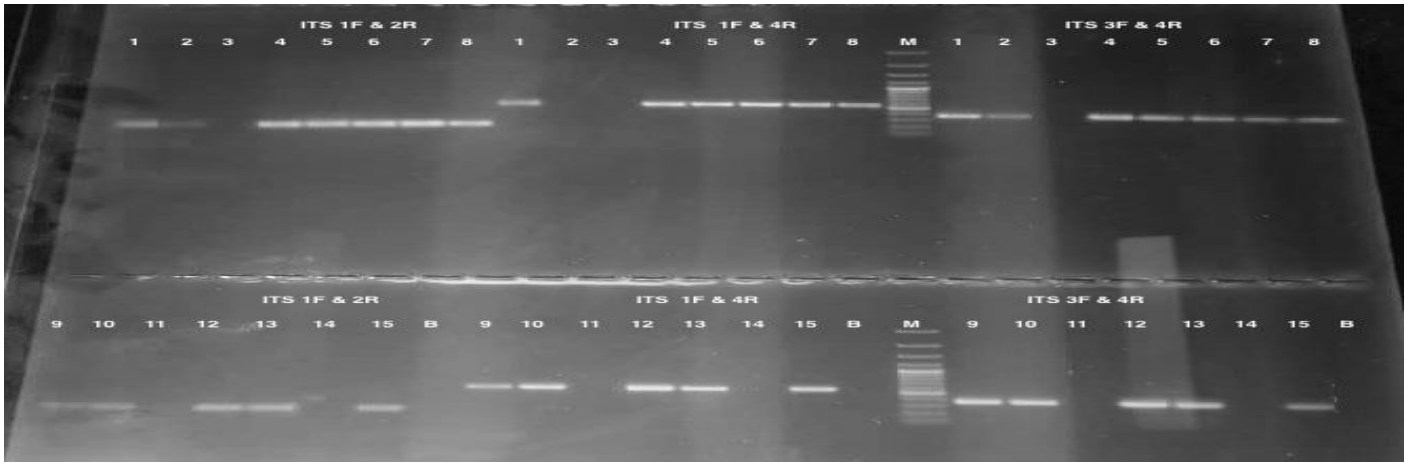


Figure 4.6: Amplification using primer ITS1F and 2R (220bp), 1F and 4R (550bp), 3F and 4R (390bp) with Sample #1 - #15 Negative control as B

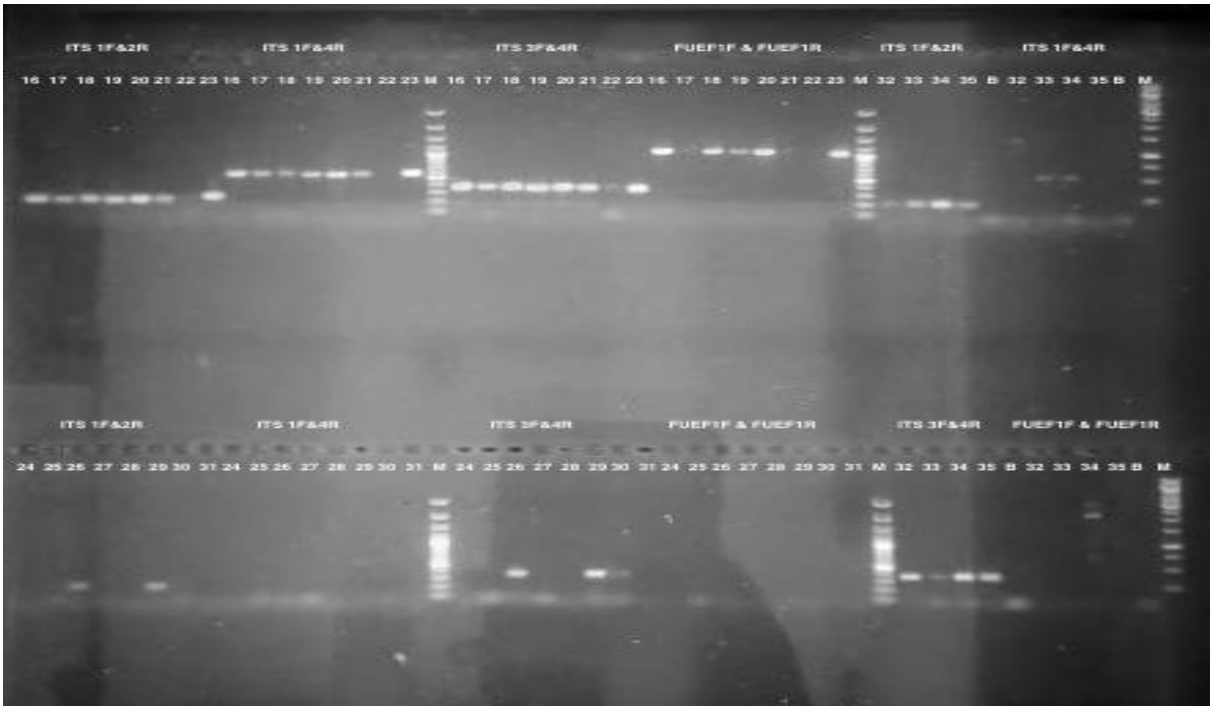


Figure 4.7: Amplification using primers EF1-983F and EF1-2218R (1235bp), ITS1F and 2R (220bp), 1F and 4R (550bp), 3F and 4R (390bp) with Sample #16 - #35 Negative control as B

The results of amplification indicated that primer EF1-983F and EF1-2218R did not amplify isolates POKGL308; POKGP2011; POKGN106; POKGP206; POKGP205; POKGP2012; POKGN101; POKGP202; POKGP201; POKGP207; POKGP2017; POKGN107; POKGP204 from HA AEZ; and isolates PORGP606, PORGP604, PORGP607; PORGP602, PORGP605 and PORGP608 from MA AEZ. ITS1Fand 2R did not amplify isolates POKGP206, POKGP205, POKGP2012; POKGP201 and POKGN107 all from HA AEZ. ITS1F and ITS4R did not amplify POKGL308, POKGP2011, POKGP206, POKGP2012, POKGP202, POKGP201, POKGP2017, POKGN107 and POKGP204 from HA AEZ with PORGP606, PORGP602 from MA AEZ. The ITS3F and ITS4R primer did not amplify isolates POKGP2011, POKGN106, POKGP206, POKGP205, POKGP2012; POKGN101; POKGP201; POKGP207; POKGN107; POKGP204 all from HA AEZ. The ITS4F and 5R primer did not amplify isolates POKGP2011; POKGN106; POKGP2013; POKGN101; POKGP202; POKGP207; POKGP2017and POKGP204 from HA AEZ with isolates PORGP606;

PORGP604; PORGP607; PORGP605 from MA AEZ. In addition, the results showed that isolates PKGP204, POKGP207, POKGN101, POKGN106 and POKGP211 all from the High altitude AEZ were not amplified by all primers.

Amplification reactions with all primers generated polymorphic bands. *Pyricularia oryzae* isolates used in the study showed differences in banding patterns. The *P.oryzae* isolates identified were polymorphic with different band sizes between 220 and 1235bp for the primers used. For the isolates amplified by primer ITS1F and 2R, the bands size was 220bp and for ITS1F and 4R primer, isolates have bands size of 550bp. The isolates amplified by primers ITS3F and 4R, ITS4F and 5R and EF1-983F and EF1-2218R, the bands size were 390bp, 350bp and 1235 bp respectively.

4.4.2 Genetic diversity of *Pyricularia oryzae* isolates from high and middle ecologies zones

The results obtained from sequencing of *Pyricularia oryzae* isoaltes by using primers ITS1F and 4 and EF1-983F and EF1-2218 indicated that the somes isolates from high altitude(Buyenzi region) and middle altitude (Mosso region) AEZ of Burundi are *Pyricularia oryzae*, specific from rice host species . Based on the results found in alignment (Appendix 2 and 4) and similarity matrix (Appendix 3 and 5), some isolates showed single nucleotide polymorphism specific of *P. oryzae*

4.4.2.1 Genetic relationship of *Pyricularia oryzae* isolates

The results obtained by sequencing of *P.oryzae* isolates for primer EF1-983 and EF1-2218 showed that the isolates belonged to *Pyricularia oryzae* with 620bp long. Isolates POKGP2010, PORGP603, POKGL304, PORGN502, PORGP608, POGL307, PORGP605 and POKGP203 belonged to the same clade and were similar to the blast pathogen from USA, China and Japan with rice being the host species, respectively

XM003716200-1, CP091461-1 and Cp034207-1. Isolates POKGN103, POKGP206, PORGP601, POKGP208, POGP209 and POKGPL301 belonged to the same clade with isolates of different host species: Wheat from Bolivia (P060333-1), *Graminis-tritici* from Brazil (KU953251-1), *Ryegrass* from USA (PO50923-1) and *Gramini Tritici* from Brazil (KU953270-1) (Figure 4.8).

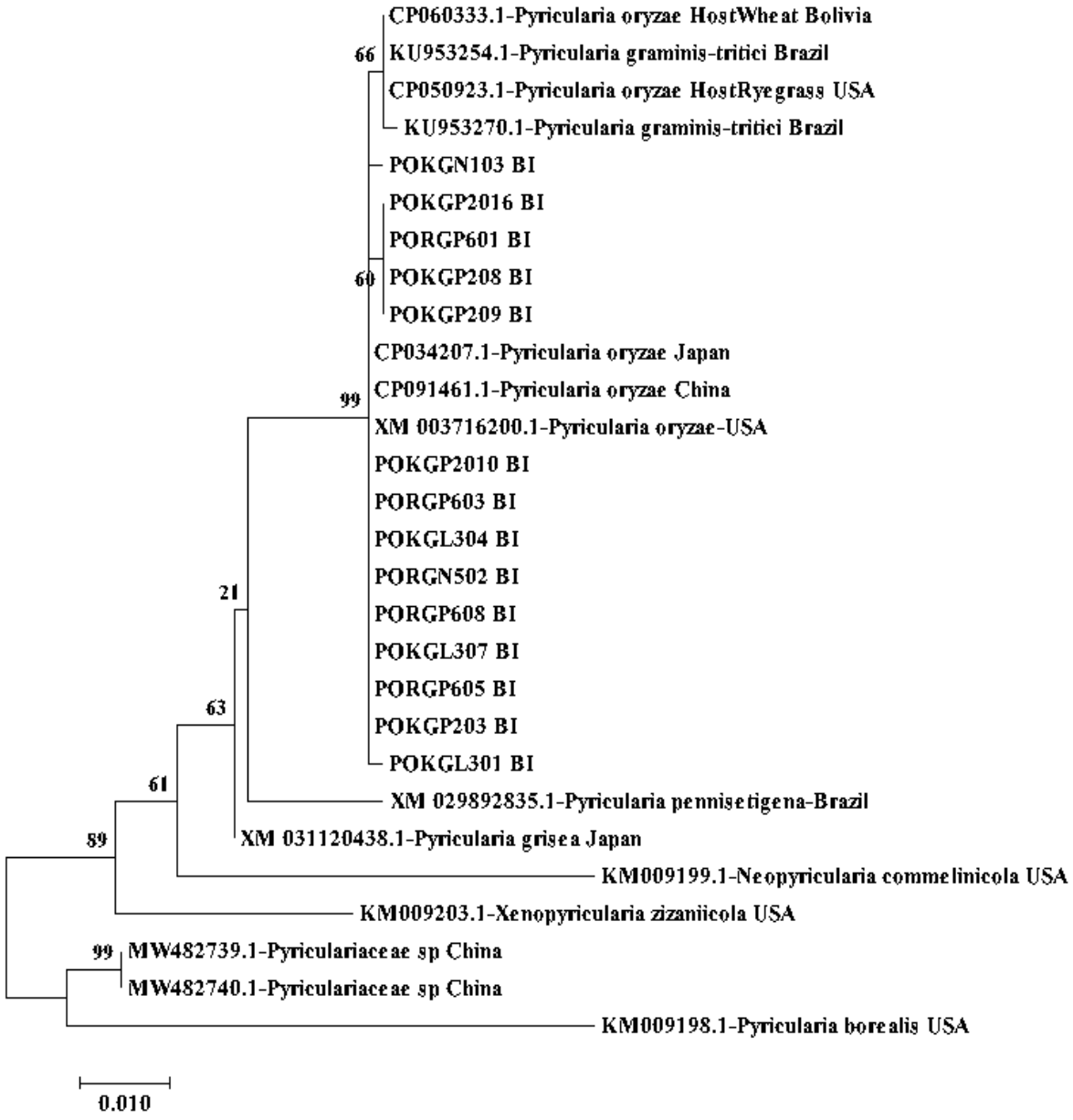


Figure 4.8: A phylogenetic tree of *Pyricularia oryzae* fungus based on dataset of the Elongation Factor (EF) constructed using the maximum likelihood with 1000 bootstrapping values in MEGA 7 software

EF alignment results indicated that the isolates POKGP2016, PORGP601, POKGP208 and POKGP209 showed substitution at position 620 (C/G), POKGN103 at position 200 (G/A) and POKGL301 at position 96 (T/C) (Appendix 2). Nucleotide similarity of isolates from Burundi range from 99.7 - 100%. *Neopyricularia* and *Xenopyricularia* have similarity of 93.9% and 95.5% respectively with *Pyricuralia* spp. *Borealis* from USA was distantly related with other *Pyricularia* species by 90.7% (Appendix 4).

4.4.2.2 Genetic relationship of *Pyricularia oryzae* isolates using ITS1F and 4R primer

The sequences of ITS1F and 4R had 240bp clean reads with high quality chromatograms. Phylogenetic analysis indicated that isolates POKGP208 and PORGP601were in the same clade with isolates of host species of rice from India, China, Thailand, Vietnam, Denmark, Iran, Srilanka and Ryegrass host species from Iran. Isolates PORGP604, POKL301, PORGP605 and PORGP203were in the same clade, showing nucleotide mutation and indicated that the isolates were introduced from Asia. The last clade composed by PORGN502, POKGP2014, PORGP603, POKGL307, POKGN103, POKGP209, PORGP608 and PORGP607 isolates have the one host specie of rice from South Korea (Figure 4.9).

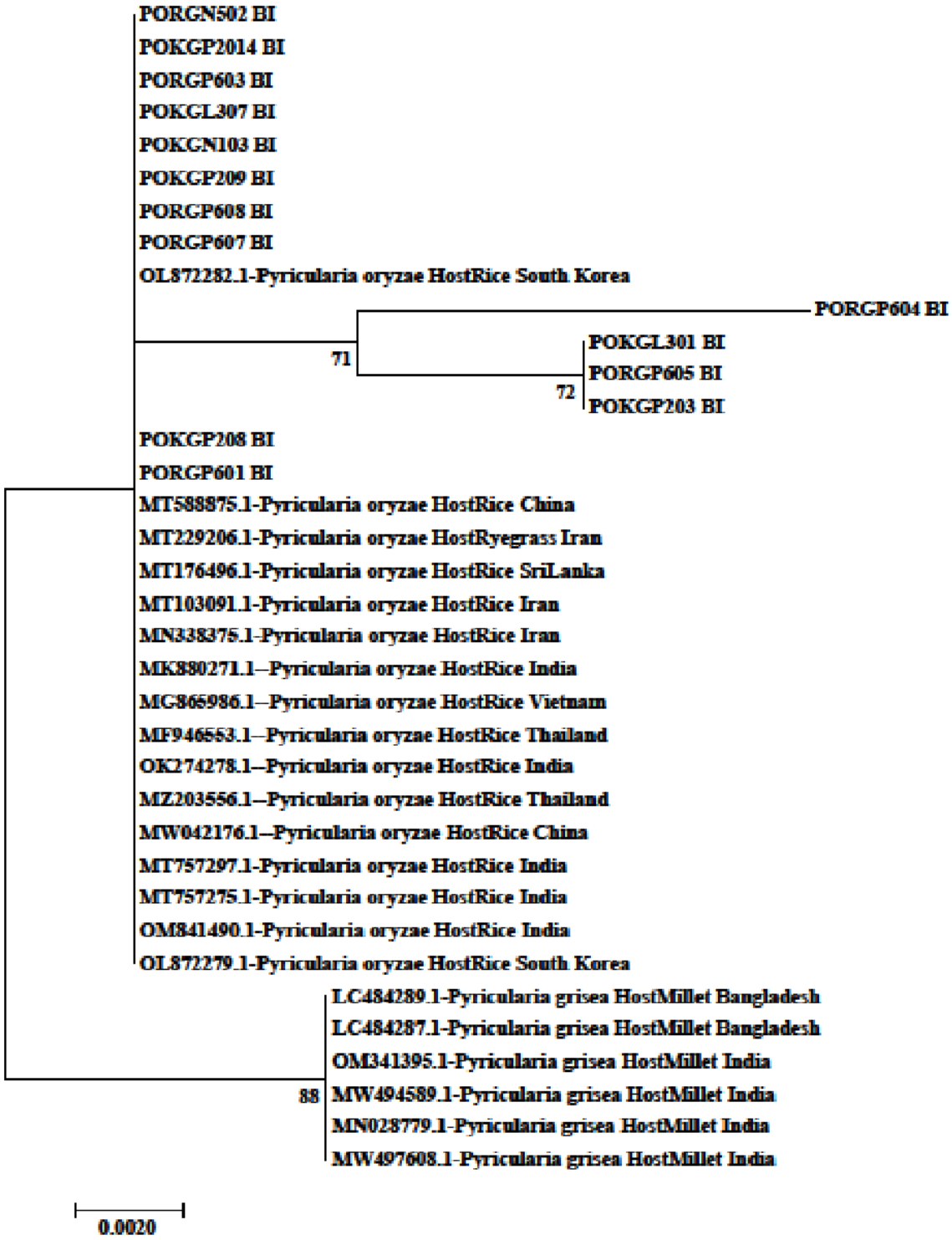


Figure 4.9: A phylogenetic tree of *Pyricularia oryzae* fungus based on dataset of Internal Transcribed Spacer (ITS) constructed using the maximum likelihood with 1000 bootstrapping values in MEGA 7 software

ITS alignment of isolates PORGN502, PORGP603 and POKGL307 showed substitution at the same position 182(G/C) and 183(C/A). While isolate PORGP604, which are the same clade of above isolates, showed nucleotide mutation at positions respectively 183(C/A), 214(A/D and 215(G/C) (Appendix 3).

Nucleotide similarity of isolates from Burundi range from 98.8 - 100%. *Pyricularia grisea* isolates from Bangladesh and India were very closely related with *P. oryzae* isolates from Burundi and for other islates from other country with nucleotide similarity ranged from 97.8-100% (Appendix 5).

4.5 Discussion

Molecular markers have been used to characterize fungal plant pathogen populations, in particular for characterization of *P.oryzae*.Based on this study, some isolates were amplified and other not amplified. The amplification reaction generated different polymorphic bands according to primer used. The isolates amplified by primers ITS1F and 2R, ITS4F and 5R and ITS3 and 4 were polymorphic with band size between 220 and 390bp. Similar results were reported by Kumar *et al*. (2010) in their study on identification of blast resistance using molecular markers such RAPD and SCAR and found that *P. oryzae* strains have band sizes ranged between 100 and 500bp. Isolates amplified by ITS1F and 4R produced band sizes of 550 bp. Similar results reported by Chuwa (2016) in his study of molecular characterization of *P.oryzae* causative agent of rice blast in Tanzania, he found that *P. oryzae* strains MOS, KIK, KAP and MSU were amplified by primers ITS1, ITS4, ACT-512, CAL-228 and CAL-737 and produced strong 550 bp bands. Another study done by Fujita *et al*. (2001) on detection and identification of yeast strains, reported that the amplification of fungi using ITS1 and ITS4 and ITS3 and ITS4 primers has released fragments 350 to 880 bp long and 233 to

432 bp long respectively. Bands size of 1235bp was produced by isolates amplified by primer EF1-983 and EF1-2218R. Similar results were found by O'Donnell *et al*. (2012) during identification of *Fasarium* spp. by EF primers, and reported bands of size 1158bp. The studies done by Hsuan *et al*. (2011) showed a single 750bp band successfully amplified from 78 isolates of Fusarium species from PCR amplification with EF primers. Also, different results were reported by Nitschkeet *et al*. (2009) in their studies when detecting DNA samples of an EF gene fragment and revealed that no amplification product was obtained for common soil and bee fungi (*Pythium ultium, Phoma betae and Rhizoctonia solani*).

Phylogenetic analysis showed that the isolates from Burundi belong to *P.oryzae* and that genetic diversity that exists in *P. oryzae* comes from different hosts or locations. Similar results were reported by Longya *et al*. (2020) when they were studying the characterization and genetic diversity of *Pyricularia oryzae* using ISSR and SRAP markers. In addition, similar results were found by Gladieux *et al*. (2018) in their study on Gene flow between divergent cereal-and grass-specific lineages of the rice blast.

The findings in this study are similar to finding by Qi *et al*. (2019) who reported that *P. oryzae* is the most important causative agent of blast disease over a wide range of hosts including rice and other grass species. This is because *P.oryzae* can infect over 50 grass host species, and infection of a new host is a major pathway for disease emergence according to Gladieux *et al*. (2018).

The results showed that the isolates vary considerably depending on the specific hosts. Some derived isolates were from hosts other than rice, *Ryegrass*, millet, wheat and *Graminis-tritic* for different locations. Similar results were found by Maciel *et al*. (2014)

and reported that the emergence of wheat blast in Brazil is the result of changes and expanding capacities of *P. oryzae* hosts. Also, Klaubauf *et al*. (2014) reported that some *Pyricularia* isolates from India can infect perennial Ryegrass.

4.6 Conclusion and Recommendations

The results revealed narrow genetic variations among the isolates of *P. oryzae* isolated from rice host and other hosts species such as *Ryegrass*, millet, wheat and *Graminis-tritic*. Some *P. oryzae* isolates showed single nucleotic mutation, no similarities to other host species, possibly due to environmental changes and are closed relate to *P. oryzae* from Asia. In addition, the *P. oryzae* from Burundi are very far related to the *P. oryzae* isolates from millet. The observed variations in the genone may lead the isolates to be more virulent or avirulent, hence calling for more studies to understand the pathogenicity level among the analyzed isolates. For this, the data obtained could be extended to other studies on the genetic diversity of *P. oryzae* isolates in Burundi. Beacuse of limitations of isotates used, further investigations in two seasons can also be carried out on the relationship between the genetic diversity of isolates, since genetic variation between strains plays an important role in blast dynamics and, therefore, in the success of integrated blast control, in particular for the selection of resistant rice varieties. The isolates sequenced in this study will be deposited in the GenBank to get their accession number for further use in other studies.

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CHAPTER FIVE

Pathogenicity of *Pyricularia oryzae* isolates obtained from cultivars grown in Middle and High altitudes zones of Burundi

Estella, Niyonkuru[1]; Madege, Richard Raphael[1]; Joseph, Bigirimana[2], Georges, Habarugira[2]*

1Sokoine University of Agriculture, College of Agriculture, Department of Crop Science and Horticulture

2International Rice Research Institute-Burundi (IRRI-Burundi)

**Correspondent author, E-mail: estelleniyonkuru@yahoo.fr Tel: +257 61388208*

Abstract

Rice blast disease caused by the fungal pathogen *Pyricularia oryzae* is an economically important disease distributed in rice-growing regions of the world. Understanding pathogenic variation of *Pyricularia oryzae* is one of the most efficient ways help in devising appropriate strategies for breeding and disease control.Thirteen isolates of *P. oryzae* from high and middle altitude rice ecosystems were inoculated into ten cultivars commonly grown by rice farmers in the middle and high-altitude areas of Burundi, and categorized for their ability to cause rice blast disease under Screen house condition. A complete randomized design (CRD) with three repetitions was used. The tested cultivars were evaluated as susceptible(S) or resistant (R) to a particular *P.oryzae* isolate based on disease severity score determined through visual observation using a standard 0-9 scale developed by IRRI (2014). Significant differences ($p = 0.000$) in blast incidence and severity were recorded between cultivars as well as isolate. Disease incidence and severity in the cultivars ranged from 11.11-33.33% and 3.70-69.14% respectively. Despite this variability in the isolate pathogenicity, three rice cultivars (Mugwiza, Rufutamadeni and V18) were less susceptible to the disease and hence can be regarded

as having traits of resistance which can be used by rice farmers for managing rice blast disease in the two agro-ecologies zones of Burundi.

5.1 Introduction

Rice is the second most important cereal crop after wheat and the most consumed major staple food cultivated worldwide (Katsantonis *et al*., 2017). Unfortunately, the cultivation is constrained by rice blast disease caused by fungus *Pyricularia oryzae* carava [synonym *P.grisea* Sacc (teleomorph Magnoporthe (Hebert) Barr] (Wang *et al*., 2015). The disease has been reported in more than 85 countries both in upland and low land rice and considered the most important worldwide (Fetene, 2019). Based on its economic importance, the pathogen is knwon as the most destructive fungus in the world (Katsantonis *et al*., 2017). The blast fungus is capable of infecting all the above-ground parts of rice plants such as leaves, neck and panicles of rice. The lesions on the leaves reduce the photosynthetic area thereby affecting the normal physiological aspects of rice growth (Bastiaans, 1991, Koutroubas *et al*., 2009, Azizi *et al*., 2015). Blast infection occurs when *P. oryzae* conidia land and attach themselves to the leaves using tip mucilage (Dutta, 2017). *P. oryzae* germinates via the development of a melanin-lined appressorium capable of producing a pressure to break open the leaf cuticle, ramify within the leaf tissue and leave the leaf dead (Dutta, 2017). *P. oryzae* is favoured by moist warm conditions and a minimum of 8 hours moisture is needed for infection to occur (Fetene, 2019). The most effective method to control rice blast disease is to plant the resistant cultivars (Fukuta *et al*., 2019).

However; the ability of a cultivar to resist the attack by a pathogen depends on the pathogen to cause a disease. Previous studies have established that different isolates of *P. oryzae* havae their pathogenicity varying with cultivars (Ali and Nadarajeh, 2014).

Hence, cultivars that can be classified as resistance, moderate resistance and susceptible depending on the cultivar response to the particular strain of the pathogen (Bakar, 2019). This can be done by establishing pathogenicity of *P.oryzae* isolates in different rice cultivars (Correa-Victoria and Zeigler, 1993). The objective of the present study was to evaluate of the pathogenicity of blast (*Pyricularia oryzae*) isolates on rice cultivars grown in middle and high altitude agro-ecological zones of Burundi.

5.2 Materials and Methods

5.2.1 Rice blast isolates

A collection of 13 monosporic isolates of *Pyricularia oryzae* isolated from samples of leaves, panicles and nodes of infected rice plants from High altitude (Buyenzi region) and Middle altitude (Mosso region) agro-ecological zones of Burundi were prepared. The isolation of blast fungi (*P.oryzae*) was done at the International Rice Research institute (IRRI) Burundi. The obtained isolates are presented in Table 5.1.

Table 5.1: *Pyricularia oryzae* isolates used in pathogenicity test

No	Name of isolates	Diseased plant parts	Origin of isolates
1	POKGL308	Diseased leaf	Mosso region
2	POKGL304	Diseased leaf	Buyenzi region
3	POKGL301	Diseased leaf	Buyenzi region
4	POKGL307	Diseased leaf	Buyenzi region
5	PORGN501	Diseased node	Mosso region
6	PORGN502	Diseased node	Buyenzi region
7	POKGP2018	Diseased panicle	Buyenzi region
8	PORGP603	Diseased panicle	Mosso region
9	POKGP2010	Diseased panicle	Buyenzi region
10	POKGP206	Diseased panicle	Buyenzi region
11	POKGP209	Diseased panicle	Buyenzi region
12	POKGP2017	Diseased panicle	Buyenzi region
13	POKGP2017	Diseased panicle	Buyenzi region

5.2.2 Inoculated plants materials

Plant materials used was composed of ten varieties most cultivated by rice farmers in Burundi. These varieties were Mugwiza, Gwizumbwu, Vuninzara obtained from IRRI. Other cultivars, Watt, Karundi, Kigori, Buname, Rufutamadeni, Kabuye and V18 were obtained from farmers (Table 5.2).

Table 5.2: Description of cultivars used in pathogenicity test

S/No	Rice cultivars	Local name	Dateof release
1	IR77713-30-1-1-3	Vuninzara	2011
2	IR79511-47-2-6-5	Gwizumwimbu	2011
3	IR91028-115-2-2-2-1	Mugwiza	2016
4	V46	Kigori	1997
5	Landrace	Watt	NA
6	V564-2-7	Kabuye (Rubabi)	2002
7	V18	Umuzambiya	NA
8	Landrace	Karundi	NA
9	Landrace	Buname	NA
10	Landrace	Rufutamadeni	NA

NA: Not Applicable

Before sowing, seeds were treated with pencozeb (2g/kg) and pre-germination was done by placing seeds in sterile petri dishes containing moist towel paper by using sterile distilled water for 6 days. Cultivars were sown in pots arranged in a completely randomized design with three replications. Five pre-germinated seeds were trans-planted in plastic pots of 20cm diameter, according to the protocol described by IRRI (2013). The experiment was carried out in the screen house of IRRI-Burundi.

5.2.3 Culture medium and inoculum preparation

Following the protocol described by IRRI (2013), the culture medium for blast fungi development was prepared using 20g of rice bran, 15g of agar and 2.5g of yeast extract in 1 liter of distilled water. The mixture was boiled using a heat-stiller-machine, sterilized at 121^{o}C for 20min. Antibiotic (streptomycin) was added to prevent bacterial contamination. The medium was then dispensed into petri dishes, left at room temperature for the medium to solidify and kept in the fridge at 3^{o}C before use.

To revive the stored culture, colonized paper disk were placed on rice flour agar (agar was manufactured by Glentham Life Science Ltd, United Kingdom) 7-10 days to allow mycelial growth and incubated for 2 weeks at room temperature (25-30^{o}C). After the second week, mycelia were scraped using a sterilized glass slide and kept under a fluorescent light for 7 days at 25^{o}C to induce fructification. This was followed by harvesting the spores using sterilized distilled water with 0.02% Tween 20 into a beaker (IRRI, 2013). The concentration of inoculum suspension was determined using a hemocytometer (manufactured by Labo MODERNE, France) and spores suspension was adjusted to 2x10^{5}spores/ml**.**

5.2.4 Inoculation

After preparation of the inoculum, 21days after sowing, plants that have 3 to 4 leaves were prepared for inoculation. 10-20ml of inoculum per pots of five test plants was prepared. Plants of differentsvarieties with similar isolates were put together and inoculated using a hand sprayer (Chuwa *et al*., 2015). Then they were placed in the same moisture chamber covered by wet blankets and plastic to promote high relative humidity at 25^{o}C of temperature. Plants were maintained in the humid chamber for 24-36 hours

after inoculation and placed in the mist room at 25-30°C (IRRI, 2013) for symptoms development.

5.2.5 Assessment of the rice blast disease

One week after inoculation, rice cultivars were assessed to observe their levels of resistance to *P. oryzae* isolates from Buyenzi region and Mosso region. The disease symptoms was determined by using the visual scales of 0 to 9 based on predominant lesions according to protocol disrobed by IRRI (2014), where 0 = no lesions; 1 = small, brown, specks of pinpoint size or larger brown specks without sporulation center; 3 = small, roundish to slightly elongated, necrotic, sporulation spots, about 1-2 mm in diameter with a distinct brown margin or yellow halo; 5 = narrow or slightly lesions, 1-2mm in breadth, more than 3mm long with brown margin; 7 = broad spindle-shaped lesion with yellow, brown or purple margin; 9 = rapidly coalescing small, whitish, grayish, or bluish lesions without distinct margins. According to Hayashi and Fukuta (2009), the plants reactions with score 0-1 were categorized as resistant (R) and 3-9 Susceptible (S).

Thereafter, disease incidence (Ghazanfar *et al*., 2009) and severity (Waller *et al*., 2002) was calculated using the following formula

Disease incidence(%) $\frac{Number\ of\ plants\ infected}{Total\ number\ of\ plants\ assessed} x100$ … (Equation 5.1)

Disease severity (%) $= \frac{n \times v}{V \times N} x100$ ……..… (Equation 5.2)

Where; (n) =Number of plants in each category

v = Numerical values of symptoms category

N = Total number of plants

V= Highest value scale

5.3 Data Analysis

Data analysis on rice blast disease incidence and severity were subjected to the analysis of variance (ANOVA), using SPSS (Statistical Package for Social Sciences) (IBM SPSS version 21) software. Statistical model: $Yijk = \mu + \alpha i + \eta k\ i + \beta j + \alpha\beta\ ij + \varepsilon k$, Where, μ: Grand mean, αi: mean effect of cultivars, $\eta k\ i$: Error plot, βj: Mean effect of Isolate, $\alpha\beta\ ij$: Interaction between Cultivar and Isolate, $\varepsilon k\ i$: Error plot. Comparison of means for rice blast disease incidence and severity were performed using Duncan's Multiple Range Test (DMRT) at 5% level of significance. In the model, rice blast disease incidence and severity were the dependent variables and Cultivar and isolate the independent variables.

5.4 Results

5.4.1 Isolate pathogenicity based on disease incidence and severity

Cultivars inoculated with *Pyricularia* oryzae isolates, showed different blast disease incidence and severity for the same isolate. Table 5.3 presents the effects of the isolates and cultivars on rice blast disease incidence and severity. The effects of isolate (p = 0.000) and cultivar (p = 0.000) were statistically significant. Similarly, effects due to interaction between cultivar and isolate were significant (p = 0.000) at 5% level of significance.

Table 5.3: Effects of cultivar and isolate on rice blast incidence and severity

Source	Incidence		Severity	
	Computed F	p.Value	Computed F	p.Value

Cultivar	15.559	0.000	63.025	0.000
Isolate	4.958	0.000	16.358	0.000
Isolate *Cultivar	4.051	0.000	19.798	0.000

5.4.2 Rice blast disease incidence and severity of cultivars

5.4.2.1 Incidence of rice leaf blast on cultivars

Incidences of rice blast in different cultivars varied significantly between isolates. In terms of isolate pathogenicity, statistically significant difference between cultivars were observed for isolate POKGP209 ($p = 0.012$); POKGL307 ($p < 001$); POKGP 206 ($p < 001$); POKGP2010 ($p = 0.008$); POKGL 301 ($p < 001$); PORGN501 ($p = 0.025$); POKGL308 ($p < 001$) (Table 5.4).

Table 5. 4: Rice blast incidence of rice cultivars inoculated with different *Pyricularia oryzae* isolates

Cultivars	Isolates												
	POKG P 2016	**POKG P 209**	**POKG L 307**	**POKG P 206**	**POKG P 2010**	**PORG P 603**	**POKG L 301**	**POKG L 304**	**PORG N 502**	**PORG N 501**	**POKG L 308**	**POKG P 2018**	**POKG P 2017**
Vuninzara	11.11	11.11	25.93	14.81	18.52	11.11	11.11	11.11	14.81	14.81	29.63	11.11	14.81
Gwizumwimbu	11.11	18.52	11.11	11.11	11.11	11.11	14.81	11.11	11.11	25.93	11.11	14.81	11.11
Mugwiza	14.81	11.11	11.11	11.11	11.11	14.81	14.81	11.11	14.81	11.11	14.81	11.11	11.11
Kigori	14.81	22.22	11.11	11.11	11.11	11.11	11.11	11.11	11.11	11.11	11.11	14.81	11.11
Watt	18.52	14.81	11.11	33.33	11.11	18.52	11.11	11.11	11.11	18.52	11.11	11.11	14.81
Kabuye	11.11	11.11	11.11	11.11	14.81	11.11	33.33	11.11	11.11	11.11	11.11	11.11	11.11
V18	11.11	14.81	11.11	14.81	18.52	11.11	11.11	14.81	11.11	14.81	11.11	11.11	11.11
Karundi	22.22	11.11	29.63	18.52	29.63	14.81	11.11	14.81	14.81	33.33	14.81	11.11	18.52
Buname	11.11	11.11	11.11	11.11	11.11	11.11	33.33	11.11	11.11	18.52	11.11	11.11	11.11
Rufutamadeni	11.11	11.11	14.81	11.11	11.11	11.11	11.11	14.81	11.11	11.11	11.11	11.11	11.11
Mean	**13.70**	**13.70**	**14.81**	**14.81**	**14.81**	**12.59**	**16.30**	**12.22**	**12.22**	**17.04**	**13.70**	**11.85**	**12.59**
CV	**35.90**	**26.6**	**24.6**	**24.6**	**36.2**	**35.6**	**18.1**	**30.3**	**28.7**	**44.2**	**25.2**	**24.9**	**25.8**
p-value	**0.117**	**0.012**	**<001**	**<001**	**0.008**	**0.474**	**<001**	**0.701**	**0.639**	**0.025**	**<001**	**0.589**	**0.115**

CV% = Percent of coefficient of variation

Rice blast disease incidence in ten cultivars ranged from 11.11- 33.33%. The highest rice blast incidence of 33.33% was recorded on Watt for isolate POKGP206; Buname and Kabuye for POKGO301 and Karundi for PORGN501. 29.63% blast incidence was observed on Karundi for POKGL307 and POKGP2010; and Vuninzara for POKGL308. While blast incidence of 25.93% was recorded on cultivars Vuninzara for POKGL and Gwizumwimbu for PORGN501. A 22.22% incidence was observed on cultivars Karundi for POKGO2016 and Kigori for POKGP209. Rice blast disease incidence of 18.52 % was also observed on Karundi cultivar for POKGP206 and POKGP2017; on Watt for POKGP2016, PORGP603 and POKRGN501, on V18 and Vuninzara cultivars for POKGP2010, Gwizumbimbu for POKGP209 and Buname for PORGN501.

Incidence of 14.81% was recorded on cultivars Vuninzara for POKGP206, PORGN502, PORGN501 and POKGP2017 isolates, on Gwizumimbu for (POKGL301 and POKGP2018 isolates. On Mugwiza for POKGP2016, PORGP603, POKGL301, PORGN502, POKGL308 isolates, on Kigori for POKGP2016 and POKGP2018 isolates, on Watt for POKGP209 and POKGP2017 isolates ; on Kabuye for isolate POKGP2010; on V18 for POKGP209, POKGP206, POKGL304 and PORGN501 isolates; on Karundi for PORGP603, POKGL304, PORGN502, POKGL2018islates; on Rufutamadeni for POKGL304 and POGP307 isolates. The lowest blast disease incidence of 11.11% was recorded on different cultivars for some isolates. For example, the cultivars Rufutamadeni, Buname and Kabuye show lowest blast incidence, except for two isolates (Table 5.4).

5.4.2.2 Mean value of rice leaf blast severity of cultivars under screen house

Among the ten (10) rice cultivars used to evaluate the pathogenicity of *Pyricularia oryzae* isolates, significant difference of disease severity were recorded between

cultivars for the following isolates: POKGP209 ($p = 0.09$); POKGL307 ($p < 001$); POKGP206 ($p < 001$); POKGP2010 ($p < 001$); POKGL301($p < 001$); PORGN501 ($p = 0.006$); POKGL308 ($p < 001$) (Table 5. 5).

Table 5.5: Rice blast disease severity on rice cultivars caused by artificially inoculated *Pyricularia oryzae* isolates

Cultivars	Isolates												
	POKGP 2016	POKGP 209	POKGL 307	POKGP 206	POKGP 2010	PORGP 603	POKGL 301	POKGL 304	PORGN 502	PORGN 501	POKGL 308	POKGP 2018	POKGP 201
Vuninzara	3.704	3.704	35.802	4.938	6.17	3.704	3.70	3.704	4.938	4.94	29.63	3.704	4.938
Gwizumwimbu	3.704	16.049	3.704	3.704	3.70	3.704	4.94	3.704	3.704	23.46	3.704	4.938	3.704
Mugwiza	4.938	3.704	3.704	3.704	3.70	4.938	4.94	3.704	4.938	3.70	4.938	3.704	3.704
Kigori	4.938	22.22	3.704	3.704	3.70	3.704	3.70	3.704	3.704	3.70	3.704	4.938	3.704
Watt	6.173	4.938	3.704	33.333	3.70	6.173	3.70	3.704	3.704	13.58	3.704	3.704	4.938
Kabuye	3.704	3.704	3.704	3.704	4.94	3.704	55.56	3.704	3.704	3.70	3.704	3.704	3.704
V18	3.704	4.938	3.704	4.938	6.17	3.704	3.70	4.938	3.704	4.94	3.704	3.704	3.704
Karundi	14.815	3.704	29.830	30.864	69.14	4.938	3.70	4.938	4.938	33.33	4.938	3.704	6.173
Buname	3.704	3.704	3.704	3.704	3.70	3.704	55.56	3.704	3.704	13.58	3.704	3.704	3.704
Rufutamadeni	3.704	3.704	4.938	3.704	3.70	3.704	3.70	4.938	3.704	3.70	3.704	3.704	3.704
Mean	**5.31**	**7.04**	**9.63**	**9.63**	**10.86**	**4.20**	**14.32**	**4.07**	**4.07**	**10.86**	**6.54**	**3.95**	**4.20**
CV	**97.5**	**84.3**	**22.1**	**36.9**	**45.6**	**35.6**	**6.9**	**30.3**	**28.7**	**81.7**	**33.4**	**24.9**	**25.8**
p.value	**0.289**	**0.009**	**<001**	**<001**	**<001**	**0.474**	**<001**	**0.701**	**0.639**	**0.006**	**<001**	**0.589**	**0.115**

CV% = Percent of coefficient of variation

The results in Table 5.5 indicated that rice blast disease severity in different cultivars varied with isolates where by the severity and ranged between 3.7- 69.14%. Karundi cultivar inoculated with isolate POKGP2010 had highest rice blast disease severity (69.14%) followed by 55.56% on Kabuye and Buname cultivars inoculated with POKGP301 and Vuninzara (35.802%) inoculated with POKGL307. Watt and Karundi cultivars which were inoculated with POKP206 and PORGN501 respectively both showed blast severity of 33.33%. Karundi registered blast severity of 30.86 and 29.83 % when inoculated with POKGL207 and POKGP206 respectively; and Vuninzara had blast severity of 29.63% when inoculated with POKGL308. Low rice blast disease severity was observed on Kigori cultivar (22.22%) for POKGP209, Gwizumwimbu (16.04- 23.46%) for POKGP209 and PORGN501 respectively, Karundi (14.81%) and Buname (13.58%) cultivars for isolates POKGP2016 and PORGN501 respectively. The lowest rice blast disease severity varied between 3.7- 6.17%, and was recorded on cultivars Mugwiza, V18 and Rufutamdeni for all isolates of *Pyrcularia oryzae*.

5.4.3 Resistance scores of rice cultivars against *Pyricularia oryzae* isolates from two agro - ecologies

Table 5.6 shows cultivars which showed less than 3 score of blast disease severity were regarded resistant (R) and those which showed blast disease score of three and above were considered susceptible (S) to a particular *P.oryzae* isolate. Among the ten rice cultivars, Rufutamadeni and V18 from high altitude and Mugwiza from middle altitude ecological zones were resistant to all isolates of *P.oryzae*. Others rice cultivars Vuninzara and Gwizumwimbu from middle altitude: Buname, Karundi, Kabuye, Kigori, and Watt from high altitude zone showed susceptible reaction for disease with at least to 1 isolates (Table 5.6).

Table 5.6: Reaction of rice cultivars to *Pyricularia oryzae* isolates from two agro ecologies

Cultivar	Isolate												
	POKG P 2016	**POKG P 209**	**POKG L307**	**POKG P 206**	**POKG P2010**	**PORG P 603**	**POKG L 301**	**POKG L 304**	**PORG N 502**	**PORG N 501**	**POKG L 308**	**POKG P 2018**	**POKG P 2017**
Vuninzara	R	R	S	R	R	R	R	R	R	R	S	R	R
Gwizumwimbu	R	S	R	R	R	R	R	R	R	S	R	R	R
Mugwiza	R	R	R	R	R	R	R	R	R	R	R	R	R
Kigori	R	S	R	R	R	R	R	R	R	R	R	R	R
Watt	R	R	R	S	R	R	R	R	R	S	R	R	R
Kabuye	R	R	R	R	R	R	S	R	R	R	R	R	R
V18	R	R	R	R	R	R	R	R	R	R	R	R	R
Karundi	S	R	S	S	S	R	R	R	R	S	R	R	R
Buname	R	R	R	R	R	R	S	R	R	R	R	R	R
Rufutamadeni	R	R	R	R	R	R	R	R	R	R	R	R	R

POKG: *Pyricularia oryzae* isolates from HA (Buyenzi region), PORG: *Pyricularia oryzae* isolates from MA (Mosso region)

With exception of the three cultivars; Mugwiza, V18 and Rufutamadeni, which showed resistance to all isolates of *P.oryzae,* the other seven cultivars reacted differently to the different isolates. Karundi showed resistance to 8 isolates but was susceptible to 5 isolates. Cultivar Vuninzara, Rwizumwimbu and Watt showed resistance to 11 isolates and susceptibility to 2 isolates. And finally, Kigori, Kabuye and Buname cultivars were resistant to 12 isolates and susceptible to 1 isolate (Table 5.6).

In addition, cultivars that showed compatible reactions (Susceptible) to rice blast disease were recorded with a high disease severity value. For example, Karundi was susceptible to disease and had a high value of rice blast severity of 69.14%; Buname and Kabuye were susceptible and had a high value of blast severity of 55.56%. However, cultivars with incompatible reactions (Resistance) to the disease had lower disease severity values. For instance, the cultivars Mugwiza, Rufutamadeni and V18 were resistant and their values of blast severity ranged from 3.7- 6.17% (Table 5.5 and 5.6).

5.5 Discussion

This study has established that reactions of different rice cultivars were different to have resistance traits to all the thirtheen isolates from both middle and high altitude rice growing zones of Burundi. Also, the study has established that for all the thirtheen *P.oryzae* isolates were diferent and the reaction of rice cultivar to a particular isolates varied depending on the region where the cultivar is grown. This observation can be linked to differences in environment characteristics reffering to the data on rainfall and temperature described in Table 3.1 in Chapter three on rice blast disease occurrence on rice cultivars in two agro-ecologies of Burundi. During this study, it was noted that the temperature of HA and MA were 18.8^{o}C and21.7^{o}C respectively. Similarly rainfall in HA and MA zones were 122.4 mm and 11.7 mmm suggesting that the activity of

P.oryzae isolates was dependent on these environmental factors. The observations are in line with Chuwa (2016) who reported that due to the variation in favourable climatic factors (temperature, relative humidity and rainfall), the rice blast disease recorded varied according to the results found during his study in Mbeya and Morogoro regions in Tanzania. Similarly, Muñoz (2008) revealed that the environmental conditions have a strong influence on the development of rice blast disease.

Differences in pathogenicity among isolates suggesting that the cultivars had different compatibilities with isolates hence different level of significance. For example (1) POKGP showed a higher level compatible reaction on cultivar karundi, while it was lowest for others cultivars (2) PORGP301 showed higher level compatible reaction on cultivar Buname and kabuye, while it is the lowest for other cultivars. (3) PORGP603, PORGN502, POKGP2017 and POKGP2018 showed the lowest level compatible reaction with all cultivars. The observation confirm previous findings by Asfasha *et al*. (2015) who found that the incidence/severity of the disease varied from low to high depending on the location where cultivars are grown.

The results found in this study showed that cultivars such as Mugwiza, Rufutamadeni and V18 cultivars were found to have traits of resistance against P.oryzae. This in line with results found by Nsanzineza (2021) in his study in Imbo plain of Burundi who reported that some cultivars had traits of resistance against rice blast disease. The variability in pathogen pathogenicity could be due to changes that frequently occur in *P. oryzae* avirulence genes due to their unstable nature (Orbach *et al*., 2000, Khadka *et al*., 2013). The virulence of the pathogen can be strongly influenced by the varieties from which they were isolated based on nutritional differences between rice varieties

(Bonman *et al*., 1987). With this variation, it is important to select resistant genotypes for use in disease management for better agricultural production.

5.6 Conclusion and Recommendations

The results of this study indicated that the strains of *P.oryzae* used differ in the pathogenic pattern. The identified strains of *P. oryzae* differed in their pathogenicity profiles between cultivars and *P.oryzae* isolates. Of the ten rice cultivars, only three were resistant (R) to all isolates of *P. oryzae*. This study allows concluding that the use of Mugwiza, Rufutamadeni and V18 cultivars in rotation could be a way out tf maintaining the Pyricularia oryzaepopulation. Also can be used as source of resistance genes for crop improvement programs to improve disease management and yield in Burundi. Further testing of these isolates should be performed in order to fully understand the phenotypic and genotypic nature of these isolates.

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CHAPTER SIX

6.0 GENERAL CONCULUSION AND RECOMMENDATIONS

6.1 General Conclusion

Rice blast disease is major constraints in all rice growing agro-ecologies including the High altitude (Buyenzi region) and Middle altitude (Mosso region) agro ecological zones of Burundi. The purpose of this study was to assess the incidence and severity of blast disease on rice cultivars in high and middle altitudes agro-ecologies. Further the study amide to establish the genetic relatedness of rice blast (*Pyricularia oryzae*) isolates from Buyenzi region and Mosso region and the pathogenicity of these isolates in different cultivated rice cultivars.

The results indicated that rice blast disease incidence and severity was significantly different between high and middle altitudes agro ecologies. However, significant difference of rice blast disease incidence and severity was registered on between cultivars at different growth stages (Tillering, Booting and Dough stage) in the two agro-ecologies of Burundi. V18, Watt and Mugwiza cultivars showed avurilence to the rice blast disease. High altitude agroecological zone showed a high incidence and severity of disease than the Middle altitude agroecological zone. The variations of incidence and severity of rice blast disease in two agroecologies zones associated to the environmental conditions factors. From the study of isolate genetic relatedness, the findings confirmed that the isolates of *Pyricularia oryzae* were of rice hosts from Burundi and there are narrow genetic variations among the isolates of *P. oryzae* isolated from rice and other host species. The observed variations in the genomic may lead the isolates to be more virulent or avirulent, hence calling for more studies to understand the pathogenicity level among the analyzed isolates.

Through the artificial inoculation, the results showed high pathogenic variation among isolates and cultivars on incidence and severity altitude. The isolates of *P. oryzae* varied in their pathogenicity profiles between cultivars and isolates. Of the ten rice cultivars, only three showed incompatible reaction (Mugwiza, Rufutamadeni and V18) can be advised to rice farmers than to exchange seeds from places unkwon.

6.2 Recommendations

Based on the current study, the following recommendations have been made**:**

i. The variations of incidence and severity of rice blast disease in two agro-ecologies zones were related to the environmental conditions factors and also the exchange seeds from places unkwon by farmers' rice. Therefore, further research is need that will develop cultivars for resistant to rice blast disease

ii. Cultivar development will need to consider environmental conditions of different locations and cultural practices because in this study, significant difference between environments on the occurrence of rice blast disease.

iii. The rice cultivars V18, Watt and Mugwiza showed less virulence to the blast disease under field conditions. Therefore, an experiments trial for another cropping season is needed to confirm its level resistance to rice blast disease fungal.

iv. The isolates of *Pyricularia oryzae* from two agro-ecologies showed genetic diversity among them and will be deposed in GenBank for their numbers for futher use. Therefore, other studies are needed to extend the genetic diversity and relationship between isolates of *P. oryzae* in Burundi.

v. The rice cultivars Mugwiza, Rufutamadeni and V18 cultivars showed incompatible reaction to rice blast disease. Therefore, those cultivars can be advised to only three showed incompatible reaction (Mugwiza, Rufutamadeni and V18) can be advised to rice farmers in rotation for the management of the rice blast disease, but also can be used as a source of resistance genes for crop improvement programs to improve disease management and yield in Burundi.

vi. Further testing of *Pyricularia oryzae* isolates are need to perform in order to fully understand the phenotypic and genotypic nature of these isolates.

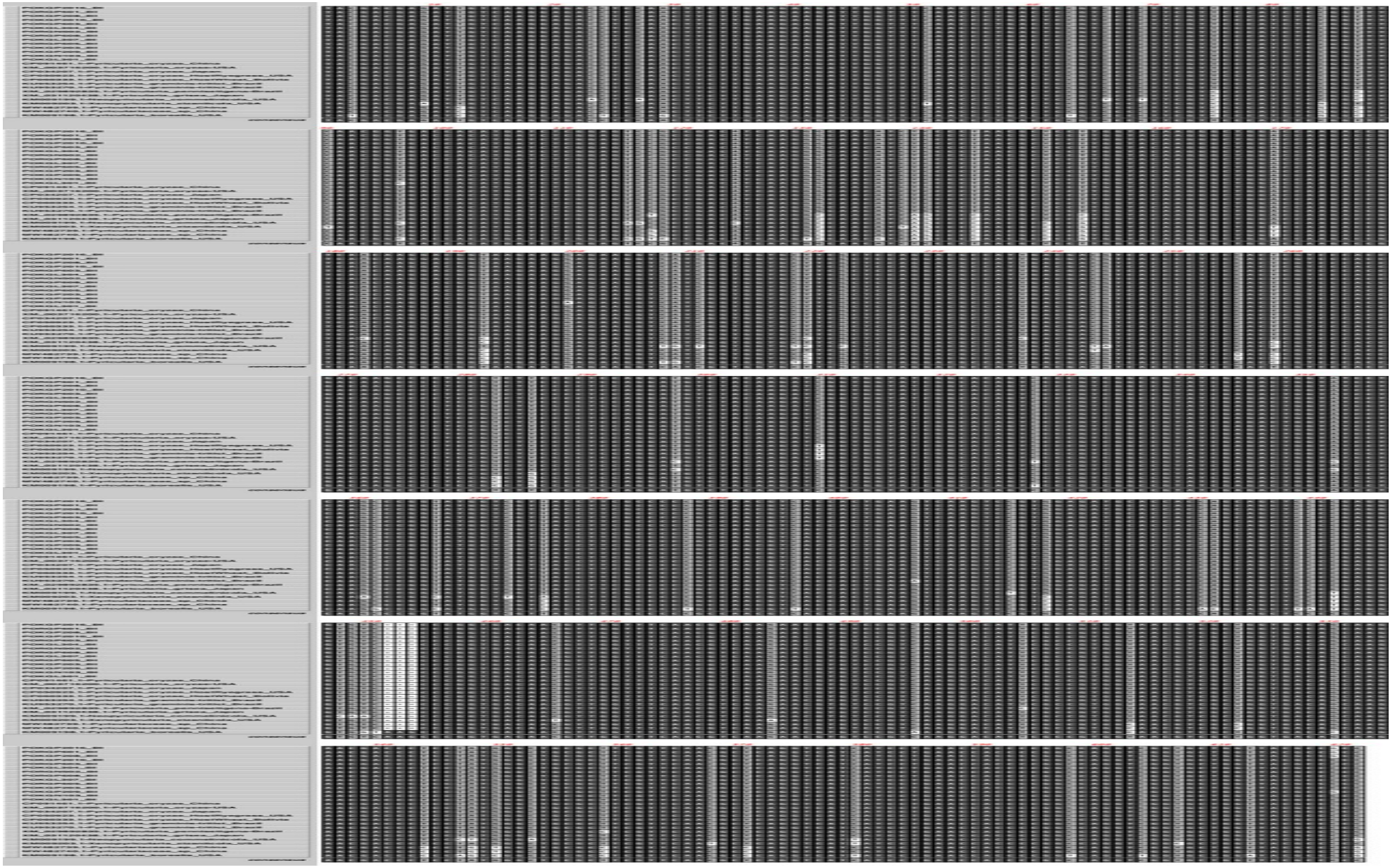
vii. Further studies to evaluate the incidence and severity of rice blast in relation to paddy yield and genetic diversity of isolates are needed to enhance the effectiveness of rice blast disease management for other locations not yet covered by the current study.

APPENDICES

Appendix 1: ***Pyricularia oryzae*** **detection from isolates collected from Rice samples of Burundi**

Sample ID	Isolate name	ITS 1F and 2R	ITS 1F and 4R	ITS3F and 4R	ITS 4F and 5R	EF1-983F and EF1-2218R
1	POKGP2018	1	1	1	1	1
2	POKGL308	1	0	1	1	0
3	POKGP2011	0	0	0	0	0
4	PORGN501	1	1	1	1	1
5	PORGN502	1	1	1	1	1
6	POKGL304	1	1	1	1	1
7	POKGP2014	1	1	1	1	1
8	POKGL301	1	1	1	1	1
9	PORGP603	1	1	1	1	1
10	POKGP208	1	1	1	1	1
11	POKGN106	0	0	0	0	0
12	PORGP601	1	1	1	1	1
13	POKGP2010	1	1	1	1	1
14	POKGP206	0	0	0	1	0
15	POKGL307	1	1	1	1	1
16	POKGN103	1	1	1	1	1
17	PORGP605	1	1	1	0	0
18	POKGP209	1	1	1	1	1
19	POKGP2016	1	1	1	1	1
20	POKGP203	1	1	1	1	1
21	PORGP608	1	1	1	1	0
22	POKGP205	0	0	0	1	0

23	POKGP2013	1	1	1	0	1
24	POKGP2012	0	0	0	1	0
25	POKGN101	0	0	0	0	0
26	POKGP202	1	0	1	0	0
27	POKGP201	0	0	0	1	0
28	POKGP207	0	0	0	0	0
29	POKGP2017	1	0	1	0	0
30	POKGN107	0	0	0	1	0
31	POKGP204	0	0	0	0	0
32	PORGP606	1	0	1	0	0
33	PORGP604	1	1	1	0	0
34	PORGP607	1	1	1	0	0
35	PORGP602	1	0	1	1	0
Negative Control	-	0	0	0	0	0
Total scores	-	25	20	25	23	16



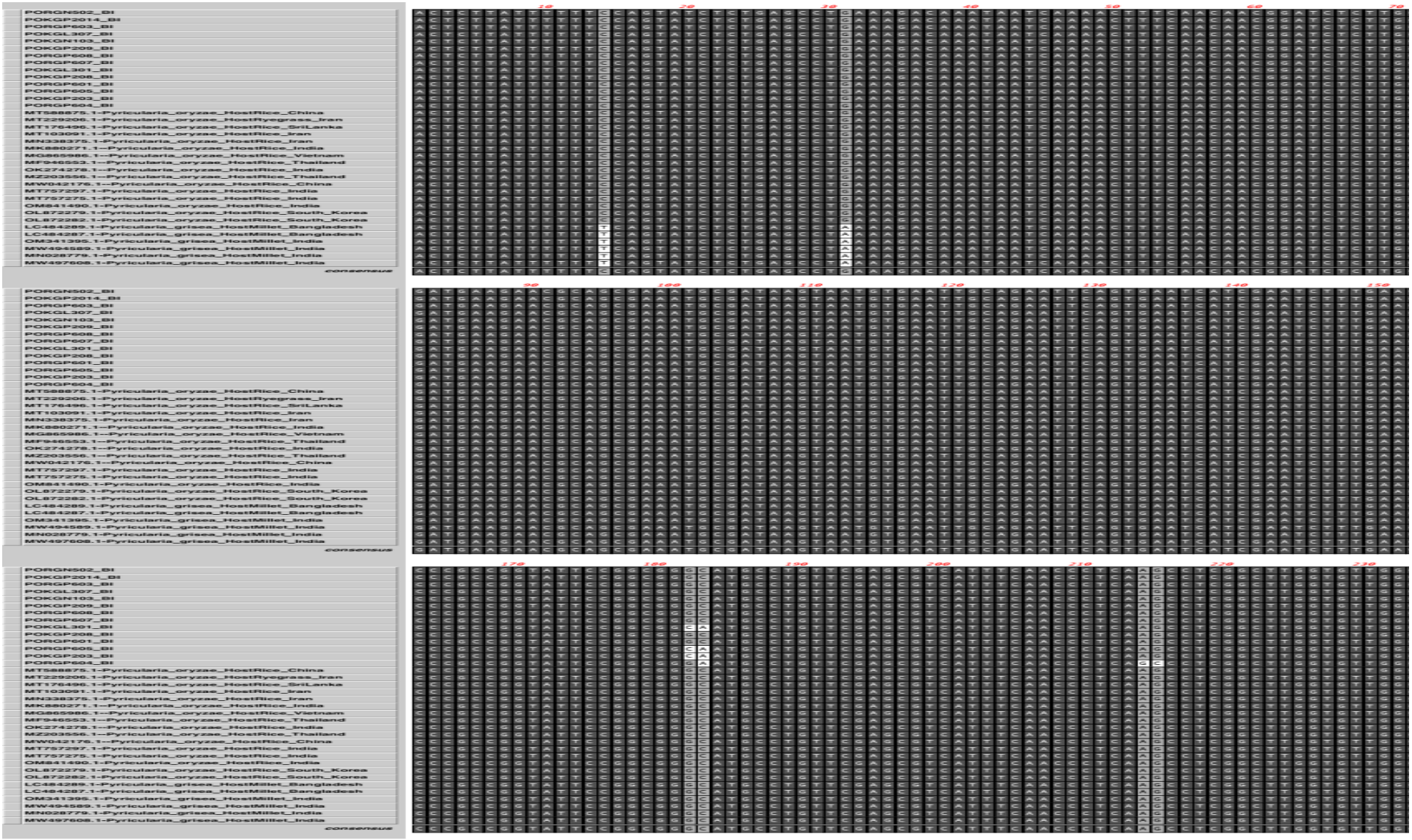
Appendix 3: Sequence Alignment obtained from ITS –DNA sequence using ITS1 and ITS4 primers

EF1- Similarity matrix

	POKGP2016_BI	PORGP601_BI	POKGP208_BI	POKGP2010_BI	PORGP603_BI	POKGL304_BI	PORGN502_BI	PORGP608_BI	POKGL307_BI	PORGP605_BI	POKGP203_BI	POKGP209_BI	POKGN103_BI	POKGL301_BI	CP091461.1-Pyricularia_oryzae_China	XM_003716200.1-Pyricularia_oryzae-USA	CP034207.1-Pyricularia_oryzae_Japan	CP050923.1-Pyricularia_oryzae_HostRyegrass_USA	CP060333.1-Pyricularia_oryzae_HostWheat_Bolivia	KU953254.1-Pyricularia_graminis-tritici_Brazil	KU953270.1-Pyricularia_graminis-tritici_Brazil	XM_029892835.1-Pyricularia_pennisetigena-Brazil	XM_031120438.1-Pyricularia_grisea_Japan	KM009199.1-Neopyricularia_commelinicola_USA	KM009203.1-Xenopyricularia_zizaniicola_USA	MW482739.1-Pyriculariaceae_sp_China	MW482740.1-Pyriculariaceae_sp_China	KM009198.1-Pyricularia_borealis_USA
POKGP2016_BI	100	100	100	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	100	99.7	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	93.9	95.5	95.1	95.1	90.2
PORGP601_BI	100	100	100	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	100	99.7	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	93.9	95.5	95.1	95.1	90.2
POKGP208_BI	100	100	100	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	100	99.7	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	93.9	95.5	95.1	95.1	90.2
POKGP2010_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
PORGP603_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
POKGL304_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
PORGN502_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
PORGP608_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
POKGL307_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
PORGP605_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
POKGP203_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
POKGP209_BI	100	100	100	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	100	99.7	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	93.9	95.5	95.1	95.1	90.2
POKGN103_BI	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7	100	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	93.9	95.5	95.1	95.1	90.2
POKGL301_BI	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7	99.7	100	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	94.2	95.5	95.1	95.1	90.5
CP091461.1-Pyricularia_oryzae_China	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
XM_003716200.1-Pyricularia_oryzae-USA	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
CP034207.1-Pyricularia_oryzae_Japan	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
CP050923.1-Pyricularia_oryzae_HostRyegrass_USA	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7	99.7	99.7	99.8	99.8	99.8	100	100	100	99.8	97.1	98.4	93.9	95.5	95.1	95.1	90.2
CP060333.1-Pyricularia_oryzae_HostWheat_Bolivia	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7	99.7	99.7	99.8	99.8	99.8	100	100	100	99.8	97.1	98.4	93.9	95.5	95.1	95.1	90.2
KU953254.1-Pyricularia_graminis-tritici_Brazil	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7	99.7	99.7	99.8	99.8	99.8	100	100	100	99.8	97.1	98.4	93.9	95.5	95.1	95.1	90.2
KU953270.1-Pyricularia_graminis-tritici_Brazil	99.5	99.5	99.5	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.5	99.5	99.5	99.7	99.7	99.7	99.8	99.8	99.8	100	96.9	98.2	93.7	95.3	95	95	90
XM_029892835.1-Pyricularia_pennisetigena-Brazil	97.1	97.1	97.1	97.2	97.2	97.2	97.2	97.2	97.2	97.2	97.2	97.1	97.1	97.1	97.2	97.2	97.2	97.1	97.1	97.1	96.9	100	98.4	94.3	95	95.8	95.8	91.3
XM_031120438.1-Pyricularia_grisea_Japan	98.4	98.4	98.4	98.5	98.5	98.5	98.5	98.5	98.5	98.5	98.5	98.4	98.4	98.4	98.5	98.5	98.5	98.4	98.4	98.4	98.2	98.4	100	95	96.3	96.6	96.6	91.6
KM009199.1-Neopyricularia_commelinicola_USA	93.9	93.9	93.9	94	94	94	94	94	94	94	94	93.9	93.9	94.2	94	94	94	93.9	93.9	93.9	93.7	94.3	95	100	92.9	93.4	93.4	90.8
KM009203.1-Xenopyricularia_zizaniicola_USA	95.5	95.5	95.5	95.6	95.6	95.6	95.6	95.6	95.6	95.6	95.6	95.5	95.5	95.5	95.6	95.6	95.6	95.5	95.5	95.5	95.3	95	96.3	92.9	100	95.3	95.3	90.7
MW482739.1-Pyriculariaceae_sp_China	95.1	95.1	95.1	95.3	95.3	95.3	95.3	95.3	95.3	95.3	95.3	95.1	95.1	95.1	95.3	95.3	95.3	95.1	95.1	95.1	95	95.8	96.6	93.4	95.3	100	100	93.7
MW482740.1-Pyriculariaceae_sp_China	95.1	95.1	95.1	95.3	95.3	95.3	95.3	95.3	95.3	95.3	95.3	95.1	95.1	95.1	95.3	95.3	95.3	95.1	95.1	95.1	95	95.8	96.6	93.4	95.3	100	100	93.7
KM009198.1-Pyricularia_borealis_USA	90.2	90.2	90.2	90.3	90.3	90.3	90.3	90.3	90.3	90.3	90.3	90.2	90.2	90.5	90.3	90.3	90.3	90.2	90.2	90.2	90	91.3	91.6	90.8	90.7	93.7	93.7	100

** Similarity Scores (%) **

Appendix 4: Matrix similarity obtained from EF-DNA sequence using EF1- 983 and EF1-2218 primers



Appendix 5: Matrix similarity obtained from ITS –DNA sequence using ITS1 and ITS4 universal primers

	POKGP2016_BI	PORGP601_BI	POKGP208_BI	POKGP2010_BI	PORGP603_BI	POKGL304_BI	PORGN502_BI	PORGP608_BI	POKGL307_BI	PORGP605_BI	POKGP203_BI	POKGP209_BI	POKGN103_BI	POKGL301_BI	CP091461.1-Pyricularia_oryzae_China	XM_003716200.1-Pyricularia_oryzae-USA	CP034207.1-Pyricularia_oryzae_Japan	CP050923.1-Pyricularia_oryzae_HostRyegrass_USA	CP060333.1-Pyricularia_oryzae_HostWheat_Bolivia	KU953254.1-Pyricularia_graminis-tritici_Brazil	KU953270.1-Pyricularia_graminis-tritici_Brazil	XM_029892835.1-Pyricularia_pennisetigena-Brazil	XM_031120438.1-Pyricularia_grisea_Japan	KM009199.1-Neopyricularia_commelinicola_USA	KM009203.1-Xenopyricularia_zizaniicola_USA	MW482739.1-Pyriculariaceae_sp_China	MW482740.1-Pyriculariaceae_sp_China	KM009198.1-Pyricularia_borealis_USA
POKGP2016_BI	100	100	100	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	100	99.7	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	93.9	95.5	95.1	95.1	90.2
PORGP601_BI	100	100	100	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	100	99.7	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	93.9	95.5	95.1	95.1	90.2
POKGP208_BI	100	100	100	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	100	99.7	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	93.9	95.5	95.1	95.1	90.2
POKGP2010_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
PORGP603_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
POKGL304_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
PORGN502_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
PORGP608_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
POKGL307_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
PORGP605_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
POKGP203_BI	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
POKGP209_BI	100	100	100	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	100	99.7	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	93.9	95.5	95.1	95.1	90.2
POKGN103_BI	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7	100	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	93.9	95.5	95.1	95.1	90.2
POKGL301_BI	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7	99.7	100	99.8	99.8	99.8	99.7	99.7	99.7	99.5	97.1	98.4	94.2	95.5	95.1	95.1	90.5
CP091461.1-Pyricularia_oryzae_China	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
XM_003716200.1-Pyricularia_oryzae-USA	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
CP034207.1-Pyricularia_oryzae_Japan	99.8	99.8	99.8	100	100	100	100	100	100	100	100	99.8	99.8	99.8	100	100	100	99.8	99.8	99.8	99.7	97.2	98.5	94	95.6	95.3	95.3	90.3
CP050923.1-Pyricularia_oryzae_HostRyegrass_USA	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7	99.7	99.7	99.8	99.8	99.8	100	100	100	99.8	97.1	98.4	93.9	95.5	95.1	95.1	90.2
CP060333.1-Pyricularia_oryzae_HostWheat_Bolivia	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7	99.7	99.7	99.8	99.8	99.8	100	100	100	99.8	97.1	98.4	93.9	95.5	95.1	95.1	90.2
KU953254.1-Pyricularia_graminis-tritici_Brazil	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.7	99.7	99.7	99.8	99.8	99.8	100	100	100	99.8	97.1	98.4	93.9	95.5	95.1	95.1	90.2
KU953270.1-Pyricularia_graminis-tritici_Brazil	99.5	99.5	99.5	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.5	99.5	99.5	99.7	99.7	99.7	99.8	99.8	99.8	100	96.9	98.2	93.7	95.3	95	95	90
XM_029892835.1-Pyricularia_pennisetigena-Brazil	97.1	97.1	97.1	97.2	97.2	97.2	97.2	97.2	97.2	97.2	97.2	97.1	97.1	97.1	97.2	97.2	97.2	97.1	97.1	97.1	96.9	100	98.4	94.3	95	95.8	95.8	91.3
XM_031120438.1-Pyricularia_grisea_Japan	98.4	98.4	98.4	98.5	98.5	98.5	98.5	98.5	98.5	98.5	98.5	98.4	98.4	98.4	98.5	98.5	98.5	98.4	98.4	98.4	98.2	98.4	100	95	96.3	96.6	96.6	91.6
KM009199.1-Neopyricularia_commelinicola_USA	93.9	93.9	93.9	94	94	94	94	94	94	94	94	93.9	93.9	94.2	94	94	94	93.9	93.9	93.9	93.7	94.3	95	100	92.9	93.4	93.4	90.8
KM009203.1-Xenopyricularia_zizaniicola_USA	95.5	95.5	95.5	95.6	95.6	95.6	95.6	95.6	95.6	95.6	95.6	95.5	95.5	95.5	95.6	95.6	95.6	95.5	95.5	95.5	95.3	95	96.3	92.9	100	95.3	95.3	90.7
MW482739.1-Pyriculariaceae_sp_China	95.1	95.1	95.1	95.3	95.3	95.3	95.3	95.3	95.3	95.3	95.3	95.1	95.1	95.1	95.3	95.3	95.3	95.1	95.1	95.1	95	95.8	96.6	93.4	95.3	100	100	93.7
MW482740.1-Pyriculariaceae_sp_China	95.1	95.1	95.1	95.3	95.3	95.3	95.3	95.3	95.3	95.3	95.3	95.1	95.1	95.1	95.3	95.3	95.3	95.1	95.1	95.1	95	95.8	96.6	93.4	95.3	100	100	93.7
KM009198.1-Pyricularia_borealis_USA	90.2	90.2	90.2	90.3	90.3	90.3	90.3	90.3	90.3	90.3	90.3	90.2	90.2	90.5	90.3	90.3	90.3	90.2	90.2	90.2	90	91.3	91.6	90.8	90.7	93.7	93.7	100