

Pathogenic Variation and Molecular Characterization of *Pyricularia oryzae*, Causal Agent of Rice Blast Disease in Tanzania

Charles Joseph Chuwa¹, Robert B. Mabagala², Mnyuku S.O.W. Reuben³

^{1,2,3}Sokoine University of Agriculture (SUA), Department of Crop Science and Production, Morogoro, Tanzania

Abstract: Rice blast disease, caused by the fungus *Pyricularia oryzae*, is one of the most devastating diseases of rice. Understanding pathogenic variation and molecular characterization of *P. oryzae* is one of the most efficient ways to manage the disease. Studies on identification, characterization and pathogenic variation of *P. oryzae* were conducted in the laboratory and screen-house at Sokoine University of Agriculture, Morogoro. Seven *P. oryzae* strains collected from Shinyanga, Kilimanjaro, Mbeya and Morogoro were tested with a set of ten rice blast differentials viz: IRBLk-Ka, IRBLkm-Ts, IRBLb-w/co, IRBLkp-K60, IRBLz-Fu, IRBLa-C, IRBLi-F5, IRBLta2-Pi, St and IRBLt-K59. Twenty one-day-old seedlings grown in pots were inoculated with spore suspension of a *P. oryzae* at a concentration of $2 \times 10^5 \text{ ml}^{-1}$ using a hand-hold sprayer in the evening. Considerable pathogenic variations among the tested strains were observed. The International rice differentials IRBLk-Ka, IRBLkp-K60, IRBLa-C, IRBLi-F5, IRBLta2-Pi, St and IRBLt-K59 were resistant to all strains, however, IRBLb-w/co, IRBLkm-Ts and IRBLz-Fu (Piz) were susceptible. Molecular analysis using four primers Bt1a and Bt1b, CAL-228F and CAL-737R, ACT-512F and ACT-783R, ITS1 and ITS4 were used for amplification of seven *P. oryzae* strains. The results showed no differences in banding patterns between strains, indicating that all strains analyzed were genetically homogeneous but pathogenically heterogeneous.

Keywords: Characterization, pathogenic variation, PCR, *Pyricularia oryzae*

1. Introduction

Rice is one of the most important cereal crops grown worldwide. In Tanzania, more than a half of the population depends on rice as the main food and source of income. Rice blast disease is the most important and destructive disease of rice. It is caused by the fungus *Pyricularia oryzae* (Teleomorph *Magnaporthe oryzae* Couch) formally known as *Pyricularia grisea* (Cooke) Sacc. [8], [38], [15] [Teleomorph *Magnaporthe grisea* (Herbert)]. The incidence and severity of rice blast disease vary yearly based on location and environmental conditions [46], [35], [3]. Symptoms of the disease can occur on all above ground parts of the plant and is observed at earlier growing stages up to maturity. Symptoms appear on the leaves, nodes and panicles [14], [38]. It has been reported to cause grain yield losses of up to 100 % [31], [11], [40].

However, *P. oryzae* [anamorph] Cavara has been reported to have high pathogenic variation with respect to host range and variety specificity [24]. The level of pathogenic variation of *P. oryzae* isolates differs with rice varieties [4]. Therefore, the studies on reaction of differential rice genotypes and molecular characterization are important in understanding pathogenic variation of *P. oryzae*. Rice blast disease can be classified into various pathotypes based on the infection pattern observed on a set of differential rice genotypes [20].

The use of resistant rice varieties is the most economical and effective means of managing blast disease in rice [6], [14]. However, sometimes resistant varieties may become ineffective due to evolutionary changes in the pathogen population [22]. Loss of resistance shortly after variety release is common in many rice growing areas [20]. Therefore, understanding pathogenic variation of *P. oryzae* is

important in overcoming constraints facing many rice breeding programs [7].

Pathogenic variation is the main cause of resistance breakdown in rice against rice blast disease [37]. Several studies have reported different sources of pathogenic variation in *P. oryzae*. Great pathogenic variation has been reported in *P. oryzae* from single-spore isolates originating from single lesions and monoconidial sub-cultures [7]. The isolates from the same lesion may differ in pathogenicity, and single-spore subcultures may also differ in pathogenicity from the original single-spore cultures [12]. However, the composition of groups of isolates that are genetically different may cause variation in the pathogen [4]. [22] reported that variation in the pathogen is caused by variation in chromosome numbers or genomic rearrangements. Similarly, [44], [45] reported that parasexual recombination is one of the means of variation in *P. oryzae*. Further understanding of pathogenic changes during sexual hybridization may provide evidence to pathogenic variation observed in the asexual stage of the fungus.

Pyricularia oryzae strains are not pathogenically homogenous, existence of pathogenic races has been reported from many major rice growing countries [1]. However, such variation in Tanzania has not yet been studied. [1] identified about five pathogenic race groups, ID-1, ID-2, IB-4, IC-17 and IC-25. Among these groups, physiologic race group IC-17 was observed to be predominant. [28] investigated 31 isolates of the fungus in India, of which 21 isolates belonged to a new race group designated as IJ, and the rest belonged to the international race groups. Their study identified races IC 3 and ID 1 as common in India. In Brazil, a great number of physiological races with distinct virulence characteristics have been identified based on reaction types on a set of eight standard

international rice differentials [29]. Races IC-1 and IB-9 were reported to be predominant in Brazil.

A culture of *P. oryzae* may change its pathogenicity but it can not be used as the criteria of assigning pathogenic race [41]. In a study of [41] categorized pathogenic isolates by assessing the reaction of rice varieties with respect to blast disease, and came up with three categories namely; resistance, moderate resistance and susceptible rice varieties. The use of differential rice genotypes of blast disease is widely adapted as the technique for distinguishing pathogenic races of pathogens.

Molecular studies are currently appropriate approaches in identification and characterization of *P. oryzae* [12]. However, the use of DNA technologies such as polymerase chain reaction (PCR) is the most important approach in detection of the pathogen [2], [6], [9]. The PCR technique is effective for distinguishing between closely related isolates. The aim of this study was to identify, characterise and determine of pathogenic variation of *P. oryzae* using a set of rice differentials as well as PCR techniques.

2. Materials and Methods

2.1 Source of isolates

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

2.2 Isolation and identification of *Pyricularia oryzae*

Infected tissues (lesions) were sterilized using 1 % Sodium hypochlorite for 1 minute to reduce saprophytes, and then rinsed in distilled water three times. Each lesion was placed on moistened filter papers in Petri dishes and incubated for 72 hours (3 days) at 25 °C to allow for fungal sporulation on the lesions [4]. Identification of *P. oryzae* was done three days after incubation based on morphological features as described by [13], [18]. Conidia were identified from the sporulating lesions using a stereomicroscope and identity confirmed following procedures of [23].

Thereafter, sterile drawing pins were dipped in potato dextrose agar (PDA) petri plates and then slightly touched on the sporulating rice blast lesions, followed by inoculation of the agar plates with the pin containing spores of the presumed *P. oryzae*. Then the pathogen was cultured in petri dishes on PDA agar, incubated at 25 ± 1°C to induce sporulation as described by [33]. After sporulation, the conidia were harvested by adding 10 ml of sterilized distilled water per petri plate and gently scraped the surface to harvest the spores. Conidial suspensions at a concentration of about 10⁴ spores/ml were prepared in sterilized distilled water with 0.1 % Tween 20 to increase spore dispersion [32]. Conidial densities were counted using a haemocytometer. Established cultures were subsequently maintained [39] and used for further studies as and when required.

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

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

Four hundred seeds for each variety as recommended by [17] were sown in eight 15 cm diameter plastic pots containing sterilized moist silt loam soil arranged in a Completely Randomised Design (CRD) with three replicates. Fifty seeds were sown in each pot, each pot represented one replication. Inoculum preparation was done as described by [36]. Rice seedlings were inoculated with *P. oryzae* at the 4-5 leaf stage (21-day-old seedlings) in the evening using a low-pressure spray bottle with a suspension of conidia 2 x 10⁵ spores/ml following procedures described by [46]. Disease reaction was assessed seven to ten days after inoculation based on the [16] standard evaluation scale of 0 – 9 where: 0= no lesions; 1= small, brown, specks of pinhead size; 3 = small, roundish to slightly elongated, necrotic, gray spots about 1-2 mm in diameter; 5 = typical blast lesions infecting < 10% of the leaf area; 7 = typical blast lesions infecting 26-50% of the leaf area; 9 = typical blast lesions infecting >51% leaf area and many dead leaves [43]. The pathogenic race of each isolate was determined by the reaction of the rice differential varieties used.

2.4 Characterization of *Pyricularia oryzae* by PCR

2.4.1 DNA extraction

Total DNA was extracted from seven isolates of *P. oryzae*. The pathogen isolates were grown on 10 g of potato dextrose agar supplemented with 2 g of yeast extract per liter in Erlenmeyer flasks for hours without agitation followed by ten days with constant agitation in the darkness at 24 °C. One or two mycelial paper discs were transferred to 250 ml Erlenmeyer flasks containing 150 ml of the culture medium. The harvested mycelia was freeze-dried, lyophilized and macerated in liquid nitrogen. DNA extraction was done following the procedure described by [29].

About 300 mg of powdered mycelia was suspended in 700 µl of extraction buffer (50 mM Tris-HCl, pH 8.0; 50 mM EDTA; 3% sodium dodecyl sulfate, wt/vol and 1% of mercaptoethanol) at 65 °C for 1 hour. The cellular proteins were precipitated with 30 µl of potassium acetate (3 M and pH 5.2). DNA was precipitated in 200 µl of cold isopropanol, washed with 70 % ethanol, dried under vacuum and re-suspended in TE buffer (10 mM Tris-HCl, pH 8.0; 1.0 mM EDTA), containing 10 mg/ml of RNase A and incubated at 37 °C for 30 min. The DNA concentration was estimated by fluorometer and adjusted to 10 ng/µl. The DNA pellets were dried overnight and dissolved in 1 ml of TE buffer (pH 8.0). Quantification of DNA was performed on 0.8 % agarose gel and diluted with sterile distilled water to a concentration of 25 ng for PCR analysis. The PCR contained

the following reaction mixture (25 μ l): 50 ng DNA, 2.5 μ l 10 x buffer reaction (200 mM Tris-HCL, Ph 8.4 and 500 mM of KCL), 2.0 μ l 50 mM, MgCL₂; 0.5 μ l dNTP (10 mM each dATP, dGTP, dCTP and dTTP); 1.25 μ l of each primer (100 mM); 5 units of Taq polymerase.

2.4.2 Polymerase chain reaction (PCR) amplification

The PCR amplification reaction was carried out in a thermocycler with the following temperature conditions as described by [5] with modifications. An initial PCR cycle

was performed at 95 °C for 4 minutes, primer annealing at 55 °C for 30 s, polymerization at 72 °C for 1 minute. This was followed by 30 cycles of 94 °C for 5 minutes, 55 °C for 30 s and 72 °C for 1 minute. Final extension was performed at 72 °C for 7 minutes. The PCR products were separated in agarose gel electrophoresis 2 % in TBE 0.5 x with 100 volts for 30 minutes (Figure 1) then visualized in UV light after soaking in ethidium bromide. Four primers (Table 1) were used to amplify the targeted DNA fragments.

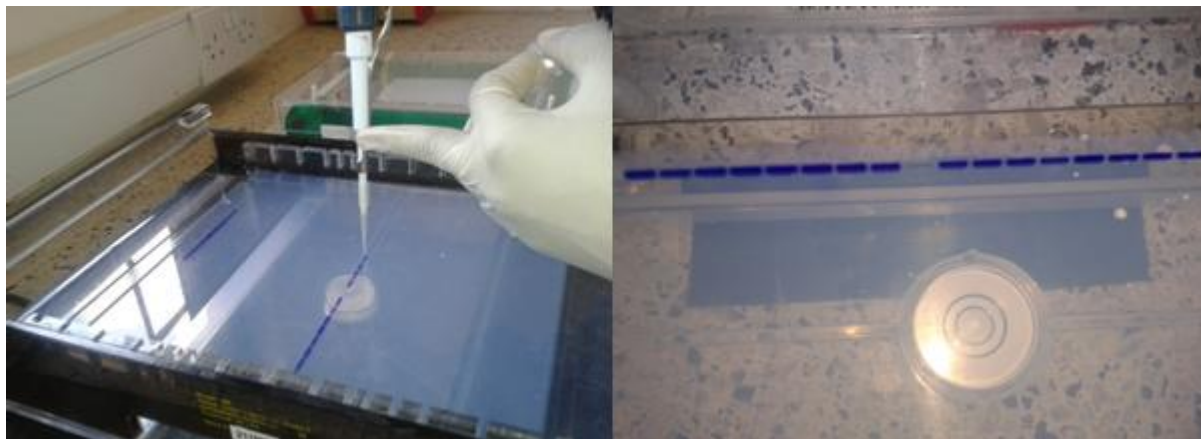


Figure 1: Loading of DNA in the agarose gel for amplification (photos: C. J. Chuwa, MARI, Dar es Salaam).

2.4.3 Statistical Analysis

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

3. Results and Discussion

3.1 Identification and pathogenicity test of *Pyricularia oryzae*

Of the 320 isolates of rice blast disease collected, only seven strains were identified as *Pyricularia oryzae* based on morphology and PCR. The pathogen *P. oryzae* obtained from the blotter method was cultured on PDA medium. The strains from leaf component DAK, KAH, KAP, KIK, MOS, MSU and SUA showed excellent sporulation and growth on PDA medium (Figure 2a). These results correspond with the study [15] found that PDA containing segments of rice leaf, better favored the growth of *P. oryzae*. Conidial variation of the *P. oryzae* isolates used was not identified in the study. Conidia were pyriform, almost hyaline to pale olive, 2-septate and 3-celled (Figure 2b).



Figure 2: a) Colony growth and b) conidia morphology of the three isolates *Pyricularia oryzae* (photos: C. J. Chuwa, SUA, Morogoro).

3.2 Pathogenic variation of *Pyricularia oryzae* using rice differentials

The *P. oryzae* strains pathotyped by inoculation on the international rice differentials showed compatible reactions (Table 1). The International rice differentials IRBLk-Ka

(*Pik*), IRBLkp-K60 (*Pik-p*), IRBLa-C, IRBLi-F5 (*Pii*), IRBLta2-Pi (*Pita-2*), St and IRBLt-K59 (*Pit*) were resistant to all *P. oryzae* strains. However, differentials IRBLb-w/co, IRBLkm-Ts (*Pik-m*) and IRBLz-Fu (*Piz*) were susceptible.

Table 1: Reaction of differential rice varieties to seven strains of *Pyricularia oryzae* collected from Kilimanjaro, Mbeya, Morogoro and Shinyanga regions in Tanzania

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

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

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

However, all seven *P. oryzae* strains were pathogenic on IRBLkm-Ts and IRBLz-Fu (Table 1). Similar contradicting results have been reported by [43] when studying molecular marker application for rice blast resistance selection on the double haploid rice population. The virulence of the strain obtained have been reported to be strongly influenced by the varieties from where they were isolated [4].

On the basis of the reaction of the ten international differentials, three races (A, B and C) of *P. oryzae* were identified (Table 1). Most of the strains belonged to race B (Table 1). Race A and C consisted of a single strain each. Race C characterized by resistant reaction of the eight international differentials viz; IRBLk-Ka, IRBLa-C, St, IRBLkp-K60, IRBLt-K59, IRBLta2-Pi, IRBLb-w/co and IRBLi-F5. Race B was characterized by susceptible reaction of three differentials IRBLb-w/co, IRBLkm-Ts and IRBLz-Fu followed by race A which was characterized by susceptible reaction of four differentials.

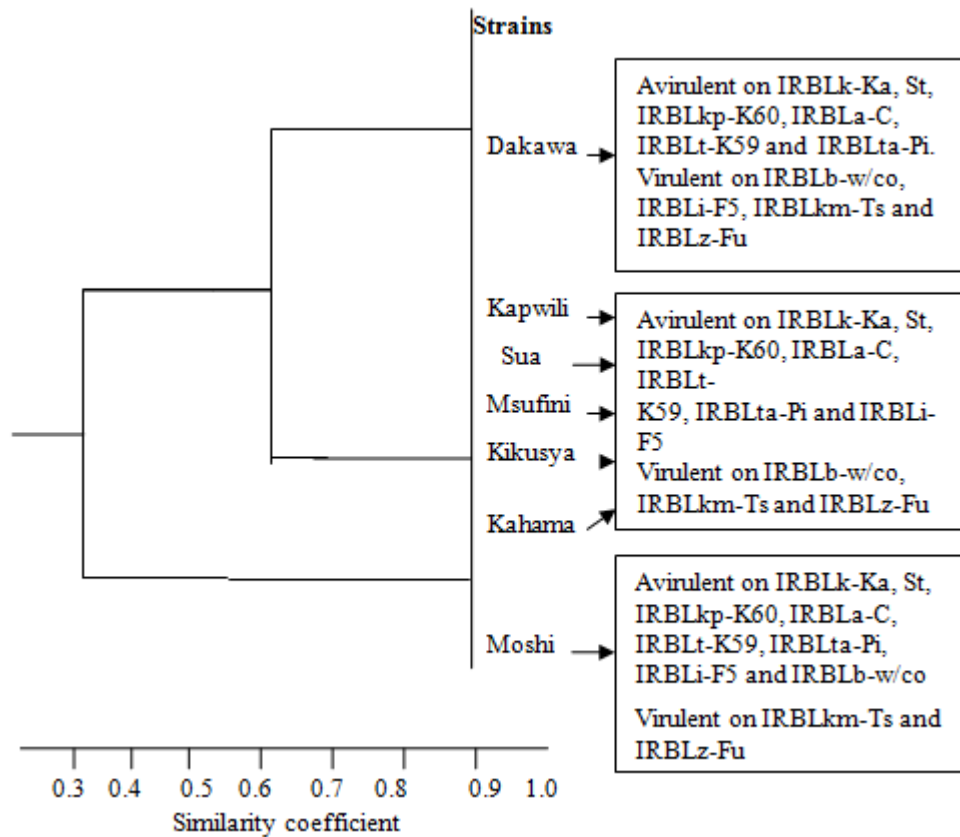
Pathogenic variation has been cited as the principal cause for the frequent breakdown of resistance shortly after monoconidial sub-cultures. [12] also found that the strains of *P. oryzae* from the same lesion may differ in pathogenicity, and single-spore sub-cultures may also differ in pathogenicity from the original single-spore cultures. Strains

from each rice-growing region were limited; therefore, further collection and evaluation of strains are needed to confirm the findings of this study.

The similarity coefficients of *P. oryzae* strains used in this study varied from 0.3 to 1.0 and clustered broadly into two groups (Figure 3). The average similarities within the cluster of strains in group one was significantly smaller than the average similarities among strains from group two. However, on the basis of the reaction types either pathogenic or non-pathogenic, the seven strains were grouped into three different pathotypes (Figure 3).

Pyricularia oryzae strains induced differential reaction on IRBLb-w/co, IRBLi-F5, IRBLkm-Ts and IRBLz-Fu. Rice differentials IRBLb-w/co, IRBLi-F5, IRBLkm-Ts and IRBLz-Fu were susceptible to all strains. This agrees with findings reported by [42] when identifying rice blast disease resistance genes using international rice differential varieties that of *P. oryzae* strains can genetically remain stable in the resistance cultivar.

Results showed that both pathogenic and non-pathogenic strains were clustered together in a closely related group. In the present study, the strains were designated into two main groups (Figure 3). The first group was designated as slightly virulent strains consisting of MOS strain. Five strains, KAP, SUA, MSU, KIK and KAH were assigned to group two designated as moderately virulent strains with the remaining DAK strains assigned to group three, a severely virulent strains. The present investigation revealed that these three groups of *P. oryzae* strains infected all the International rice differentials. These strains could overcome a great number of resistance genes because each of the differential genotype possessed different resistance gene against rice blast disease.



Differences in pathogenicity between individual strains have been used for a long time to determine pathogenic variation of *P. oryzae* [34].

In pathotype one, only Dakawa strain was included, while a maximum of five strains (Kapwili, Sua, Kikusya, Msufini and Kahama) were included in pathotype 2 (Figure 4) and the remaining Moshi strains were grouped in pathotype three. Among the host differentials, IRBLk-Ka, St, IRBLkp-K60, IRBLa-C, IRBLt-K59 and IRBLta-Pi showed resistance to all seven strains and IRBLi-F5 to five strains followed by IRBLb-w/co being resistant to one strain.

These results are supported by the study of [21] on the virulence characteristic analysis and identification of new pathotypes of the rice blast fungus (*Magnaporthe grisea*) in India.

Strains of *P. oryzae* were grouped in different pathotypes based on their reaction on rice varieties known to have

sources of resistance. However, the resistance genes *Pik*, *Pik-p*, *Pii*, *Pita-2* and *Pit* remained effective against rice blast strains while genes *Pik-m* and *Piz* were not effective against the disease. Such observations have also been reported by [4], [7], [10], [27].

3.3 Characterization of *Pyricularia oryzae* strains using molecular technique

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

Table 2: *Pyricularia oryzae* primers for reductase gene amplification used in the current study.

Primer	Primer sequence (5' – 3')
Forward - ACT-512	ATGTGCAAGGCCGTTTCGC
Reverse - ACT-783	TACGAGTCCTTCTGGCCCAT
Forward - Bt1a	TTCCCCGTCTCCACTTCTTCATG
Reverse - Bt1b	GACGAGATCGTTCATGTTGAACTC
Forward - CAL-228	GAGTTCAAGGAGGCCTTCTCCC
Reverse - CAL-737	CATCTTCTGGCCATCATGG
Forward - ITS1	TCGGTAGGTGAACCTGCGG
Reverse - ITS4	TCCTCCGCTTATTGATATGC
Forward - Pot2-1	CGGAAGCCCTAAAGCTGTTT
Reverse - Pot2-2	CCCTCATTCGTCACACGTTT

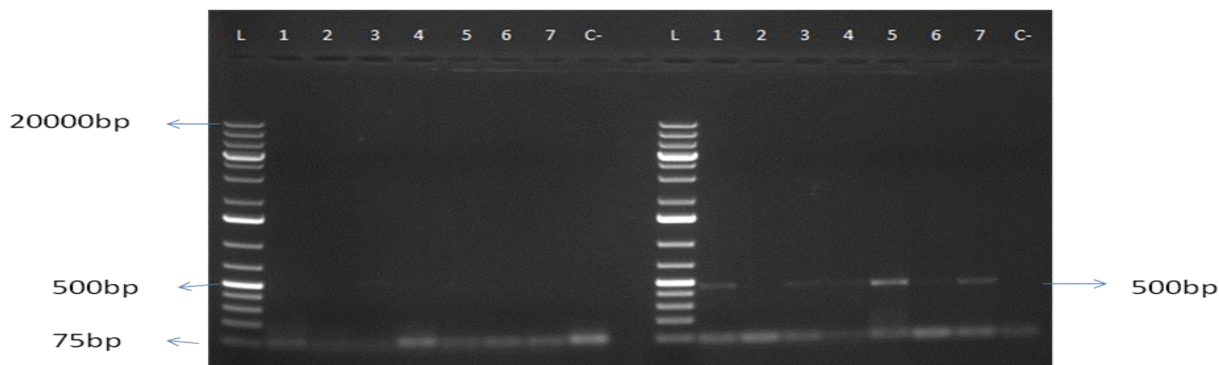


Figure 4: Agarose gel electrophoresis of PCR amplification products of *Pyricularia oryzae* genomic DNA using primers BTta & BTtb (left) and CAL-228F & CAL-737R (right). Strains from left to right were MSU, SUA, DAK, KAH, KAP, KIK and MOS. L = Ladder (1kb+) and C = control. Arrows indicate the two fragments that are characteristic of *P. oryzae*. Molecular sizes are shown in base pairs (bp).

Of the ten (10) primers used, Bt1a and Bt1b, Pot2-1 and Pot2-2 did not amplify DNA from all *P. oryzae* strains, suggesting problems with the primers' specificity for the *Pyricularia oryzae* pathogen. However, the primers CAL-228F and CAL-737R amplified genomic DNA of *P. oryzae* strains MSU, DAK, KAP and MOS while SUA, KAH and KIK strains were not amplified (Figure 4 & 5).

Furthermore, the primer ACT amplified KAP, KIK and MOS strains while MSU, SUA, DAK and KAH were not amplified. The primers ITS amplified *P. oryzae* strains MSU, SUA, KAH, KAP, KIK and MOS. The *P. oryzae* strains SUA and KAH were very faintly amplified. The strain DAK was not amplified by both ACT and ITS primers, suggesting lack of similarity with the fungal DNA structure (Figures 4 & 5). Similar results have been reported by [19] in the investigation of an expedited method for isolation of DNA for PCR from *Magnaporthe oryzae* stored on filter paper, who characterized strains from rice and indicated that the strains of *P. oryzae* attacking rice leaves and panicles were genetically distinct from location to location.

The amplification reactions with the three primers generated polymorphic bands. A single 500 bp product was exhibited by all the strains of *P. oryzae* amplified by CAL-228F & CAL-737R (Figure 4). [23] reported similar results when they were identifying blast resistance expression in rice genotypes using molecular markers (RAPD & SCAR). They found that most of the *P. oryzae* strains identified were polymorphic in nature with band sizes of between 100 and 500 bp.

The primers ACT-512 and ACT-783 amplified *P. oryzae* strains KAP, KIK and MOS collected from Mbeya and Kilimanjaro regions, respectively (Figure 5). Primers ITS 1 and ITS 4 amplified strains MSU, SUA, DAK, KAH, KAP, KIK and MOS of *P. oryzae*, and strains also appeared similar with polymorphic banding pattern of 550 bp. These amplification values were higher than those reported by [23] which ranged of from 40 bp to 420 bp. Similar results were reported by [34] using REMAP markers and amplification values ranged from 490 to 600 bp.

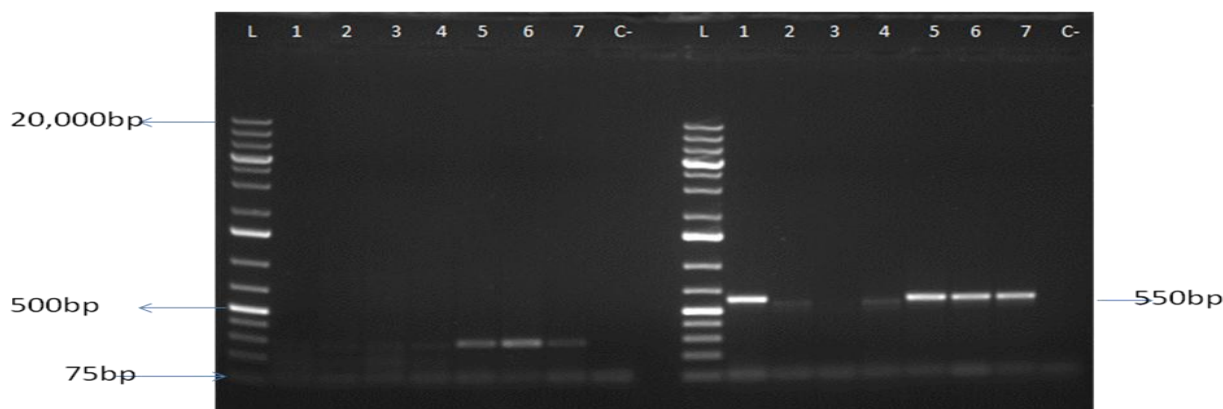


Figure 5: Agarose gel electrophoresis of PCR amplification products of *Pyricularia oryzae* genomic DNA using primers ACT-512F & ACT-783 (left) and ITS1 & ITS4 (right). The *P. oryzae* strains from left to right in the photograph were MSU, SUA, DAK, KAH, KAP, KIK and MOS, respectively. L = Ladder (1kb+)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

The *Pyricularia oryzae* strains did not show differences in banding patterns. The banding patterns of seven *P. oryzae* strains from major rice growing areas in Tanzania were all similar (Figure 4 & 5). This study revealed that the *P. oryzae* strains collected from different major rice growing areas of Tanzania were not significantly different genetically.

PCR products of seven *P. oryzae* strains produced a strong band of 550 bp and very weak bands at ≤ 500 bp (Figures 4 & 5). *Pyricularia oryzae* strains MOS, KIK, KAP and MSU were amplified by primers ITS1, ITS4, ACT-512, CAL-228 and CAL-737 and produced strong bands of 550bp. This confirmed these isolates to be *P. oryzae* and the PCR with

ITS1, ITS4, ACT-512, CAL-228 and CAL-737 primers proved to be a reliable method to differentiate the rice blast pathogen. The number of amplification products obtained was specific to each primer. Overall, the random amplified polymorphic DNA patterns did not show high level of polymorphism. The results indicate that the *P. oryzae* strains genome were genetically stable, but varied in pathogenic [20], [25], [47]. These results can be used for screening resistance commercial rice varieties for planting in areas where there is high risk of *P. oryzae* infection. This information however, is imperative to develop effective breeding rice varieties resistance to rice blast disease strategies in the country.

4. Conclusion

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

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

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

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Author Profile



Charles J. Chuwa received the Bsc, Msc and PhD degree in Agriculture from Sokoine University of Agriculture in 1997, 2004 and 2015, respectively. During 2004 to- date, he is a researcher in the Ministry of Agriculture and Food security of Tanzania.