Genetic Relatedness of Rice Blast Fungus (*Pyricularia oryzae*) Isolates from Two Agro Ecologies of Burundi

Niyonkuru, E.[1]*; R.R. Madege[1]; J. Bigirimana[2]; G. Habarugira[2] and D.P. Massawe[3]

[1]Sokoine University of Agriculture, College of Agriculture, Department of Crop Science and Horticulture
[2]International Rice Research Institute-Burundi (IRRI-Burundi)
[3]Plant Molecular Biology Laboratory at Sokoine University of Agriculture (SUA).

*Corresponding author's email: *richmadege@sua.ac.tz Tel: +255 688248994*

Abstract

Rice blast disease caused by fungus Pyricularia oryzae is one of the most destructive diseases in rice-producing areas of Burundi. 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 a 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.

Keywords: Rice blast, DNA extraction, PCR, Molecular characterization, Genetic diversity

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 is rice blast pathogen Devi *et al*., 2015, Miah *et al*., 2013,). Rice blast disease 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 along with good agricultural practices is the most effective and economical way to fight against the disease. Unfortunately, genetic resistance is often confronted with resistance braking due to various environmental and biological factors (Fukuta, 2014). In Philippines, *P. oryzae* exhibits high genetic diversity, narrow host range and even varietal specificity (Leung *et al*., 1988). Pathogenicity of *P. oryzae* isolates differs between rice varieties (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 difference in virulence between the rice isolates makes the selection for resistance even more difficult (Marangoni *et al*., 2013). Another constraint to developing rice varieties with complete resistance to rice blast is the pathogen's characteristic of the sexuality and mitosis which allow them to adapt very quickly to the changing environmental condition (McDonald and Linde, 2002). Similarly, parasexual recombination has been identified as one of the means of variation of *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 each 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 technique for distinguishing between closely related strains of *P. oryzae*. This study aimed to determine the genetic diversity of *P. oryzae* isolates to establish their phylogenetic relation of *P. oryzae* in the high and middle altitude agro-ecological zones of Burundi.

Materials and Methods

Sampling

Sixty (60) household farms were randomly selected from each of the two agro ecological zones (high and middle altitude) (Fig. 1), using a Stratified Random Sampling procedure (Boschetti *et al*., 2006). Sampling plant materials was done by collecting plants with symptoms of blast: 60 leaves, 60 nodes and 60 panicles from both Buyenzi (High altitude or HA) and and Mosso (Middle Altitude or MA) regions (Fig. 2). Samples were kept in labeled envelops gathered in transparent bags at low temperature (cool box) and later in refrigerator maintained at 3-5°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.

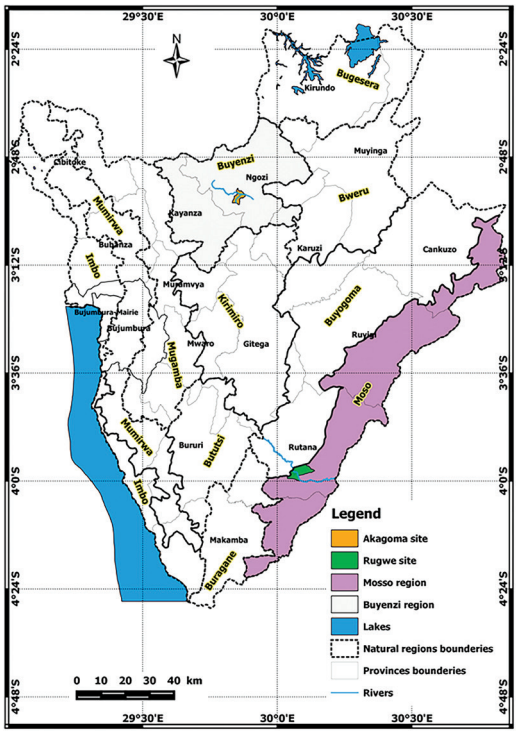

Figure 1: Map of Burundi showing the study locations (Buyenzi and Mosso regions)

Isolation of *Pyricularia oryzae*

Isolation of *P. oryzae* was done by cutting infected leaves, nodes, and panicles into small pieces (5cm) small piece 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 24 hours, fungal spores were harvested, with a glass needle, plated on Water Agar medium and incubated for 24 hours at room temperature 25°C). This was followed by microscopic observation to identify germinating spores after which a monosporic culture was established in Potato-Dextrose-Agar (PDA) medium (IRRI, 2013).

Molecular characterization of *Pyricularia oryzae*

DNA extraction

Total DNA was extracted from 35 isolates

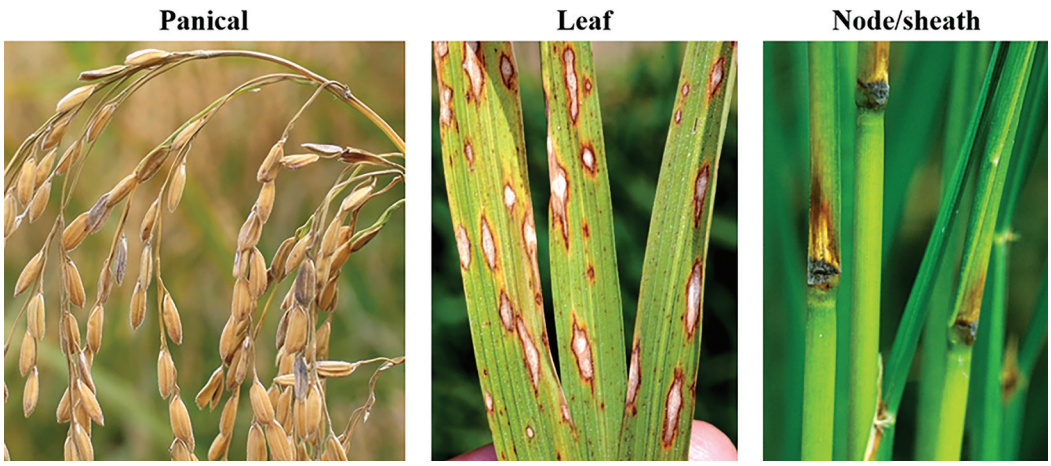

Figure 2: Panicles, leaves and node/sheath with rice blast symptoms

of *P. oryzae* (Table 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 into the 2 ml Eppendortf tube. Mortar and pestle were used to macerate 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 the 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 25°C. The DNA pellets were washed with 500 µl of ethanol and centrifuged at 1300xg for 3 min at 25°C (the process was repeated 3 times). The pellets were dried under vacuum and the DNA was dissolved using the hotplate at 65°C for 30 min by adding 50 µl Tris (10 µl).

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 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 cycler machine. Thermal cycling followed: 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. After adding loading buffer in each DNA sample, an agarose gel was prepared according to Longya *et al*. (2020) and fater solifying the gel box with 1xTAE (or TBE) were filled until the gel is covered followed by loading a molecular weight ladder into the first lane of the gel. The PCR products were separated in a 1.2% agarose gel at 120 volts for 1hour 30min (Muni *et al*., 2014), stained with ethidium bromide and results scored in a gel documentation system. 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 1: Geographical origin of isolates and the diseade plant parts from which isolates were obtained

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

Table 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

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 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.

Results

PCR amplicons for each isolate

Thirty-five (35) isolates 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 and 7 isolates from MA (Fig. 3-6).

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 High altitude Agroecological zone (HA AEZ); and isolates PORGP606, PORGP604, PORGP607; PORGP602, PORGP605 and PORGP608 from Middle altitude Agroecological zone (MA AEZ). ITS1F and ITS2R 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*

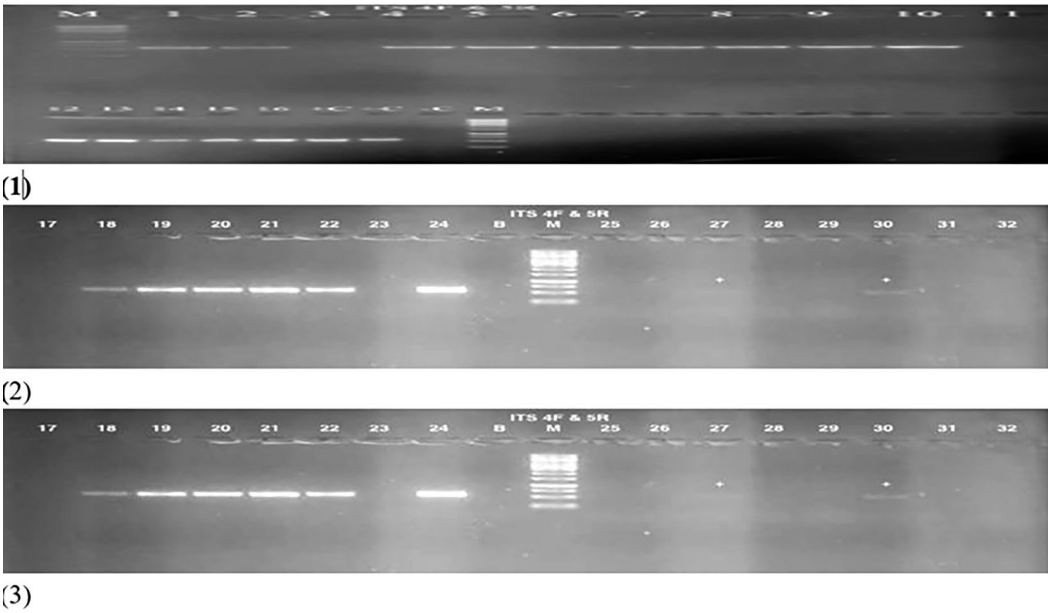

Figure 3: Amplification using primer ITS 4F and 5R (350bp) with (1) Sample number number1 - 16 Negative control as –C; and positive control as +C, (2)Sample number number 17 - number 32 Negative control as B; and (3) Sample number33 - 35 Negative control as B and positive controls as F1-F5

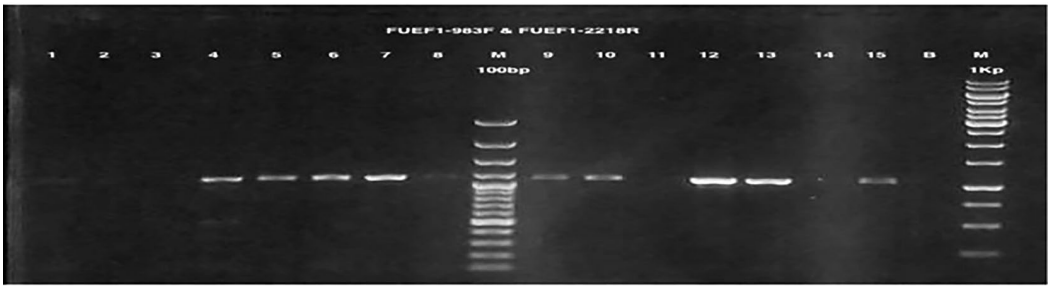

Figure 4: Amplification using primer EF1-983F and EF1-2218R (1235) with Sample number1 - 15 Negative control as B

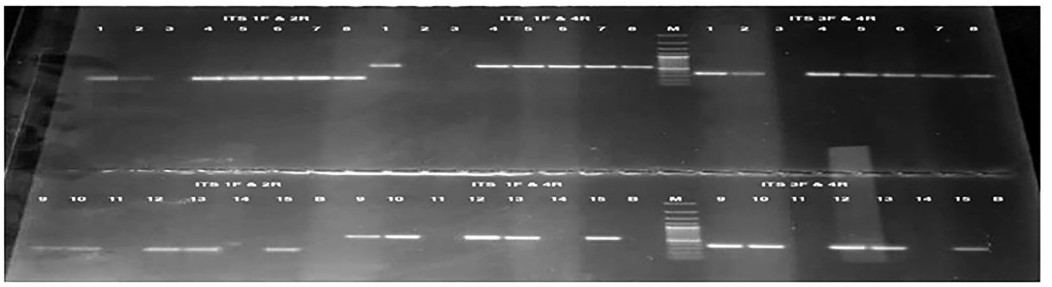

Figure 5: Amplification using primer ITS1F and 2R (220bp), 1F and 4R (550bp), 3F and 4R (390bp) with Sample number1 - #15 Negative control as B

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

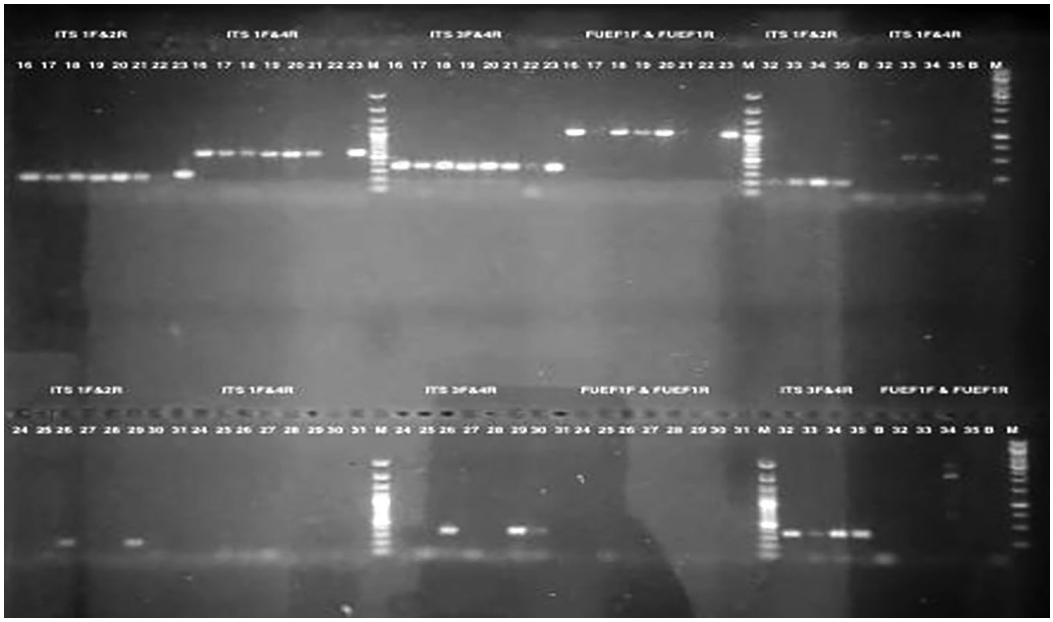

Figure 6: Amplification using primers EF1-983F and EF1-2218R (1235bp), ITS1F and 2R (220bp), 1F and 4R (550bp), 3F and 4R (390bp) with Sample number 16 - 35 Negative control as B

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 was 390bp, 350bp and 1235 bp respectively.

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

The sequencing results for *Pyricularia oryzae*, confimed that somes isolates (POKGP2010, POKGL304, POKGL307, POKGP203 and POKGL301) from high altitude (Buyenzi region) and somes isolates (PORGP603, PORGN502, PORGP608, PORGP605) from middle altitude (Mosso region) AEZ of Burundi are *Pyricularia oryzae,* specific from rice host species. Based on the results found in alignment and similarity matrix, the isolates POKGP2016, PORGP601, POKGP208, POKGL301, POGN103, PORGP605, POKGP203 and PORGP604 showed single nucleotide polymorphism specific of *P. oryzae*.

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) (Fig. 7).

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). The nucleotide similarity of isolates from Burundi ranges from 99.7 - 100%. Neopyricularia and Xenopyricularia have similarities of 93.9% and 95.5% respectively with Pyricuralia spp. Borealis from USA was distantly related with other Pyricularia species by 90.7%.

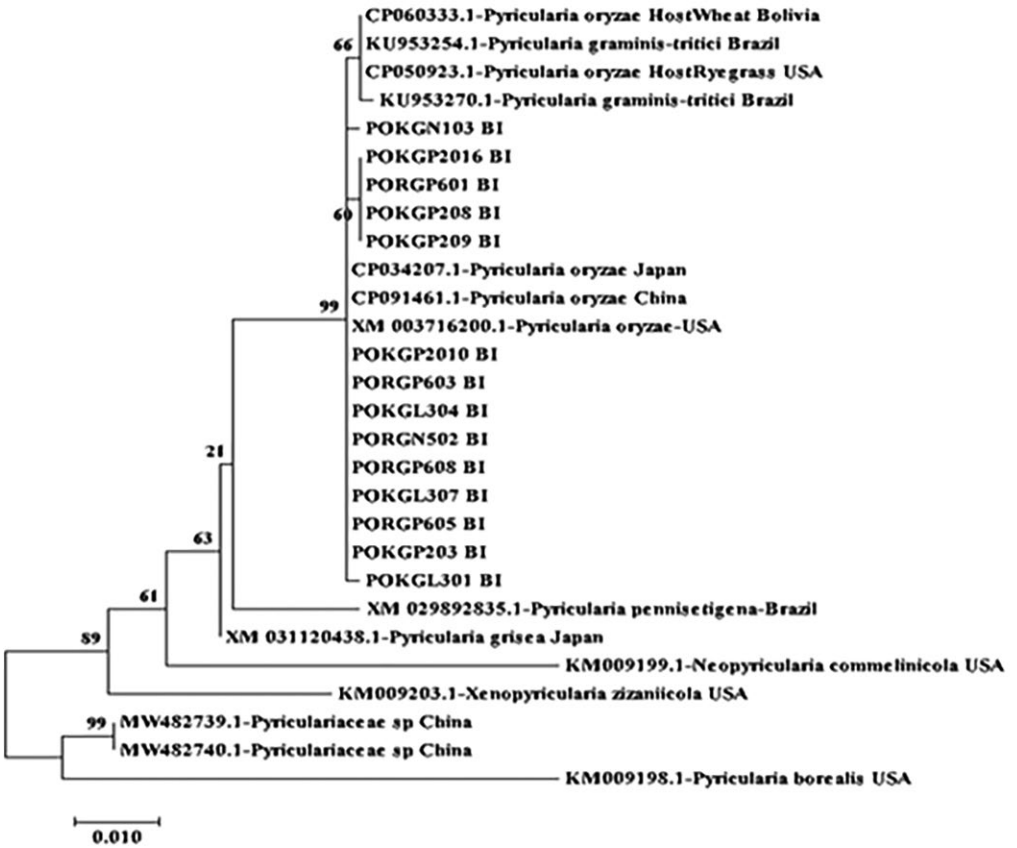

Figure 7: A phylogenetic tree of *Pyricularia oryzae* fungus based on dataset of the Elongation Factor (EF) constructed using the maximum likelihood with 1000 bootstrapping values in MEGA 7 software

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 (Fig. 8).

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).

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 isolates from other country with nucleotide similarity ranged from 97.8-100%.

Discussion

Molecular markers have been used to characterize fungal plant pathogen populations, for characterization of *P. oryzae*. Based on this study, some isolates were amplified and other not

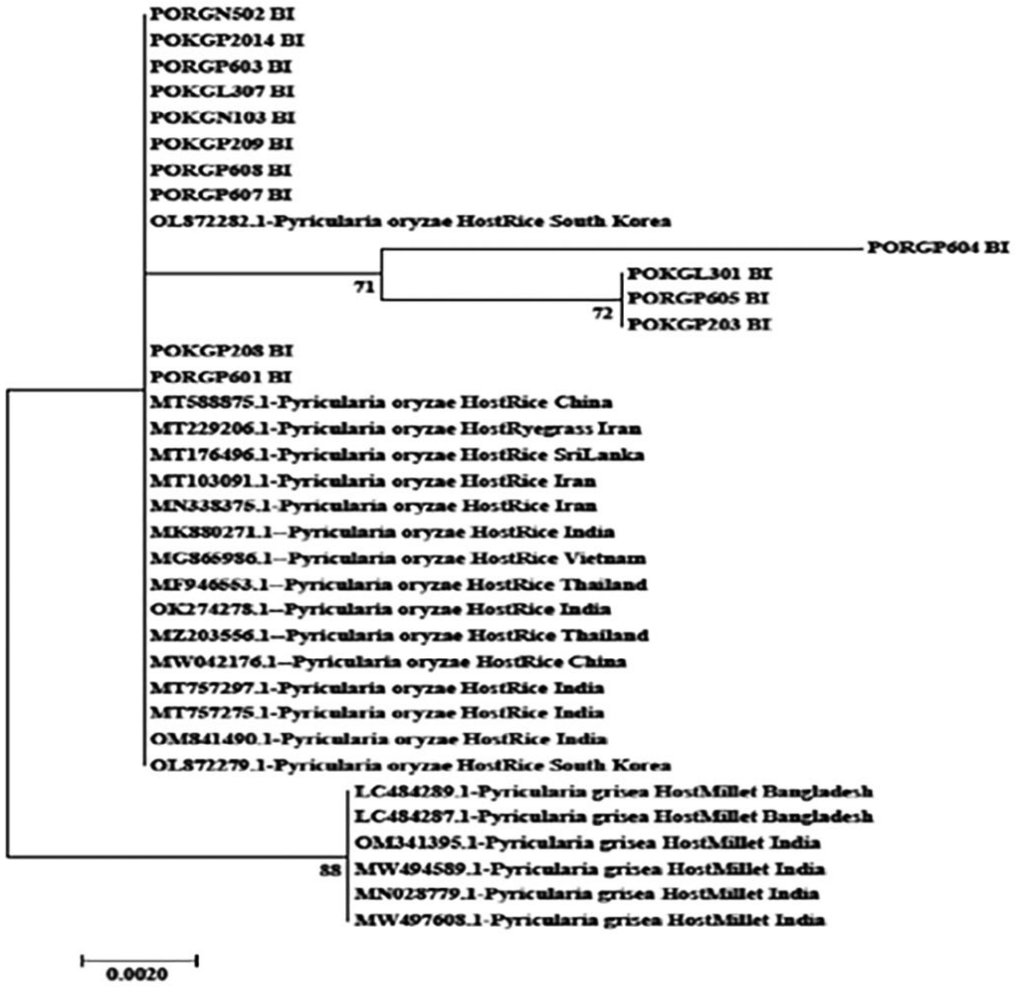

Figure 8: A phylogenetic tree of *Pyricularia oryzae* fungus based on dataset of Internal Transcribed Spacer (ITS) constructed using the maximum likelihood with 1000 bootstrapping values in MEGA 7 software

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) on molecular characterization of *P. oryzae* causative agent of rice blast in Tanzania, 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 had 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 the genetic diversity of *P. oryzae* in Burundi is due to their origin as they comes from different hosts or locations. The findings are in line with several previous reports. Longya *et al*. (2020) when they were studying the characterization and genetic diversity of *Pyricularia oryzae* using ISSR and SRAP markers Gladieux *et al*. (2018) in their study on Gene flow between divergent cereal-and grass-specific lineages of the rice blast. Qi *et al*. (2019) 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 in the two regions. Some derived isolates were from hosts other than rice, Ryegrass, millet, wheat and Graminis-tritic for different locations conforming with the findings Maciel *et al*. (2014) 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) who reported that some Pyricularia isolates from India can infect perennial Ryegrass.

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, 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.

Acknowledgements

The authors thank the International Rice Research Institute for financial support and the technical assistance of laboratory staff in IRRI Burundi and SUA Tanzania.

Declaration of competing for interest

The manuscript has no competing interests.

Funding

This work was supported by the World Bank for the Regional Project for Integrated Agricultural Development of the Great Lakes Region (PRDAIGL) through the International Rice Research Institute (IRRI-Burundi).

References

Abed-Ashtiani, F., Kadir, J., Nasehi, A., Hashemian-Rahaghi, S.R., Vadamalai, G. and Rambe, S.K. (2016). Characterisation of Magnaporthe oryzae isolates from rice in peninsular Malaysia. *Czech Journal of Genetics and Plant Breeding* 52(4): 145-156. http://dx.doi.org/10.17221/102/2016-CJGPB

Baramburiye, J. (2010). Baseline seed study for Burundi harmonization of seed policies, laws and regulations. [http://afsta.org/wp content/uploads/documents/Burundiseed sector baseline study.pdf]. Site visited on 22/3/2022.

Bonman, J.M., De Dios, T.V., Bandong, J.M. and Lee, E.J. (1987). Pathogenic variability of monoconidial isolates of *Pyricularia oryzae* in Korea and in the Philippines. Plant Disease 71(2): 127-130. https://doi.org/10.1094/pd-71-0127

Boschetti, L., Brivio, P.A., Eva, H.D., Gallego, J., Baraldi, A. and Grégoire, J.M. (2006). A sampling method for the retrospective validation of global burned area products. IEEE Transactions on Geoscience and Remote Sensing 44(7): 1765-1773. https://DOI: 10.1109/TGRS.2006.874039/.

Chuwa, C.J. (2016). Rice blast disease caused by *Pyricularia oryzae*: epidemiology, characterization and yield loss in major rice growing areas of Tanzania. Doctoral dissertation, Sokoine University of Agriculture. 69-88pp. https://www.suaire.sua.ac.tz/handle/123456789/2178/.

Dar, M.S., Hussain, S., Darzi, A.B. and Bhat, S.H. (2011). Morphological variability among various isolates of Magnaporthe grisea collected from paddy growing areas of Kashmir. *International Journal of Pharmaceutical Sciences Review and Research* 8: 90-92. https://globalresearchonline.net/journalcontents/

Devi, S.R., Singh, K., Umakanth, B., Vishalakshi, B., Renuka, P., Sudhakar, K. V. and Madhav, M.S. (2015). Development and identification of novel rice blast resistant sources and their characterization using molecular markers. Rice Science 22(6): 300-308. https://doi.org/10.1016/j.rsci.2015.11.002

Fujita, S.I., Senda, Y., Nakaguchi, S. and Hashimoto, T. (2001). Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. *Journal of clinical microbiology* 39(10): 3617-3622, https://doi.org/10.1128/jcm.39.10.3617-3622.2001

Fukuta, Y., Koga, I., Ung, T., Sathya, K., Kawasaki-Tanaka, A., Koide, Y. and Hayashi, N. (2014). Pathogenicity of rice blast (*Pyricularia oryzae* Cavara) isolates from Cambodia. Japan Agricultural Research Quarterly 48(2): 155-166. https://doi:10.6090/JARQ.48.155

Gaddeyya, G., Niharika, P.S., Bharathi, P. and Kumar, P.R. (2012). Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Advances in Applied Science Research 3(4): 2020-2026 https://www.researchgate.net/publication/325854664

Gladieux, P., Condon, B., Ravel, S., Soanes, D., Maciel, J.L.N., Nhani Jr, A. and Fournier, E. (2018). Gene flow between divergent cereal-and grass-specific lineages of the rice blast fungus Magnaporthe oryzae. MBio 9(1): e01219-17. https://doi.org/10.1128/mBio.01219-17

Hosseyni-Moghaddam, M. and Soltani, J. (2013). An investigation on the effects of photoperiod, aging and culture media onvegetative growth and sporulation of rice blast pathogen *Pyricularia oryzae*. Progress in Biological Sciences 3(2): 135-143. https://doi.org/10.22059/PBS.2013.35843

Hsuan, H.M., Salleh, B. and Zakaria, L. (2011). Molecular identification of *Fusarium* species in Gibberella fujikuroi species complex from rice, sugarcane and maize from Peninsular Malaysia. *International Journal of Molecular Sciences* 12(10):6722-6732. http://dx.doi.org/10.3390/ijms12106722

IRRI (2013). Protocols de laboratoire. PBGB, Los Banos, Philippines. 58pp.

Kassankogno, A. I., Ouedraogo, I., Adreit, H., Milazzo, J., Ouedraogo, L.S., Sankara, P. and Tharreau, D. (2016). Analyse de la diversité génétique des isolats de Magnaporthe oryzae du Burkina Faso et du Togo par les marqueurs microsatellites (SSRs). *International Journal of Biological and Chemical Sciences* 10(5): 2259-2267. http://dx.doi.org/10.4314/ijbcs.v10i5.25

Khush, G.S. (2013). Strategies for increasing the yield potential of cereals: case of rice as an example. Plant Breeding 132(5): 433-436. https://doi.org/10.1111/pbr.1991

Kumar, A., Kumar, S., Kumar, R., Kumar, V., Prasad, L., Kumar, N. and Singh, D. (2010). Identification of blast resistance expression in rice genotypes using molecular markers (RAPD and SCAR). *African Journal of Biotechnology*, 9(24), pp. 3501-3509, 14

June 2010 http://www.academicjournals.org/AJB

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution 33(7): 1870-1874. https://doi.org/10.1093/molbev/msw054

Leung, H., Borromeo, E.S., Bernardo, M.A. and Notteghem, J.L. (1988). Genetic analysis of virulence in the rice blast fungus. Magnaporthe grisea. Phytopathology 78(9): 1227-1233. https://doi: 10.1094/Phyto-78-1227

Liu, Y.J. and Hall, B.D. (2004). Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. Proceedings of the National Academy of Sciences 101(13): 4507 - 4512. https: //doi 10.1073/pnas.0400938101.

Longya, A., Talumphai, S. and Jantasuriyarat, C. (2020). Morphological characterization and genetic diversity of rice blast fungus, *Pyricularia oryzae*, from Thailand using ISSR and SRAP markers. *Journal of Fungi* 6(1): 38. https://doi: 10.3390/jof6010038.

Maciel, J.L.N., Ceresini, P.C., Castroagudin, V. L., Zala, M., Kema, G.H. and McDonald, B.A. (2014). Population structure and pathotype diversity of the wheat blast pathogen Magnaporthe oryzae 25 years after its emergence in Brazil. Phytopathology 104(1): 95-107. https://doi.org/10.1094/PHYTO-11-12-0294-R

McDonald, B.A. and Linde, C. (2002). Pathogen population genetics, evolutionary potential and durable resistance. Annual review of phytopathology 40(1): 349-379. Https://doi10.1146/annurev.phyto.40.120501.101443

Miah, G., Rafii, M.Y., Ismail, M.R., Puteh, A. B., Rahim, H.A., Asfaliza, R. and Latif, M.A. (2013). Blast resistance in rice: a review of conventional breeding to molecular approaches. Molecular biology reports 40(3): 2369-2388. http://dx.doi.org/10.1007/s11033-012-2318-0

Mitchell, J.I., Roberts, P.J. and Moss, S.T. (1995). Sequence or structure: a short review on the application of nucleic acid sequence information to fungal taxonomy. Mycologist 9(2): 67-75. https://doi:10.1016/s0269-915x(09)80212-7

Muni, N.M. and Nadarajah, K. (2014). Morphological and molecular characterization of Magnaporthe oryzae (fungus) from infected rice leaf samples. In AIP Conference Proceedings 1614(1): 756-760. Https://doi 10.1063/1.4895296

Murray, M.G. and WF324241 Thompson. (1980). "Rapid isolation of high molecular weight plant DNA" Nucleic acids research 8(19): 4321 - 4326. https://doi.org/10.1093/nar/8.19.4321

Navajas, M., Lagnel, J., Fauvel, G. and De Moraes, G. (1999). Sequence variation of ribosomal internal transcribed spacers (ITS) in commercially important Phytoseiidae mites. Experimental and Applied Acarology 23(11): 851-859. https://doi.org/10.1023/a:1006251220052

Nguyet, N.T., Long, H.H., Ngoc, N.B., Nhai, N. T., Thuy, N.T., Hayashi, N. and Fukuta, Y. (2020). Diversity and distribution of rice blast (*Pyricularia oryzae* Cavara) races in Vietnam. Plant Disease 104(2): 381-387. https://doi.org/10.1094/pdis-05-19-1008-re

Nitschke, E., Nihlgard, M. and Varrelmann, M. (2009). Differentiation of eleven *Fusarium* spp. isolated from sugar beet, using restriction fragment analysis of a polymerase chain reaction–amplified translation elongation factor 1α gene fragment. Phytopathology, 99(8): 921-929. https:// doi:10.1094/ PHYTO-99-8-0921

Noronha, A., Mota, A., Moraes, G.J. and Coutinho, L.L. (2003). Molecular characterization of mite populations of Euseius citrifolius Denmark and Muma and Euseius concordis (Chant) (Acari: Phytoseiidae) using sequences of the ITS1 and ITS2 regions. Neotropical entomology 32: 591-596. https://doi.org/10.1590/S1519-566X2003000400009

O'Donnell, K., Humber, R.A., Geiser, D. M., Kang, S., Park, B., Robert, V.A. and Rehner, S.A. (2012). Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-

ID and Fusarium MLST. Mycologia 104(2): 427-445. https://doi.org/10.3852/11-179

Ou, S.H. (1985). Rice Diseases, 2nd ed. Common wealth Mycological. https://doi.org/10.4236/jacen.2021.103021

Qi, H., Yang, J., Yin, C., Zhao, J., Ren, X., Jia, S. and Zhang, G. (2019). Analysis of *Pyricularia oryzae* and *P. grisea* from different hosts based on multilocus phylogeny and pathogenicity associated with host preference in China. Phytopathology 109(8). https://doi.org/10.1094/PHYTO-10-18-0383-R

Rehner, S.A. and Buckley, E.P. (2005). Cryptic diversification in *Beauveria bassiana* inferred from nuclear its and ef1-alpha phylogenies. Mycologia 97(8). http://dx.doi.org/10.3852/mycologia.97.1.84

Sharma, T.R., Chauhan, R.S., Singh, B.M., Paul, R., Sagar, V. and Rathour, R. (2002). RAPD and Pathotype Analyses of Magnaporthe grisea Populations from the north western Himalayan Region of India. *Journal of phytopathology,* 150(1112): 649-656. https://doi.org/10.1046/j.1439-0434.2002.00812.x

Stielow, J.B., Levesque, C.A., Seifert, K. A., Meyer, W., Irinyi, L., Smits, D. and Robert, V. (2015). One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia-Molecular Phylogeny and Evolution of Fungi 35(1): 242-263. https://doi.org/10.3767/003158515X689135

Thon, M. R., Pan, H., Diener, S., Papalas, J., Taro, A., Mitchell, T. K. and Dean, R.A. (2006). The role of transposable element clusters in genome evolution and loss of synteny in the rice blast fungus Magnaporthe oryzae. Genome biology 7(2): 1-9. http://dx.doi.org/10.1186/gb-2006-7-2-r16

Urayama, S., Kato, S., Suzuki, Y., Aoki, N., Le, M.T., Arie, T. and Moriyama, H. (2010). Mycoviruses related to chrysovirus affect vegetative growth in the rice blast fungus Magnaporthe oryzae. *Journal of General Virology,* 91(12): 3085-3094. https://doi.org/10.1099/vir.0.025411-0

White, T.J., Bruns, T., Lee, S.J.W.T. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1): 315-322. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhu, Y.Y., Fang, H., Wang, Y.Y., Fan, J.X., Yang, S.S., Mew, T.W. and Mundt, C.C. (2005). Panicle blast and canopy moisture in rice cultivar mixtures. Phytopathology 95(4): 433-438.