Pathogenicity of *Pyricularia oryzae* Isolates Obtained from Cultivars Grown in Middle and High Altitudes Zones of Burundi

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Abstract

Rice blast disease caused by the fungal pathogen called Pyricularia oryzae is an economically important disease distributed in rice-growing regions of the world. Understanding pathogenic variation among isolates paves a way towards the most effective ways to manage the disease. Thirteen isolates of P. oryzae from high and middle altitude rice ecosystems were inoculated into ten (10) cultivars most preferred by rice farmers in the middle and high-altitude areas of Burundi. The isolates were then evaluated for their ability to cause rice blast disease under screen house condition. A complete randomized design (CRD) with three replications 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. Highly significant differences (p=0.000) between location were observed in rice blast disease incidence and severity. 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 rice ecosystems of Burundi but also as parent materials for development of rice cultivars with resistance to rice blast.

Keywords: Pathogenicity, rice blast, severity, rice cultivars.

Introduction

Rice is the second most important cereal crop after wheat and the most consumed major staple food cultivated worldwide (Katsantonis *et al.,* 2017). In Burundi, rice is an important staple food and income for millions people in rural and urban areas. 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). This biotic constrain can be linked to low (2.5 to 3 t/ha) national average of rice yield in Burundi when compared to the potential yield of 5.5 to 8 t/ha (MINEAGRIE, 2016). 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). In Burundi, rice blast is known to be a common disease in all agro-ecological zones (Nizigiyimana, 1986) and can cause yield losses of more than 10% (MINEAGRIE, 2016). Based on its economic importance, the pathogen is known 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).

To control rice blast disease, different methods are used such as timely sowing, proper use of nitrogen fertilizers, tillage, weed control and crop rotation. The 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). In Burundi (Imbo plain), a recent study done on the evaluation of rice blast disease severity, indicated high variation between cultivars grown in different agro ecological zones and cropping seasons (Nsanzineza, 2021).

Previous studies have established that different isolates of *P. oryzae* have different virulence in different rice 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 the pathogenicity of *P. oryzae* isolates in different rice cultivars (Correa-Victoria and Zeigler, 1993). In Burundi, apart from Imbo plain region having the information on rice blast disease, in other regions, current information on rice blast disease occurrence, *P. oryzae* strains variability and resistance levels of commonly cultivated rice cultivars are still limited. Hence, the objective of this study was to evaluate the pathogenicity of blast (*Pyricularia oryzae*) isolates on rice cultivars grown in middle and high 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). The two agro ecological zones had differences in temperature, rainfall and relative humidity (Table 1) which are key drivers of rice blast disease occurrence and severity.

Sampling plant materials was done by collecting plants with symptoms of rice blast whereby for each of the symptomaticleaves, nodes and panicles, 60 samples were collected from both Buyenzi and Mosso regions. Samples were kept in labeled envelops gathered in transparent bags at low temperature (cool box)

Table 1: Monthly temperature, precipitation and relative humidity at high (Buyenzi region) and middle (Mosso region) altitudes zones

	Medium altitude					High altitude				
Month	**Temperature**		**Mean**	**Precipi-tation (mm)**	**Relative humidity (%)**	**Temperature**		**Mean**	**Precipi-tation (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)

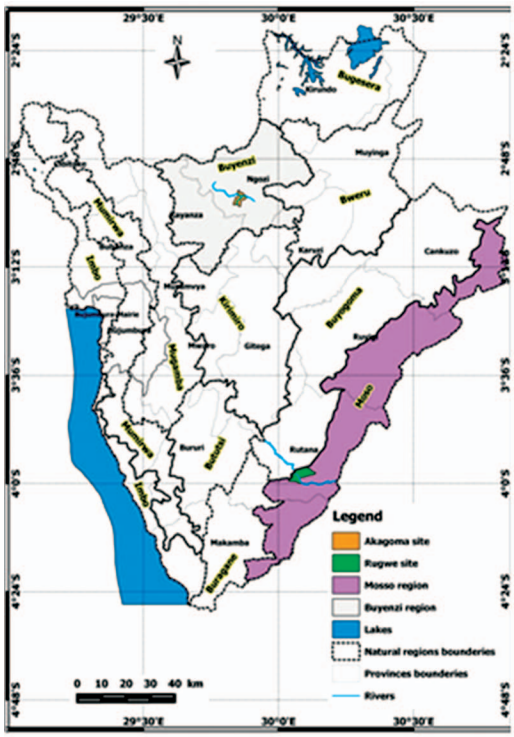

Figure 1: Map of Burundi showing the study locations (Buyenzi and Mosso regions)

and later in refrigerator maintained at 3-5°C for a systematic isolation of *Pyricularia oryzae* at IRRI Burundi laboratory. During isolation of fungi, the stored leaves, panicles and nodes were randomly selected from the while the remaining were kept in the same cold condition for future uses.

Isolation of *Pyricularia oryzae*

The isolation of blast fungi (*P. oryzae*) was done at the International Rice Research institute (IRRI-Burundi). For isolation of *P. oryzae* the symptomatic leaves, nodes, and panicles were processed according to Landschoot *et al.* (2011) with little modification. Briefly, the samples were cut into small (5mm) pieces, followed by sterilization by putting them into Hydrogen peroxide (H_2O_2) for 3 minutes to eliminate saprophytes. Then the samples were rinsed three times using 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 for mycelial growth. To induce rapid sporulation, the rapidly growing cultures were subjected to sporulation medium and light regimes according to Guochang and Shuyuan (2001) for 4 days. This was followed by harvesting the spores using a glass needle, plating on Water Agar Medium and incubating for 24 hours at room temperature 25°C (IRRI, 2013). Pure cultures were obtained using single spore isolation method (IRRI, 2013), which consist of microscoping observation to identify germinating spores by using binocular microscope, after which a monosporic culture was established in Potato-Dextrose-Agar medium (PDA) (manufactured by Santa Maria, USA) until the petri dishes were fully covered by the blast fungus.

The obtained 13 monosporic isolates of *Pyricularia oryzae* isolated from leaves, panicles and nodes of infected rice plants from high altitude (Buyenzi region) and middle altitude (Mosso region) agro-ecological zones of Burundi are presented in Table 2.

The test plant materials

The plant materials which were used to evaluate *P. oryzae* isolates for their pathogenicity consisted of ten (10) cultivars that are mostly cultivated by rice farmers in Burundi. These cultivars were Mugwiza, Gwizumbwu, Vuninzara obtained from IRRI. Other cultivars, Watt, Karundi, Kigori, Buname, Rufutamadeni, Kabuye and V18 were obtained from farmers (Table 2).

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

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

Table 2: ***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

liter of distilled water. The mixture was boiled using a heat-stiller-machine, sterilized at 121°C for 20min. Antibiotic (streptomycin at 500mg/l) 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°C before use.

To revive the stored culture, colonized paper disk were placed on rice flour agar (Manufactured by Glentham Life Science Ltd, United Kingdom) for 7-10 days to allow mycelial growth at room temperature. One best Petri dish was selected for multiplication. For each isolate, twelve Petri dishes were prepared and were incubated at room temperature (25-30°C) for 2 weeks. After the second week, mycelia were scraped using a sterilized glass slide and kept under a fluorescent light for 7 days at 25°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 spore suspension was adjusted to 2x105spores/ml.

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. To avoid cross contamination, the rice cultivars to be inoculatedwith the same isolate were put together and inoculated using a hand sprayer (Chuwa *et al.,* 2015). The plants which were inoculated by the same isolate were then placed in the same humid chamber covered by wet blankets on all sides of the chamber and plastic bag to promote high relative humidity (>90%) and the temperature was adjusted to 25°C. Plants were maintained in the humid chamber for 24-48 hours after inoculation and placed in the mist room at 25-30°C (IRRI, 2013) for symptoms development.

Assessment of the rice blast disease

Based on rice blast symptom development, checking for disease incidence and severity began one week after inoculation. While disease incidence was established as number of plants infected per treatment, the disease severity was determined by scoring using the visual scales of 0 to 9 based on predominant lesions according to protocol 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. The disease resistance or susceptibility of each entry was determined 10–15 days after the artificial inoculation and taken 2–3 times at 5 days intervals by following the Standard Evaluation System 2002 (SES) of International Rice Research Institute, Manila, Philippines as in Sahu *et al.* (2022). According to Hayashi and Fukuta (2009), the plants reactions with score 0-1 were categorized as resistant (R) and 3-9 Susceptible (S). Thereafter, the disease incidence (Ghazanfar *et al.,* 2009) and severity (Waller *et al.,* 2002) was calculated using the following formula

$$Disease\ incedence = \frac{n}{N} \times 100 \quad (1)$$

$$Disease\ severity = \frac{v \times n}{V \times N} \times 100 \quad (2)$$

Where: (n) =Number of plants in each category
v = Numerical values of symptoms category
N = Total number of plants
V= Highest value scale

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: $Yijk = \mu + \alpha i + \eta ki + \beta j + \alpha\beta ij + \varepsilon k$, *Where,* μ*:* Grand mean, αi: mean effect of cultivars, ηki: Error plot, βj: Mean effect of Isolate, $\alpha\beta ij$: Interaction between Cultivar and Isolate, εki: 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.

Results

Isolate pathogenicity based on disease incidence and severity

Cultivars inoculated with *P. oryzae* isolates, showed different blast disease incidence and severity for the same isolate. Table 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 3: Description of cultivars used as materials for pathogenicity test of different isolates

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

NA: Not Applicable

Rice blast disease incidence and severity of cultivars

Incidence of rice leaf blast

Incidences of rice blast in different cultivars varied significantly among 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 4).

Table 4: 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

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).

Rice leaf blast severity

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 6).

The results in Table 6 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 (35.80%) 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.*

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

Table 6 shows cultivars which showed less than 3 score of blast disease severity were regarded resistant (R) and those which showed

Table 5: Rice blast incidence (%) of rice cultivars inoculated with different *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 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

Table 6: 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 2017
Vuninzara	3.70	3.70	35.80	4.94	6.17	3.704	3.70	3.70	4.94	4.94	29.63	3.70	4.94
Gwizumwimbu	3.70	16.05	3.70	3.70	3.70	3.704	4.94	3.70	3.70	23.46	3.70	4.938	3.70
Mugwiza	4.94	3.70	3.70	3.70	3.70	4.938	4.94	3.70	4.94	3.70	4.94	3.70	3.70
Kigori	4.94	22.22	3.70	3.70	3.70	3.704	3.70	3.70	3.70	3.70	3.70	4.94	3.70
Watt	6.17	4.94	3.70	33.33	3.70	6.173	3.70	3.70	3.70	13.58	3.70	3.70	4.94
Kabuye	3.70	3.70	3.70	3.70	4.94	3.704	55.56	3.70	3.70	3.70	3.70	3.70	3.70
V18	3.70	4.94	3.70	4.94	6.17	3.704	3.70	4.94	3.70	4.94	3.70	3.70	3.70
Karundi	14.82	3.70	29.83	30.86	69.14	4.938	3.70	4.94	4.94	33.33	4.94	3.70	6.17
Buname	3.70	3.70	3.70	3.70	3.70	3.704	55.56	3.70	3.70	13.58	3.70	3.70	3.70
Rufutamadeni	3.70	3.70	4.94	3.70	3.70	3.704	3.70	4.94	3.70	3.70	3.70	3.70	3.70
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

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 isolate (Table 7).

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 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 and 6).

Discussion

This study has established that the pathogenicity of the different *P. Oryzae* isolates inoculated in the same cultivar, were different in the two agro ecological zones. This observation can be linked to differences in environment characteristics reffering to the data on rainfall and temperature described in Table 1. Based on climatic records, it was noted that the temperature of high altitude (Buyenzi region) and middle altitude (Mosso region) were 18.8°C and 21.7°C respectively. Similarly, the rainfall in high and middle agroecological zones were 122.4 mm and 11.7 mm. These differences can have significant influence on he pathogenicity of *P. Oryzae* (Muñoz, 2008). Similarly, Muñoz (2008) revealed that the environmental conditions have a strong influence on the development of rice blast disease. In Tanzania, Chuwa (2016) reported that due to the variation in climatic factors (temperature, relative humidity and rainfall), the rice blast disease varied between Mbeya and Morogoro regions.

Isolate pathogenicity did not only vary between locations but also between cultivars. Differences in isolate pathogenicity between cultivars suggest that he cultivars had different resistance compatibilities. Few rice cultivars demonstrated that they have resistance traits to all the thirtheen isolates from both middle and high-altitude rice growing zones of Burundi. 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 confirms previous findings by Asfasha *et al.* (2015) that the incidence/severity of the disease varied from low to high depending on the location where cultivars are grown. These explanations comply with the report of Groth and Bond (2007), in which the incidence and severity of the blast disease depended on the number of inoculums, the growth stage of culture, and varietal resistance.

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 in Nsanzineza (2021) whose study in Imbo plain of Burundi 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 cultivars from which they were isolated based

Table 7: Reaction of rice cultivars to *Pyricularia oryzae* isolates from two agro ecologies

Cultivar	Isolate												
	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 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

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

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 oryzae* population. 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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