RICE LEAF BLAST (*Pyricularia oryzae*) CAVARA PATHOGEN DISTRIBUTION, CULTIVAR RESISTANCE AND YIELD LOSS IN ZANZIBAR

ALI KHATIB BAKAR

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2019

EXTENDED ABSTRACT

Rice leaf blast is a rice disease caused by fungus *Pyricularia oryzae* Cavara. The disease is widely spread in all rice ecological systems in Zanzibar viz; rainfed upland, rainfed lowland and irrigated. In Zanzibar rice is a staple food crop that serves 3.1 millions people but the crop is highly infected with rice leaf blast disease a major producton constraint. A comprehensive survey was conducted in six districts of Zanzibar islands covering three districts in Unguja viz: North A, Central and West and Pemba districts were Micheweni Chake chake and Wete. Survey was conducted during two cropping seasons 2014-2015 and 2015-2016. Purposive sampling technique was used to select districts that carries three agro-ecologies viz; rainfed upland, rainfed lowland and irrigated which were considered as treatments. Two islands (Unguja and Pemba) were considered as blocks. Overall objective of this study was to establish rice genotypes with effective rice blast resistant genes and environmental conditions for increased rice productivity in Zanzibar and confirm current losses caused by (*Pyricularia oryzae*) pathogen. During survey, incidence, severity, prsevalence and control measures taken by farmers were recorded for the six districts within the islands.

Results showed that the disease incidence and severity of rice leaf blast disease varied considerably across the surveyed districts. The highest rice blast incidence (68.57%) and severity (20.70%) were recorded in Unguja Island while in Pemba island incidence and severity were registered (46.85%) and (18.53%) respectively. Low disease rate were recoded within majority of farmers in Pemba using cultural practices as a disease reductiona measure. It was found that cultural practices in combination with fungicides were more effective in rice leaf blast disease control. Chake chake district registered lowest rice leaf blast incidence (35.96% and severity (16.58%), while the highest disease was record in North A and Central but did not differ significantly ($P \leq 0.05$). On the three agro-ecologies irrigated agro-ecology registered lowest rice leaf blast disease rate while highest disease rate was recorded in the rainfed upland egro-ecology. Disease incidence records were higher in 2015-2016 than 2014-2015 cropping season.

Study was conducted in the laboratory of mycology in the Department of Crop Science and Horticulture involving isolation of pathogen from rice leaf samples showing rice leaf blast disease (*Pyricularia orayzae*) that was used for identification and molecular characterization of the pathogen. Three types of medium were used for culturing of *Pyricularia oryzae* and compared mycelia growth rates and other culture characteristics. Petri dish inoculated with *Pricularia oryzae* were laid in randomized complete randomized design and replicated three times. Data were collected at 4, 8 and 12days. Results showed that media differ signifycantly ($p \leq 0.001$) in influencing growth of fungi. Similarly, medium interacted significantly ($p \leq 0.001$) with days after inoculation. Oatmeal Agar Medium was suitable for mycelia growth followed by Potato dextrse agar medium while patato carrot agar was the lowest. For molecular analysis six strains were identified and characterized viz ZNZ/2017/CENTRAL, ZNZ/2017/CHK_2, ZNZ/2017/MICH, ZNZ/2017/NORTH_ A2, ZNZ/2017/NORTH_ A8 and ZNZ/2017/CHK_1.

Using 19 rice blast differential genotypes and ten traditional varieties their seeds were sown in the screen house in completely randomized design and inoculated with the spores suspension of *Pyricularia oryzae* to evaluate the effect of rice blast disease. Disease symptoms appeared after two weeks after inoculation and data on disease severity were collected at the interval of ten days for sixty days. During the experimental duration temperature in the screen house ranged from 21.5 to 38.4^{o}C with the average of 27.4 ^{o}C. Relative humidity ranged from 31.626 to 100 with the average of 71.11. Results showed that 13 rice blast differential genotypes were identified as resistant viz; IRBL11-Zh *(Pi11(t),* IRBL12-M *(Pi12(t)),* IRBLa-C *(Pia),* IRBLb-IT13[CO] *(Pib),* IRBLkh-K3 *(Pik-h),* IRBLkp-K60 *(Pik-p),* IRBLkp-K60[CO] *(Pik-p),* IRBLta-Me[CO] *(Pita),* IRBLta2-Pi *(vbfchfdj),* IRBLta2-Re[CO] *(Pita-2),* IRBLz5-Ca *(Piz5),* IRBLzt-IR56[CO] *(Piz-t)* and Moroberekan *(Pi5(t), Pi7)*. Most of traditional varieties were susceptible to the disease. The lowest total disease severity and total area under disease progress curve were 8.63 % and 271.5 % respectively were recorded in the differential variety Moroberekan. The highest total disease severity and total area under development progress curve recorded on CO39 was (95.05%).

Rice yield loss assessment was conducted in Zanzibar at two locations viz; Pemba Island and Unguja island during two rce cropping seasons of 2016 – 2018 using t-test. There were two sets of plots, one set its plots were protected by spraying with fungicide to prevent rice leaf blast disease and another set its plots were not protected. Results reported *Pyricularia oryzae* cause higher rice grain yield loss in Unguja Island at 70.670 % and in Pemba Island was 56.671 %. Unguja island had higher grain yield loss due to high disease severity than in Pemba island. Application of fungicide is an alternative measure of rice blast resistant variety in controlling rice leaf blast disease. From the results, in correlation coefficient we concluded positive correlated from two relationship between protected plots and unprotected plot that the increases of severity the increase yield loss and vice versa.

Key words: *Pyricularia oryzae*, characterization, correlation, inoculation and conidia.

DECLARATION

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

Ali Khatib Bakar Date
(PhD Candidate)

The above declaration is confirmed by:-

Prof. H.J.F. Lyimo Date
(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

First and foremost, I would like to extend my unshared thanks to the Almighty God for smoothening all aspects of my life and my studies in particular.

I would like to extend my heartfelt thanks to my research supervisors Prof. S.O.W.M Reuben and Prof. H.J.F. Lyimo for their patient guidance, encouragement and useful critiques in this study. I also acknowledge the Commission of Science and Technoogy (COSTECH) for financial assistance to undertake the research. I would also like to thank all staffs working in the Ministry of Agriculture, Natural Resources, Livestoks and Fisheries in Zanzibar for their assistance during field survey and sample collection for the study. Special thanks are due to International Rice Research Institute (IRRI) Tanzania for supplying rice blast diffential lines. I also like acknowledge Miss Silyvia Mlemba for assisting me on Molecular studies and Mr. Jackson Nahson on mycology for studies conducted in the laboratories of the Sokoine University of Agriculture (SUA) Morogoro in the Depertment of Crop Science and Horticulture.

Finally, yet very importantly special thanks go to my wife and children for their emotional and material support and patience during my entire study period.

DEDICATION

This work is dedicated to my wife Fatma Ali Msaaji who laid the foundation for my Education and also to my beloved children.

TABLE OF CONTENTS

LIST OF TABLES

TABLE IN MANUSCRIPT 1

TABLE IN MANUSCRIPT II

TABLE IN MANUSCRIPT III

TABLE IN MANUSCRIPT IV

LIST OF FIGURES

FIGURE IN MANUSCRIPT 1

FIGURES IN MANUSCRIPT 1I

FIGURES IN MANUSCRIPT III

FIGURES IN MANUSCRIPT IV

LIST OF PLATES

PLATES IN MANUSCRIPT I

PLATES IN MANUSCRIPT II

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

<	Smaller than
>	Greater than
≤	Less than or equal
ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
bp	base pair
cm	centimeter
COSTECH	Commission of Science and Technology
CRD	Complete Randomized Design
CV	Coefficient of variation
DNA	Dioxy Rebonucleus Acid
dNTPs	Deoxynucleoside triphosphates
ERPP	Expanding Rice Production Project
g	grame
IRRI	International Rice Research Institute
JICA	Japan International Cooperation Agency
KATI	Kizimbani Agricultural Training Institute
KCl	Potassium chloride
LSD	Least Significant Difference
m	meter
MAFC	Ministry of Agriculture Food Security and Cooperative
MALE	Ministry of Agriculture, Livestock and Environment
ml	mils
mm	millimeter
N	Nitrogen

^{O}C	Centrigrade
OMA	Oat Meal Agar
PCA	Potato Carrot Agar
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PDI	Percentage Disease Index
RCBD	Randomized Complete Block Design
RH	Relative humidity
rpm	round per minute
SEm±	Standard error due to mean
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TAE	Tris-acetate-Ethylenediaminetetraacetic acid
TANRICE	Rice Industry Development in Tanzania
TAUDPC	Total Area Under Development Progress Curve
TMRT	Turkey Multiple Range Test
TSP	Triple Super Phospate
UV	Ultraviolet
WP	Wateble Powder
YL	Yield loss
µl	Micro-litre

CHAPTER ONE

1.0 INTRODUCTION, JUSTIFICATION AND OBJECTIVES

1.1 Introduction

Rice (*Oryza sativa* L.) is one of the important cereal food crops supplying more than 50% of all calories consumed by the entire human population worldwide (Duan *et al*., 2013). It is second to wheat in production with area harvested each year being over 154 million ha compared to 214 million ha for wheat and 140 million ha for maize (Sheikh *et al.*, 2011). The crop feeds more than 3 billion people on a daily calorie intake of 50 to 80% (Khush, 2005). Rice is a very popular food almost everywhere in Africa but large consumption of rice is said to be in the Asian continent (Bishwajit *et al*., 2013).

In Tanzania, rice ranks second to maize in production and it is consumed by many people in the country (Nicholas, 2010). It is more preferred than maize; consumers' preferences being contributed by changes in eating habits, population increase and urbanization. The important rice producing regions in Tanzania are Shinyanga, Morogoro, Mbeya and Tabora (MAFC, 2009).

The demand for rice in Tanzania is increasing due to the growing population and changing of life style and incomes (Kihupi *et al*., 2008). In Zanzibar, rice is considered as one of the most important food crops ranking first in consumption. The agricultural sector mainly dominated by rice production is very important to the economy and livelihood of people in Zanzibar. About 80% of the population lives in rural areas and depends on agriculture, mainly rice farming and clove orchards for livelihood (MALE, 2009). Farmers in Zanzibar cultivate rice in three agro – ecosystems i.e. rain fed lowland (74%), rain fed upland (20%) and irrigated (6%) (Kanyeka, 1994; MAFC, 2009, but production are still very low and do not satisfy the demand for consumption. There is a need therefore, to increase rice production to sustain the demand of growing population.

1.2 Justification

Rice is a staple food in Zanzibar. Current rice consumption is estimated to be 120 000 tons per year; but, production stands at 20 000 tons per year. In order to fill the consumption deficit, 80% of the required rice is imported from Tanzania mainland and overseas (MALE, 2009; MALE, 2010). Increased production to meet rice consumption demand is constrained by several factors including low soil fertility, low yielding varieties, insect pests and diseases. Yield per unit area is very low (0.5 - 1.0 ton/ha) for upland rice with the average of 1.5tons/ha and for irrigated the average yield is 2.1 tons/ha. The potential yield of 5 tons/ha is for rainfed upland and 8 tons/ha for irrigated (Khatib and Makame, 2009).

Rice blast caused by a fungus *Pyricularia oryzae* is a major disease of rice in Zanzibar and it is a leading rice production constraint. However, the extent of losses due to the disease have not been determined in Zanzibar. The disease is widespread in all rice growing ecologies as most of the available varieties are susceptible to the disease (Khatib *et al.*, 2013). Lack of effective rice blast resistant varieties is the cause of wide spread of the disease and notoriously low yields. No research has been carried to study the pathogen to reduce the damage caused by the disease. Several elite varieties have been introduced in Zanzibar but none have been able to reduce the disease to a significant level due to lack of adequate knowledge on pathogen gene diversity of the area; as a result, most of the introduced varieties become susceptible within a short period of time. Efforts to combat the disease worldwide have been based on identifying appropriate resistant genes that effectively control the disease at a particular area taking into account the highlly variability nature of the pathogen (Siluê *et al*., 1992). Study of genes carried by the pathogen becomes a necessity in a particular area as there is a gene relationship between the host and the pathogen that control expression of host plant resistance based on Flor gene-for gene concept (Flor, 1971).

The viable option to increase rice production in Zanzibar is through the application of improved production technologies. These include identifying rice genotypes with resistance to blast which would require comprehensive studies of pathogenic genetic diversity and virulence potentials in the populations of the pathogenic fungus in different rice-agro-ecologies of Zanzibar (Dennis and Geoffrey, 2011). Initial work has been done by Africa Rice Project in mainland Tanzania where appropriate genes to control rice blast in the Southern Tanzania (Mbeya, Rukwa and Ruvuma regions) have been identified (Maganga, 2014). Extension of this work to Zanzibar will assist in identifying appropriate genes for resistance for future breeding programs. Moreover, the actual crop loss due to rice blast disease has not been established, neither isolation nor characterization of the pathogen, despite rice being an important food crop in Zanzibar (MALE, 2010).

Rice blast differential lines donated by Africa Rice Project with known genes for blast resistance will be tested against population of *Pyricularia oryzae* where suitable resistance genes for Zanzibar will be identified (Rajan et *al.*, 2013). These will be recommended to breeders for future rice breeding to improve local rice cultivars.The objective of this research therefore, is to study the prevalence, distribution, virulence potentials of *Pyricularia oryzae* and yield losses caused by the pathogen in Zanzibar (Pemba and Unguja).

1.3 Objectives

1.3.1 Overall objective

To identify rice genotypes with effective rice blast resistance genes for increased rice productivity in Zanzibar and establish losses caused by the *Pyricularia oryzae* pathogen.

1.3.2 Specific objectives

i. To determine prevalence and distribution of *Pyricularia oryzae* in Zanzibar.
ii. To conduct molecular characterization of strains of *Pyricularia oryzae* in Zanzibar.
iii. To identify effective rice blast resistance genes in Zanzibar using differential lines.
iv. To determine yield loss caused by rice blast disease in Zanzibar.

1.4 Organization of the thesis

This thesis is organized in publishable manuscripts format consisting of six chapters. Chapter one is general introduction of the thesis, chapter two, three, four, five, consist of publishable manuscripts. Chapter six is general conclusion and recommendations.

REFERENCES

Bishwajit, G., Sajeeb, S., Marce, A. K., Hui, G., Liu, J., Daogen, Y. and Sharmistha, G. (2013). Self-sufficiency in rice and food security: A South Asian Perspective. *Agriculture and Food Security* 2(10): 1-6.

Dennis, O. and Geoffrey, T. (2011). Survey on incidence and severity of rice yellow mottle virus disease in Eastern Uganda. *Journal of Plant Pathology* 2(1): 15-25.

Duan, M., Zhizhong, S., Liping, S., Yanning, T., Dong, Y., Xuewu, S., Ruifen, L.,Yujie, L., Siyu, G. and Dingyang, Y. (2013). Genetic analysis of an elite super hybrid rice parent using high-density SNP markers. *Rice Journal* 6(1): 21.

Flor, H.H. (1971). Current status of the Gene - for- Gene Concept. *Annual Review of Phytopathology* 9(1): 275- 296.

Kanyeka, Z.L., Msomba, S. and Kihupi, A.N. (1994). Rice ecosystem in Tanzania, characterization and classification. Tanzania. *Agricultural Research and Training Newsletter* 9(1-2): 13-15.

Khatib, J.K. and Makame, S.M. (2009). Evaluation of rice mutant lines for improving small holder crop productivity in Zanzibar. Paper presented in Ist Annual Agriculture Review Workshop, Zanzibar, 30-31 July, 2009.

Khatib, K.J., Mohammed, B.K. and Idarous, F.M. (2013). ParticipatoryVariety Selection of Elite Genotypes for Improving Rice Productivity in Zanzibar. *Paper* *presented at 3rd African Rice Congress* 21-24 Yaunde Cameroon October, 2013: 1-12 pp.

Khush, G.S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology* 59: 1-6.

Kihupi, A., Zakayo, J. A., Tusekelege, H., Mkuya, M., Kibanda, N.J.M., Khatib, K.J. and Maerere, A. (2008). Mutation breeding for rice improvement in Tanzania. *International Atomic Energy Agency* 40 (03): 385-387.

MAFC (Ministry of Agriculture Food Security and Cooperatives), (2009). National Rice Development Strategy final draft: 1-32 pp.

Maganga, R.M. (2014). Population structure of *pyricularia grisea* and reaction of rice cultivars in southern highlands of Tanzania. MSc. Dissertation Sokoine University ofAgriculture Morogoro, Tanzania. 112pp.

MALE (Ministry of Agriculture Livestock and Environment), (2009). Zanzibar Food Balance Sheet Report for the Year 2007: 1- 35pp.

MALE (Ministry of Agriculture Livestock and Environment) (2010). Zanzibar Agricultural Transformation for Sustainable Development, 2010-2020: 1-48 pp.

Nicholas, M. (2010). Staple Food Prices in Tanzania. African Agricultural Marketing Project (AAMP) pp. 1-15.

Rajan, H. D., Upadhyaya, S. V., Manjunatha, K. N. and Rai, S. K. (2013). Pathogenic Variation in the Pearl Millet Blast Pathogen *Magnaporthe grisea* and identification of resistance to diverse pathotypes. *Journal of Plant Disease* 97(2): 189-195.

Sheikh, G.A., Garg, V.K., Pandit, A.K., Ali, A. and Salim, A. (2011). Disease Incidence of Paddy Seedlings in Relation to Environmental Factors under Temperate Agroclimatic Conditions of Kashmir. *Journal of Research and Development* 11: 29 – 38.

Siluê, D., Notteghem, J. L. and Tharreau, D. (1992). Evidence of a gene-for-gene relation ship in the Oryza *sativa-Magnapor* the grisea pathosystem *Phytopathology* 82(5): 577–580.

CHAPTER TWO

MANUSCRIPT I

Severity, Prevalence and Control of Rice Leaf Blast Disease (*Pyricularia Oryzae Cavara*) In Rice Agro-Ecosystems of Zanzibar

[1]Ali KhatibBakar., [2]S.O.W.M. Reuben., [3]H.J.F. Lyimo

[1]**Department of Crop Science and Horticulture,** P. O. Box 97, Wete-Pemba, Email: alikhatibbakar@yahoo.com

[2]**Department of Crop Science and Horticulture,** Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Email: smreuben@yahoo.com

[3]**Department of Crop Science and Horticulture,** Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Email: flyimo_1999@yahoo.com

Target Journal and Status: International Journal of Science and Research (IJSR). Manuscript is under review process.

Abstract

Rice leaf blast disease incited by *Pyricularia oryzae Cavara* is a major disease of rice in Zanzibar wide spread in all rice growing agro-ecologies. Survey was conducted in two rice cropping seasons; 2014/15 and 2015/16 to assess the rice leaf blast disease incidence, severity, prevalence and control measures used by farmers. Six districts viz: Micheweni, Wete and Chake Chake in Pemba island and North "A", Central and West in Unguja island were assessed. The sample size was 288 farmers. In each district had 3 agro-ecologies and each provided 16 farmers which were selected randomly and forming 48 farmers for each district. Nested design was used for statistical analysis. Means were separated by Duncan's multiple range tests at ($P \leq 0.05$). Results indicated significant differences between islands for severity, incidence, prevalence, awareness, cultural control, cultural combined with fungicides. Pemba island had the lowest mean disease incidence (46.85%) and severity (18.53%) while the highest disease incidence (68.57%) and severity (20.70%) were recorded in Unguja Island during the study. About 87% of farmers interviewed were aware of the disease. Cultural methods and use of fungicides were the main means of disease control used by farmers. Percent use of fungicides was low in Pemba (21.7%) while the use of cultural control methods was higher in Pemba Island (68.84%) than in Unguja Island (59.30%). Incidence of rice blast in six different districts varied ranging from 35.96% to 74.01%, Chake chake was the lowest and North A was the highest. For the severity, Chake chake registered lowest (16.58%) and Micheweni was the highest (21.43%). The rainfed upland agro-ecologies showed highest rice blast incidence (69.34%) and the lowest (46.11%) was recorded in irrigated rice-agro-ecology. Highest rice blast disease severity (23.24%) was recorded in rainfed upland rice agro-cologies, and lowest (16.41%) was in irrigation rice agro ecologies. The use of cultural control methods was high in rainfed upland (71.3%) while combined use of cultural and fungicide was highest in irrigation rice agro-ecologies 39.3% and lowest in rainfed upland rice agro-ecologies 18.7%. In 2014-15 rice cropping season rice blast disease incidence was significantly lower 51.4% compared to 64.1% recorded in 2015-16. Disease incidence, severity, cultural control and cultural combined with fungicide showed significant interactive effect with islands and agro-ecologies. Disease incidence ranged from 31.69% in Pemba irrigation to 78.50% in Unguja rain fed upland while the severity records ranged from 15.29% in Pemba irrigation to 24.72% in rainfed upland. In Pemba 2015-2016 disease incidence recorded was lower 44.23% compared to 49.47% in 2014-2015, diseases severity significantly differed 18.23% in 2014-2015 and 18.83% in 2015-2016. Temperature showed positive and significant correlation with rice blast disease incidence ($r = 0.954$, $P< 0.01$) and severity ($r = 0.834$, $P< 0.05$). However, negative and significant correlation axhibited between temperature with incidence ($r = - 0.841$, $P< 0.05$), severity ($r = -0.842$, $P< 0.05$), temperature with relative humidity ($r = - 0.900$, $P< 0.05$) was recorded in Wete district. In West district, relative humidity showed positive and significant correlation with blast severity ($r =0.834$, $P< 0.05$). On the other hand, significant negative correlation were observed between relative humidity with disease severity ($r = - 0.836$, $P< 0.05$) and wind run with relative humidity ($r = -0.909$, $P< 0.05$).

Key words: Distribution, Prevalence, severity, *Pyricularia oryzae*, relative humidity, wind run, ratoon crops and correlations.

2.0 INTRODUCTION

Rice blast caused by a fungus *Pyricularia oryzae* Cavara, [(synonym *P. grisea* Sacc *(teleomorph: Magnaporthe grisea* (Hebert) Barr)] is a serious yield reducing disease of rice worldwide (Merketa *et al.,* 2014; Kohli *et al.,* 2011). *Pyricularia oryzae* Cavara specifically attacks rice, although some strains attack weeds belonging to the Poaceae family (Deepti *et al.,* 2014). The fungus infects other important agriculture cereal crops including barley, wheat and pearl millet (Khatib *et al.,* 2013).

Rice blast affects all aerial parts of the rice plant during vegetative phase while leaves and neck are affected during reproductive phase (Anushree *et al.*, 2016). In Africa, rice blast disease was first reported in 1922 and mostly widespread in Sub Saharan Africa (Bidaux *et al.,* 1978). *Pyricularia oryzae* Cavara overwinters on diseased straw residues that fall during crop harvest, in seeds, or on ratoon crops. These are the sources of primary inoculum for subsequent infection during growing season and from one crop season to another season (Miahet *et al.*, 2017). Under favorable conditions, conidia can survive on the host for more than a year and mycelium can survive up to three years (Ou, 1985). It is reported that high rice blast intensity is favored by periods of warm temperatures (25°C to 28°C), high relative humidity from 89% to 93% and long periods of high moisture conditions (Rosangela *et al.,* 2014). The infected leaves reduce the photosynthetic area of the plant that reduce yield (Castejón-Muñoz, 2008). Rice yield losses ranging from 20-100% have been reported in Japan, Brazil and Kenya (Kush and Jena 2009; Prabhu *et al.,* 2009; Kihoro *et al.,* 2013).

Rice production in Zanzibar suffers from many diseases caused by fungi, bacteria, virus, nematodes and other non parasitic agents. Rice leaf blast disease caused by *Pyricularia oryzae* is considered as a major threat to rice production in Zanzibar because it is wide spread and destructive in all agro-ecological zones with favourable conditions (Chuwa *et al.*, 2015; Akhilesh *et al.*, 2017). Most of the available rice varieties are susceptible to the disease (Khatib *et al.*, 2013). In Zanzibar, rice yield per unit area is very low on average being 0.5 – 1.0 ton/ha for upland rice and 2.1 tons/ha for irrigated rice compared to the potential yield of 5 and 8 tons/ha, respectively (Sekiya *et al.*, 2013). Despite wide spread occurrence of the disease in both islands of Zanzibar, no detailed study has been carried out to evaluate the incidence, severity, prevalence and available disease control measures. This study evaluates the incidence, severity, prevalence and control of rice leaf blast disease (*Pyricularia oryzae Cavara*) in the rice agro-ecosystems of Zanzibar.

2.1 MATERIALS AND METHODS

2.1.1 Description of the study area

The study was conducted in Zanzibar located at 6°09′50″S and 39°11′52″E during 2014/ 2015 and 2015/2016 rice cropping seasons. Zanzibar receives bimodal rainfall and long rains start in March to June range from 1500mm to 1800mm. Short rains start in mid-September to November with average. The driest period of the year is February – March receives about 20mm of rainfall. Mean monthly/annual temperature range from 26 -32^{o}C. The soils in Zanzibar are grouped into three categories, namely: loamy soils (‘Kinongo’), sand soil (‘Mchanga’) and clayey soils (‘Kinamo’) (Piyasiri, 1990).

2.1.2 Methodology

Field survey was carried out during 2014 and 2015 cropping seasons covering Unguja and Pemba Islands of Zanzibar. In this study, the experimental units consisted of two Islands (Pemba and Unguja) as a factor; each island had 3 districts considered as blocks nested within islands. In each block, there were 3 agro-ecologies regarded as treatments (Irrigated, Rainfed Lowland and Rainfed upland rice agro-ecologies).

2.1.3 Sample size determination

The sample size was statistically determined using the Fox *et al.* (2009) formula; Assuming that the precision proportion of respondents involved in paddy production must be plus or minus 5% which is a confidence interval (E). In this case the standard error calculated by dividing the confidence interval (E) with 1.96 which is 5/1.96 = 2.55. The estimated percentage proportion (P) of the respondents involved in paddy production is 75%. In order to calculate sample size (N) uses the following formula:

$$\mathrm{N} = \frac{P(100\% - P)}{(\mathrm{SE})^2}$$

Where: N = Sample size;

P = population percentage (75%)

E = Confidence interval (0.05)

SE = Standard error (2.55)

Therefore:

$$\mathrm{N} = \frac{75(100 - 75)}{(2.55)^2} = \frac{1875}{6.50} = 288.461 \approx 288 \text{ (Rounded up)}$$

For each agro-ecology (treatment) 16 rice fields were allocated making 48 rice fields in a block (district). Thus, each island had 48x3= 144 rice fields making a total of 288 rice fields for the two islands. The two islands are shown in Figure 1.

2.1.4 Sampling procedures

A purposive sampling technique (Mussa, 2015) was used to collect data in the three rice agro- ecologies viz., rainfed upland, rainfed lowland and rrigated. Three districts were selected randomly from each Island making a total of six districts (Micheweni, Wete and Chake Chake for Pemba and for Unguja are North “A”, Central and West) and from each district 48 rice fields were selected randomly, 16 rice fields for each agro-ecology. A total of 288 farmers' fields were surveyed when rice was at tillering stage. This is the growth stage that the rice blast symptoms are expected to reach their maximum severity levels (Mebratu *et al.*, 2015).

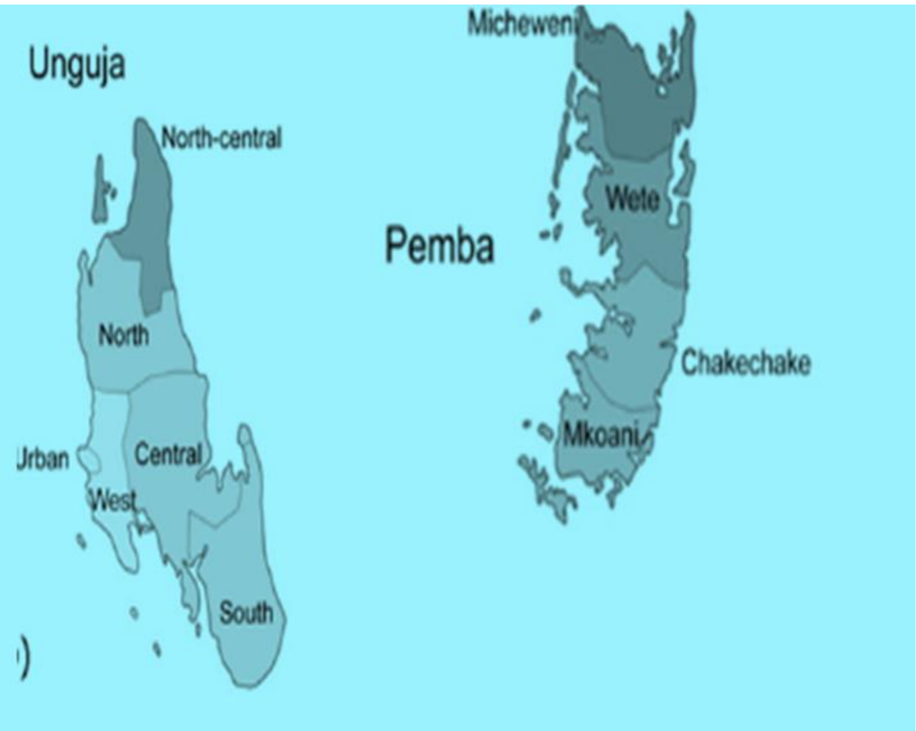


Figure 2.1: Map showing surveyed districts in Zanzibar

2.1.5 Sampling technique for disease assessment

Assessment of rice leaf blast disease severity and incidence in the field was done randomly using 1m x 1m quadrant in double diagonal fashion (Delp *et al.*, 1986).

2.1.6 Disease incidence

Disease incidence was calculated by counting the number of plants in the quadrant showing symptoms of rice blast infection over the total number of all plants in the quadrant expressed in percentage (Gatachew *et al.*, 2014).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100\%$$

2.1.7 Disease severity

Disease severity was assessed using a scale of 0-9 (IRRI, 2002). Ten infected leaves in the quadrant were selected randomly and their lesions were scored for their infection rates and percentage disease severity was calculated using formula given by Rini *et al.* (2017) and Ghimire *et al.* (2017).

$$\text{Disease severity (\%)} = \frac{\text{Sum of the scores}}{\text{Number of observations x highest number in rating}} \times 100\%$$

2.1.8 Prevalence of rice leaf blast disease

This was recorded by counting all rice fields showing typical symptoms of rice blast expressed as percentage of all fields surveyed. The percentage rice leaf blast prevalence was determined by counting the number of rice fields in each district showing typical symptoms expressed as percentage of all fields studied (Wasihun and Flagote, 2016). The prevalence was determined separately in each agro-ecology.

$$\text{Rice leaf blast disease prevalence (\%)} = \frac{\text{Number of infected fields}}{\text{Total number of fields assessed}} \times 100$$

2.1.9 Disease awareness

Disease awareness was assessed using questioners interviewing farmers if they were aware of the disease symptoms found in the field. The percentage disease awareness for the area was calculated as:

$$\text{Percentage disease awareness (\%)} = \frac{\text{Number of farmers aware of disease}}{\text{Total number of farmers interviewed}} \times 100$$

2.1.10 Assessment of current disease control measures

Due to high infection of the rice blast disease, farmers use indigenous control measures to reduce disease attacks to some extent, yet the level of disease is still very high with very little success. Every farmer (males and females of different age) applied control measures. Control measures that were used included: proper spacing, timely planting, timely weeding, avoiding excessive nitrogen fertilizer application, slashing and burning of rice straws (cultural), chemical and cultural control in combination. A questioneer was used to interview farmers on control measures used in the areas.

2.1.11 Data collection and statistical analysis

Data collected in each district included temperature, rainfall, relative humidity, wind run, sun shine, disease incidence, disease severity, disease prevalence and disease control measures currently used by farmers. Data for disease severity, incidence and prevalence were analyzed using GenStat version 16 computer statistical packages for ANOVA test. Nested design was used to determine the significant differences between factors. Means were separated by Turkeys Test at ($P \leq 0.05$). All percentage data were transformed to Arc Sine and Logarithmic transformation to make them normally distributed before analysis.

2.2 RESULTS

2.2.1 Disease infection variation

There was disease infection variation from different agro-ecologies. In irrigated field, disease severity rate was lower compared to rainfed upland agro-ecology (Plate 2.1 and Plate 2.2).

Plate 2.1: Rice leaf blast symptoms

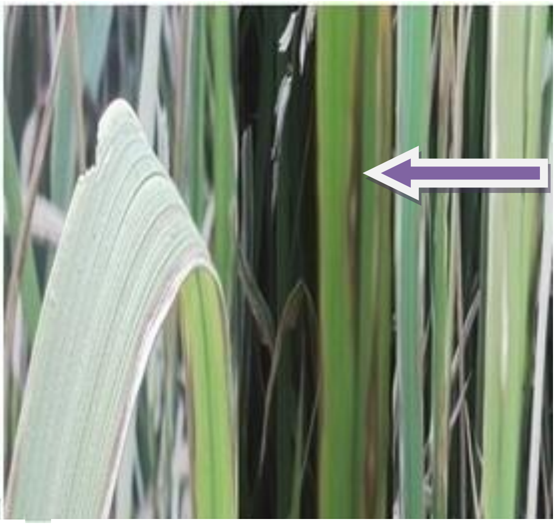


Plate 2.2: Rice leaf blast symptoms

2.2.2 Mean effects of islands on studied variables

Pemba island recorded the significancy ($P \leq 0.05$) lowest disease incidence (46.85%) and severity (18.53%). On the other hand, high percentage of farmers used cultural control methods in Pemba (68.84%) than in Unguja Island (59.30%). Overall means of variables recorded in Unguja were significantly higher than recorded in Pemba except for cultural control method. (Table 2.1).

Table 2.1: Mean effect of Islands on studied variables

Island	Incidence (%)	Severity (%)	Disease prevalence (%)	Disease awareness (%)	Use of Cultural control (%)	Use of Cultural and fungicides. Control (%)
Pemba	46.85^{b}	18.53^{b}	9.53^{b}	44.0^{b}	68.84^{a}	21.7^{b}
Unguja	68.57^{a}	20.70^{a}	10.00^{a}	56.1^{a}	59.30^{b}	31.0^{a}
Mean	**57.71**	**19.62**	**9.76**	**50.10**	**64.1**	**26.3**
$SE_{\bar{x}}$ (±)	1.18	0.34	0.09	2.02	2.35	2.39
LSD at 0.05	3.28	0.95	0.28	5.96	6.92	7.05

Means followed by the same letter are not statistically different $P \leq 0.05$ according to DMRT

2.2.3 Mean effect of districts on studied variables

The mean scores for the studied variables varied significantly (P≤0.05) between districts. The mean disease incidence recorded in Micheweni and Wete districts were not significantly (P= 0.05) different. Highest mean disease incidence score was recorded in North A (74.01%) then in Central (70.80%) districts but did not differ significantly. Disease incidence recorded at West (60.91%) and Chake chake (35.96%) were significantly different. The least severity was recorded at Chake chake (16.58%) followed by West (18.97%) and North A (19.09%). Micheweni recorded the highest severity attack (21.23%), while the least least disease prevalence (9.3%) was recorded at Chakechake followed by Micheweni (9.72%). For disease awareness, observations revealed that North A, Central and West had the highest scores (54.2-59.7%) and Chakechake and Micheweni had the least (40.6 - 41.8%). For cultural disease control variable, Chakechake and Micheweni had the highest scores (73 and 76%) respectively while West (53.8%) and Wete (57.5%) had the least. For cultural and fungicide control records West (37.1%) and Wete (32.5%) had the highest rating while Chakechake had the least (16.9%). (Table 2.2).

Table 2.2: Mean effects of districts on studied variables

Districts	Disease incidence (%)	Disease severity (%)	Disease prevalence (%)	Disease awareness (%)	Cultural control (%)	Cultural and Fungicide. Control (%)
Micheweni	52.57^{c}	21.43^{b}	9.72^{ab}	41.8^{b}	76.00^{a}	15.58^{b}
Wete	52.03^{c}	20.84^{ab}	9.61^{ab}	49.6^{ab}	57.49^{c}	32.51^{a}
Chakechake	35.96^{d}	16.58^{c}	9.28^{b}	40.6^{b}	73.04^{ab}	16.95^{b}
North A	74.01^{a}	19.09^{b}	10.00^{a}	54.4^{a}	61.60^{bc}	28.39^{ab}
Central	70.80^{a}	20.78^{a}	10.00^{a}	54.2^{a}	62.52^{bc}	27.48^{ab}
West	60.91^{b}	18.97^{b}	10.00^{a}	59.7^{a}	53.79^{c}	37.08^{a}
$\bar{\mathbf{x}}$	**57.71**	**19.62**	**9.76**	**50.10**	**64.10**	**26.33**
$SE_{\bar{x}}$ (±)	2.04	0.59	0.17	3.50	4.06	4.14
LSD at 0.05	5.68	1.65	0.49	10.32	11.99	12.21

Means followed by the same letter are not statistically different P≤0.05 according to DMRT

2.2.4 Effect of rice-agro ecosystems on studied variables

Rainfed upland rice had highest incidence (69.34%) of rice blast followed by rainfed lowland (57.69%) and the lowest disease incidence (46.11%) was recorded in irrigation rice agro-ecologies (Table 2.3). The highest rice blast disease severity (23.24%) was recorded in rainfed upland agro ecologies followed by (19.20%) in rainfed lowland rice agro-ecologies and lowest rice blast disease severity (16.41%) was recorded in irrigation rice agro ecology. On the other hand, rice blast disease prevalence and percentage disease awareness by farmer's rice agro-ecologies revealed no significant differences. Highest percentage use of cultural control methods was recorded in rain fed upland (71.3%) agro ecologies followed by rainfed lowland (69.07%). Combined use of cultural and fungicide control methods was highest (39.3%) in irrigation rice-agro-ecologies and lowest in rained upland rice agro-ecologies (18.7%). (Table 2.3).

Table 2.3: Main effect of rice agro-ecologies on the studied variables

Ecologies	Disease incidence (%)	Disease Severity (%)	Disease Prevalence (%)	Disease awareness (%)	Cultural control (%)	Cultural and Fungici Control (%)
Irrigation	46.11^{c}	16.41^{c}	9.77^{a}	55.6^{a}	51.91^{b}	39.28^{a}
Rainfed lowland	57.69^{b}	19.20^{b}	9.86^{a}	45.3^{a}	69.07^{a}	20.98^{b}
Rainfed upland	69.34^{a}	23.24^{a}	9.66^{a}	49.3^{a}	71.26^{a}	18.74^{b}
$\bar{x}$	**57.71**	**19.62**	**9.76**	**50.10**	**64.1**	**26.33**
SE$_{x}$ (±)	1.45	0.42	0.12	2.47	2.87	2.92
LSD at 0.05	4.01	1.16	0.35	7.29	8.48	8.63

Means with different superscript letters down the column differ significantly at $P \leq 0.05$

2.2.5 Effect of years (cropping seasons) on studied variables

Rice blast disease incidence was significantly ($P \leq 0.05$) lower in 2014-15 season (51.4%) compared to 2015-16 season (64.1%). Non-significant effects were observed between means of the rest of the variables (Table 2.4).

Table 2.4: Means effect of years for the studied variables

Years	Disease incidence (%)	Disease severity (%)	Disease prevalence (%)	Disease awareness (%)	Cultural control (%)	Cultural &Fungicide.control (%)
2014-2015	51.37^{b}	19.45^{a}	9.79^{a}	48.9^{a}	61.77^{a}	28.54^{a}
2015-2016	64.06^{a}	19.78^{a}	9.74^{a}	51.2^{a}	66.37^{a}	24.12^{a}
$\bar{\mathbf{x}}$	**57.71**	**19.62**	**9.76**	**50.1**	**64.1**	**26.3**
$SE_{\bar{x}}$ (±)	1.18	0.34	0.09	2.02	2.35	2.39
LSD at 0.05	3.28	0.95	0.28	5.96	6.92	7.05

Means with different superscript letters down the column differ significantly at $P \leq 0.05$

2.2.6 Interaction effect of island (location) x rice agro-ecologies

Lowest rice blast disease incidence (31.7%) and severity (15.29%) were recorded in Pemba under irrigation rice agro-ecologies. On the other hand, Unguja rainfed upland registered the highest rice blast disease incidence (78.50%) and severity (24.72%). The effect of rice ecologies x island interaction on rice blast disease prevalence was not significant. The percent use of cultural control methods for control of rice blast was highest in Pemba island under rainfed upland system (78.1%) and lowest (46.8%) in Unguja under irrigation systems. The highest use of cultural and fungicide control methods was registered in Unguja under irrigation system (44.1%) while the lowest (11.9%) was recorded in Pemba under rainfed upland systems. The percentage disease awareness by farmers was highest in Unguja under irrigation (58.4%) system and lowest in Pemba under rainfed lowland (36.2%) systems. (Table 2.5).

Table 2.5: Interaction effect of island (location) x rice agro-ecologies

Island and Ecology	Disease incidence (%)	Disease severity (%)	Disease Prevalence (%)	Disease awareness (%)	Cultural control (%)	Cultural and Fungicide. Control (%)
Pemba irrigation	31.69^{a}	15.29^{a}	9.558^{a}	52.72^{b}	56.97^{ab}	34.53^{bc}
Pemba rainfed lowland	48.68^{b}	17.53^{b}	9.727^{a}	36.23^{a}	71.47^{bc}	18.63^{ab}
Pemba rainfed upland	60.17^{c}	18.55^{bc}	9.330^{a}	43.01^{ab}	78.10^{c}	11.90^{a}
Unguja irrigation	60.53^{c}	19.86^{c}	10.000^{a}	58.38^{b}	46.84^{a}	44.05^{c}
Ungujarainfed lowland	66.69^{c}	21.75^{d}	10.000^{a}	54.33^{b}	66.67^{bc}	23.33^{ab}
Ungujarainfed upland	78.50^{d}	24.72^{e}	10.000^{a}	55.60^{b}	64.42^{abc}	25.58^{ab}
$\bar{\mathbf{x}}$	**57.71**	**19.62**	**9.76**	**50.1**	**64.1**	**26.3**
SE_{x} (±)	2.044	0.59	0.165	3.50	4.06	4.14
LSD at 0.05	5.677	1.65	0.488	10.32	11.99	12.21

Means with different superscript letters down the column differ significantly at $P \leq 0.05$

2.2.7 Interaction effect of rice agro-ecologies and cropping seasons (years)

In Irrigation 2014-2015, disease incidence registered lowest (35.73%) and highest (74.33%) was recorded in rainfed upland 2015-2016. There was no significant difference between irrigation 2015-2016 and rainfed lowland 2015-2016. Lowest disease severity (15.32%) was recorded in irrigation 2014-2015 and the highest (24.24%) was recorded in rainfed upland 2015-2016. For disease prevalence and disease awareness, values did not differ significantly. In irrigated 2014 – 2015, lowest value (48.11%) was recorded for cultural control and highest (75.69%) was for rainfed upland, while for cultural and fungicide control lowest value (14.31%) was recorded in rainfed upland in 2015-2016 and highest (42.77%) was for irrigated 2014-2015 (Table 2.6).

Table 2.6: Interaction effect of rice agro-ecologies x cropping seasons (years)

Ecology and year	Disease incidence (%)	Disease severity (%)	Disease Prevalence (%)	Disease awarenes s (%)	Cultural control (%)	Cultural and Fungicide. Control (%)
Irrigation 2014-2015	35.73^{a}	15.32^{a}	9.612^{a}	56.59^{a}	48.11^{a}	42.77^{c}
Rainfed lowland 2014-2015	54.03^{b}	17.49^{b}	10.000^{a}	41.74^{a}	70.39^{bc}	19.70^{ab}
Irrigation 2015-2016	56.48^{bc}	18.79^{bc}	9.947^{a}	54.21^{a}	55.70^{ab}	35.80^{bc}
Rainfed lowland 2015-2016	61.36^{bc}	19.62^{c}	9.727^{a}	48.82^{a}	67.74^{bc}	22.26^{ab}
Rainfed upland 2014-2015	64.34^{c}	22.24^{d}	9.777^{a}	47.95^{a}	66.84^{bc}	23.16^{ab}
Rainfed upland 2015- 2016	74.33^{d}	24.24^{e}	9.553^{a}	50.67^{a}	75.69^{c}	14.31^{a}
$\bar{X}$	**57.71**	**19.62**	**9.76**	**50.1**	**64.1**	**26.3**
$SE_{\bar{x}}$ (±)	2.044	0.59	0.165	3.50	4.06	4.14
LSD at 0.05	5.677	1.65	0.488	10.32	11.99	12.21

Means with different superscript letters down the column differ significantly at $P \leq 0.05$

2.2.8 Interaction effect of Island (location) and cropping seasons (years) on the studied variables

The highest disease incidence (83.89%) was recorded in Unguja and the lowest disease incidence (44.23%) was recorded in Pemba during 2015-2016. In 2014-2015 cropping season Pemba registered significantly lowest rice blast disease severity (18.23%). On disease prevalence, results showed that disease prevailed more or less at equal levels in both islands and years. Percentage disease awareness by farmers was significantly lowest (40.13%) in Pemba for 2014-2015 cropping season while the highest was recorded in Unguja 2014-15 (57.6%) and 2015-16 (54.6%). The use of cultural control methods for control of rice blast was significantly lowest (52.24%) in Unguja for 2014-2015 and highest in Pemba for 2014-15 (71.3%) followed by Unguja and Pemba for 2015-16 (66.4%). The percentage use of cultural and chemical control methods was highest in Unguja for 2014-15 (38.4%) and lowest in Pemba during 2014 – 2015 (18.8%). (Table 2.7).

Table 2.7: Interaction effect of Island and cropping seasons (years)

Islands and year	Disease incidence (%)	Disease severity (%)	Disease Prevalence (%)	Disease awareness (%)	Cultural control (%)	Cultural and fungicide. Control (%)
Pemba 2015-2016	44.23^{a}	18.83^{ab}	9.484^{a}	47.85^{ab}	66.37^{b}	24.63^{a}
Pemba 2014-2015	49.47^{ab}	18.23^{a}	9.592^{a}	40.13^{a}	71.32^{b}	18.75^{a}
Unguja 2014-2015	53.26^{b}	20.67^{b}	10.000^{a}	57.59^{b}	52.24^{a}	38.35^{b}
_Unguja 2015-2016	83.89^{c}	20.74^{b}	10.000^{a}	54.61^{b}	66.38^{b}	23.62^{a}
X	**57.71**	**19.62**	**9.76**	**50.1**	**64.1**	**26.3**
$SE_{\bar{x}}$ (±)	1.669	0.48	0.135	2.86	3.32	3.38
LSD at 0.05	4.635	1.34	0.399	8.42	9.79	9.97

Means with different superscript letters down the column differ significantly at $P \leq 0.05$

2.2.9 Correlations of incidence and severity with weather factor at Central district Unguja

Disease incidence showed positive and significant r = .0507, P<0.01 corrilation with disease severity, disease incidence showed negative significant r = -.293, P<0.01 correlation with rainfall; incidence showed positive significant r = .423, P<0.01 corrilation with remperature. On the other hand, disease severity was negative significant r = -202, P<0.05, whilest negative significant r = -.260, P<0.05 corrilation was observed between rainfall and temperature. Table 2.8.

Table 2.8: Correlation coefficients for disease incidence, severity and weather factors in Central district Unguja

Variables	Incidence	Severity	Prevalence	Rainfall	Temperature	RH	Wind run	Sun shine
Incidence	1							
Severity	.507**	1						
Prevalence	-.020	-.034	1					
Rainfall	-.293**	-.202*	.049	1				
Temperature	.423**	.044	.014	-.260*	1			
RH	-.099	-.035	-.197	.101	-.084	1		
Wind run	.171	.348	.029	-.166	-.191	-.034	1	
Sun shine	-.191	.093	.071	-.046	.022	.066	.035	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

2.2.10 Correlations of incidence and severity with weather factor in Micheweni district Pemba

Incidence was positive and significant r = .791, P<0.01 corrilated with severity, incidence with relative humidity r = .275, P<0.01. Severity was registered positive and significant r = .335, P<0.01, correlation with relative humidity; severity with wind run r = .260, P<0.05; severity with sun shine r = .228, P<0.05. Temperature showed positive and significant r = .202, P<0.05 corrilation with relative humidity. Relative humidity showed positive and significant r = .228, P<0.05 corrilation with wind run, relative humidity with sun shine r = .328, P<0.01.

Table 2.9: Correlation coefficients of incidence and severity with weather factor in Micheweni district Pemba

Variables	Incidence	Severity	Prevalence	Rainfall	Temperature	RH	Wind run	Sun shine
Incidence	1							
Severity	.791**	1						
Prevalence	-.106	-.114	1					
Rainfall	.090	-.001	.119	1				
Temperature	.056	.074	-.204*	.023	1			
RH	.275**	.335**	-.158	.016	.202*	1		
Wind run	.082	.260*	.020	-.085	-.088	.228*	1	
Sun shine	.064	.228*	-.048	-.110	.023	.328**	.372**	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Wind run also showed positive and significant r = .372, P<0.01 corrilation with sun shine. On the other hand, prevalence showed negative and significant r = -.204, P<0.05 corilation with temperature Table 2.9.

2.2.11 Correlations of incidence and severity with weather factor in Chake Chake district Pemba

Results exhibited positive significant r = .779, P<0.01 relationship between incidence with severity, incidence with temperature r = .215, P<0.05. Severity exhibited positive significant r = .269, P<0.01 corrilation with temperature, also severity with wind run r = .270, P<0.01. Not only but also temperature showed positive significant r = .312, P<0.01. Contrary rainfall showed negative significant r = -.223, P<0.05 corrilation with temperature. Another negative significant r = -.355, P<0.01 relationship was registered between temperature and wind run (Table 2.10).

Table 2.10: Correlations of incidence and severity with weather factor in Chake Chake district Pemba

Variables	Incidence	Severity	Prevalence	Rain fall	Temperature	RH	Wind run	Sun shine
Incidence	1							
Severity	.779**	1						
Prevalence	.001	.024	1					
Rain fall	-.044	-.105	-.107	1				
Temperature	.215*	.269**	-.022	-.223*	1			
RH	.243*	.046	-.200	-.165	.312**	1		
Wind run	.163	.270**	.077	-.107	-.355**	-.081	1	
Sun shine	.147	.157	.073	.095	.123	.086	.076	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

2.2.12 Correlations of incidence and severity with weather factor in north A district Unguja

There were positive significant r = .238, P<0.05 correlations between incidence with rain fall; also rainfall had positive significant r = .247, P<0.05.

Morover, negative significant r = -.342, P<0.01 relationship exhibited between temperature and wind run (Table 2.11).

Table 2.11: Correlation of incidence and severity with weather factor in North A district Unguja

Variable	Incidence	Severity	Prevalence	Rain fall	Temperature	Rh	Wind run	Sun shine
Incidence	1							
Severity	-.107	1						
Prevalence	.155	.033	1					
Rainfall	.238*	-.058	-.046	1				
Temperature	.056	-.064	-.044	.247*	1			
Rh	-.109	.125	.070	.034	.030	1		
Wind run	.147	.139	.062	-.110	-.342**	-.035	1	
Sun shine	.108	-.022	-.079	.092	-.009	.046	.023	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

2.2.13 Correlations of incidence and severity with weather factor in Wete district Pemba

Positive significant r = .582, P<0.01, correlations were observed between incidence with severity and between incidence with relative humidity r =.508, P<0.01. Severity showed positive significant r = .408, P<0.01 corrilation with relative humidity. (Table 2.12). Rain fall exhibit negative significant r = -.377, P<0.01 corrilation with windrun. However, temperature showed significant r = -.270, P<0.01 corrilation with relative humidity and rain fall with wind run r = -.234, P<0.05 respectively.

Table 2.12: Correlations of incidence and severity with weather factor in Wete district

Variable	Incidence	Severity	Prevalence	Rain fall	Temperature	Rh	Wind run	Sun shine
Incidence	1							
Severity	.582**	1						
Prevalence	-.133	-.073	1					
Rainfall	-.133	-.152	-.001	1				
Temperature	-.141	-.154	-.033	.144	1			
Rh	.508**	.408**	-.005	-.138	-.270**	1		
Wind run	.076	.172	.085	-.377**	-.234*	.131	1	
Sun shine	.064	-.065	.048	-.039	-.002	.007	.120	1

**. Correlation is significant at the 0.01 level (2-tailed)
*. Correlation is significant at the 0.05 level (2-tailed)

2.2.14 Correlations of incidence and severity with weather factor in West district Unguja

Results revealed incidence positive significant r = .241, P<0.05 correlations with severity, positive significant r = .210, P<0.05. Incidence had showed positive significant r = .241, P<0.05 corrilation with wind run and positive significant r =.249, P<0.05 corrilation with sun shine. Rainfall had significant r = .201, P<0.05 relationship with temperature. On the other hand, negative significant r = -.266, P<0.01 relationship was observed between severity with temperature however temperature had also negative significant r = -.237, P<0.05 corrilation with wind run (Table 2.13).

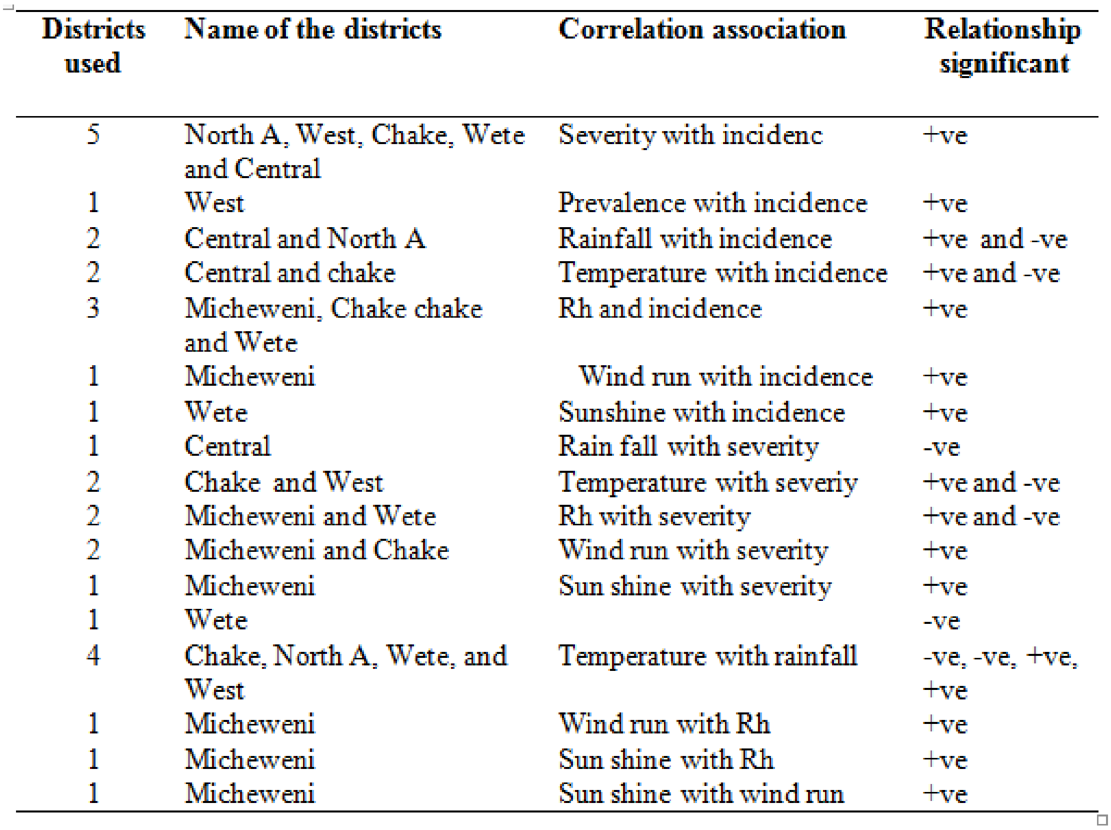
Table 2.13: Correlation of incidence and severity with weather factor in West district Unguja

Variable	Incidence	Severity	Prevalence	Rainfall	Temperature	Rh	Wind run	Sun shine
Incidence	**1**							
Severity	.241*	1						
prevalence	.210*	.169	1					
Rainfall	.017	-.051	.070	1				
Temperature	-.130	-.266**	-.047	.201*	1			
Rh	.041	-.088	.096	-.031	-.011	1		
Wind run	.241*	.147	.085	-.011	-.237*	-.118	1	
Sun shine	.249*	-.121	.010	-.094	.094	.160	.035	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

The six districts studied, variables tested reacted different significant correlation reactions. In summary the reactions against districts are shown in (Table 2.14).

Table 2.14: Correlations in the respective districts



Districts used	Name of the districts	Correlation association	Relationship significant
5	North A, West, Chake, Wete and Central	Severity with incidenc	+ve
1	West	Prevalence with incidence	+ve
2	Central and North A	Rainfall with incidence	+ve and -ve
2	Central and chake	Temperature with incidence	+ve and -ve
3	Micheweni, Chake chake and Wete	Rh and incidence	+ve
1	Micheweni	Wind run with incidence	+ve
1	Wete	Sunshine with incidence	+ve
1	Central	Rain fall with severity	-ve
2	Chake and West	Temperature with severiy	+ve and -ve
2	Micheweni and Wete	Rh with severity	+ve and -ve
2	Micheweni and Chake	Wind run with severity	+ve
1	Micheweni	Sun shine with severity	+ve
1	Wete		-ve
4	Chake, North A, Wete, and West	Temperature with rainfall	-ve, -ve, +ve, +ve
1	Micheweni	Wind run with Rh	+ve
1	Micheweni	Sun shine with Rh	+ve
1	Micheweni	Sun shine with wind run	+ve

2.3 Discussion

Rice leaf blast disease is widely spread in Zanzibar islands. In Unguja island disease intensity was registered higher than Pemba island that was associated with environmental factors favor disease development and spread including temperature and relative humidity. During the study in Pemba Island temperature ranged from 25.8 to 29.3^{0}C and for Unguja, ranged from 25.2 to 31.7^{0}C. Relative humidity Pemba ranged from 72 to 78% and for Unguja ranged from 64 to 89% all of which might have aggravated the disease severity. Similarly, Rajendra *et al.* (2015), Mebratu *et al.* (2016), Rini *et al.* (2017) reported that temperature of 20 to 35^{0}C and relative humidity of 90% had been reported to be favorable for the rice leaf blast development. Lowest disease in Pemba was associated with the 68.84% of farmers use cultural practices (timely weeding, use of proper spacing, destruction of crop residues). It is cheap and effective cost which leads to reduced disease distribution as it discourages distribution of inoculums however, the method will not provide complete eradication of the disease. Similar findings were supported by Willium *et al.* (2013), Tingting (2015), Raveloson *et al.* (2017), Christina and Cruz (2017); Miah *et al.* (2017). Grasses of the same family with rice were found accumulated in some of the fields which can be alternative hosts of the rice blast pathogen as reported by Hajime (2001), Jitendiya and Chhetry (2014). Application of fungicides in controlling diseases of rice are not widely used by small-scale farmers due to the non availability and high cost which make farmers not able to afford. Furthermore, fungicide application has the side effect of pollution of the environment and the toxic effects of synthetic chemicals on non-target organisms including insect pests, animals and humans as reported by Sukanya *et al.* (2011).

Farmers were interviewed on the perceptions of rice leaf blast disease to know their skills on how much they know about leaf blast disease (*Pyricularia oryzae*) particularly on whether their field had been affected by the rice leaf blast or not. From the comprehensive survey, findings revealed that many farmers had knowledge of a rice leaf blast disease despite their farms infected by the disease, they did not take any control measures to reduce infection in rice fields. This suggests that extension personell need to make close follow up of implementing control measures in rice field; including timely weeding, use of proper spacing, and destruction of crop residues. Chake Chake districts had the least rates of disease intensity.

The situation was associated with Environmental conditions are less favorable to disease and adopted cultural practices in their rice field. Groth and Bond (2007) reported that variation of rice leaf blast disease intensity depended on inoculums amount availability, cultural practices and agro-meteorological factor conditions. Low disease intensity in

Chake chake district is influenced by the existence international rice projects such as ERPP (Expanding Rice Production Preoject) which is funded by the World Bank, TANRICE, funded by JICA Japan and Construction of Zanzibar Irrigation Infrastructure. It is funded by the government of South Korea. Running projects causing farmers to be closer to the experts and agriculture extension officers in disease prevention measures.

2.3.1 Mean effect of Islands and ecologies with their interactions

Disease incidence and severity in Pemba were lower in irrigated agro-ecology. Several factors lead to low disease rating including more uses cultural practices and other management application in irrigated agro- ecology. In irrigation agro-ecology water seeding (planting on very wet soil) is recommended as this reduces disease transmission from the seed to the seedlings because of the anaerobic condition that is unfavorable to the *Pyricularia oryzae* pathogen as reported by Hajime (2001) Joseph (2014) and Miah *et al.* (2017). In irrigation, continuous flooding is recommended to limit rice leaf blast disease development as a water management strategy compared to situation under water stress. Draining water for long periods is not recommended as may allow the formation of nitrates resulting to drought stress. Shallow water in the irrigated rice field favors the disease, so water should be flooded deeper ranging from 4to 8inches (Tingting, 2015).

In irrigation when fields are flooded with water they have shorter dew periods than rainfed upland due to the sun heating the water in the field to 42^0C. At night, the warm water releases the absorbed heat gradually, hence dew formation is delayed. Rice plant leaf closer to the water surface shortens the dew period. Extended dew period on the plant leads to high disease infection. (Tingting, 2015; Magar *et al.*, 2015).

2.4 Mean effect of rice ecologies and cropping seasons and their interactions

2.4.1 Mean effect of seasons

Lower disease incidence and severity in irrigation agro-ecology 2014-2015 was associated with medium level of cultural method and combination of cultural with fungicide application. These two applications contributed low disease incidence and severity. The result suggests that the effectiveness of a control method depends of factors prevailing including weather variables, such as air temperature, soil temperature, relative humidity, spore dissemination and leaf wetness (Katsantonis *et al.*, 2017). These are among the most critical factors, since these play important roles in rice leaf blast disease caused by *Pyricularia oryzae* pathogenesis and rice blast development. Irrigation system farmers transplant seedlings after nursery establishment Jitendiya and Chhetry (2014) reported that transplanting of rice seedlings in irrigated field reduces the disease incidence and disease severity of rice leaf blast over broadcasting. In addition having weeded two to three times over hand weeding indicating role of certain weeds which serves as the alternate host for the rice leaf blast pathogens. Application o f cultural practices, it could be concluded to positive effect on reducing the rice leaf blast disease and may provide a useful means to the small scale farmers who cannot afford costly chemicals for the disease control. Agricultural field extension worker have to work harder insisting farmers employing cultural practice in rainfed upland rice but number of extension workers are very little to cover the area.

2.4.2 Interaction of island and cropping season

Interaction of island and cropping season indicated that low disease incidence, severity and prevalence in Pemba during 2015-2016 were associated with relatively high level of cultural control methods. On the other hand, highest incidence, severity and prevalence in Unguja during 2015-2016 was associated with medium levels of cultural and fungicide use and cultural methods. Combination of island and year, cultural method and combine together with fungicide do not seem to help much. Thus, lowest infection rate were observed in Pemba during the two seasons but with medium rate of the control practices. Adoption of integrated field management control practices of rice leaf blast disease could be an import and appropriate tool for the pathogen.

2.4.3 Correlations of incidence and severity with weather factor at central district Unguja

In the present investigation in Central district revealed that, weather parameters levels used as indices in disease status, in such, incidence was positively significant correlation with severity and temperature respectively. It showed that Increase incidence led to an increase in severity and increase in temperature led to increase in incidence. Therefore, disease incidence influenced disease seerity. This was attributed to favourable weather levels during rice growth and development stages in the field (Nyang'au *et al.,* 2014). Severity was negatively significant related with the rainfall and rainfall was negative and significant with temperature. Severity and rainfall are interrelated in disease development (rice leaf blast disease) and are highly influenced by environmental factors. Unreliable rainfall during rice growth development led high night temperature and influence rice blast disease severity. Heavy rainfall causes temperature to fall hence low disease severity.

Micheweni district the results reveal that there was a strong and positive relationship between incidence with severity and relative humidity. Severity had positive and significant correlation with relative humidity, wind run and sun shine. The increase of one variable led to increase of the other variable. The increase of incidence led to increase of severity hence plant suffer for disease. High speed wind movement caused blast existence spores to move far distance to another field for infection. Presence water dew on rice leaf for long time was associated to the sunshine. Temperature and relative humidity was positive significant related. The two variables are interdependent in disease development. High temperature leads to low level of relative humidity and vice versa. Exceeded temperature ranged 20 to 35^0C influence blast disease incidence and severity and extreme relative humidity above 90 influence blast disease (Miah *et al.* (2017).

Chake chake district, results reveal highly positive significant correlation relationship between incidence and severity, incidence had positive significant with temperature, and relative humidity (Gururaj, 2013). On the other hand, severity revealed positive and significant correlation with temperature and wind run. Temperature found positive significant correlated with relative humidity. Negative significant correlated showed between temperature with wind run and between rainfalls with temperature. In chake ckake district disease rate was registered low that was associated with significant low levels of weather factors.

North A district, incidence showed positive significant correlation with rainfall. Rainfal showed positive significant correlation with temperature while wind run showed negative significant with temperature. This implies that high wind speed lead temperature to be low which causes the blast spores reaching long distance to infect other rice field from inoculums source while low wind speed temperature raised higher leading blast spore reaching short distance from inoculums sources (Vincent, 2008). However, Wete district, incidence exhibited positive significant correlation with severity and relative humidity. These factors are interdependent in disease development. Presence of high temperature lead high rice leaf blast rate. Severity had positive significant correlated with relative humidity, meaning that high relative humidity in the atmosphere leading high disease severity rate. Rajendra *et al.* (2015), Mebratu *et al.* (2016); Rini *et al.* (2017). Negative significant correlation was observed between rainfall with wind run. Temperature exhibited negative significant correlation with relative humidity and wind run. When temperature is high, relative humidity becomes low, and if relative humidity is high temperature goes low. Those two factors influence blast disease to occur.

West district in Unguja incidence revealed positive significant correlation with severity, wind run and sun shine. Rainfall showed positive significant correlation with temperature. Further analysis resulted negative significant relationship between severity with temperature. Wind run showed negative significant correlation with temperature. High temperature rate led high disease severity rate. The rest of the variable did not show any significant corrilations with other variable due to unfavourable conditions to match. In some districts some corrilations are different from other districts, which caused by variations of weather factors not being similar from other districts.

2.5 Conclusion

In Pemba Island, disease incidence, severity and prevalence were significantly lower than in Unguja. In the six districts surveyed, significant disease variation occurred on disease incidence, severity, cultural and cultural in combination with fungicide while North A district had highest disease incidence and lowest was in chake chake district. On the other hand, in irrigation agro-ecology, diseased incidence and severity were significantly lower. Maximum incidence and severity were exhibited in raifed upland ecology. Within the two years of survey, significant difference was registered on disease incidence in 2014-2015 cropping season. Combination of ecology with island, year with island, ecology with year and three interaction of island, ecology and year showed significant variation in disease incidence while in disease severity showed no significant different except for ecology with year which was highly significantly different. Rice leaf blast disease is widely spread in all districts. Correlation coefficient analysis, weather factors such as temperature, rain fall, wind run, relative humidity and sun shine were associated with disease incidence, severity and prevalence rate.

2.6 Recommendations

i. Farmers should adopt cultural and combination with fungicides for reduction of infection rate particularly under irrigated ecologies of Zanzibar.

ii. Zanzibar island should encouraged to participate more in rice irrigation farming. It is more efficient, economically production and efficient in their resource allocation than those in rain fed production.

References

Akhilesh, K.K., Neha, S., Mukesh, K.S., Roshan, K., Kushram, T. and Sanath, V.B.K. (2017). Growth of Rice Blast Fungus Pyricularia oryzae (Cav.) on Different Solid and Liquid Media. *International Journal of Current Microbiology and Applied Sciences* 6(6): 1154-1160.

Anushree, P.U., Naik, R.M., Satbhai, R.D., Gaikwad, A.P. and Nimbalkar, C.A. (2016). Differential biochemical response of rice (*Oryza sativa* L.) genotypes against rice blast (*Magnaporthe oryzae*). *Cogent Biology* (2016) 2: 1-15.

Bidaux, J.M., Buddenhagen, I.W. and Persley, G.J. (1978). Screening for horizontalresistance to rice blast (*Pyricularia oryzae*) in Africa. International Institute of Tropical Agriculture, Ibadan, Nigeria, London, New York, San Francisco. pp. 159–174.

Castejón-Muñoz, M. (2008). The effect of temperature and relative humidity on the airbone concentration of *Pyricularia oryzae* spores and the development of rice blast in Southern Spain. *Spanish Journal of Agricultural Research* 6(1): 61-69.

Chuwa, C.J. Mabagala, R.B. and Mnyuku, S.O.W.R. (2015). Pathogenic Variation and Molecular Characterization of *Pyriculariaoryzae*, Causal Agent of Rice Blast Disease in Tanzania. *International Journal of Science and Research* 4(11): 1131 – 1139.

Christina, D.C. and Barbar, V. (2017). Wheat disease: danger on the move. *Tropical plant pathology* 42: 210-222.

Deepti, S., Shamim, M.D., Deepak, K., Pramila, P., Khan, N.A. and Singh, K.N. (2014). Morphological and Molecular Characterization of Pyricularia Oryzae Causing Blast Disease in Rice (*Oryza Sativa*) from North India. *International Journal of Scientific and Research Publications* 4(7): 1-9.

Delp, B.R., Stowell, L.J. and Marois, J.J. (1986). Field Runner: A Disease Incidence, Severity and Spatial Pattern Assessment System. *Plant disease* 70 (10): 954 - 957.

Fox, N., Hunn, A. and Mathers, N. (2009). *Sampling and sample size calculation.* TheNationaInstitute of Health Research, Research Design Services for the East Midlands/Yorkshire and the Humber. 41pp.

Getachew, G., Tesfaye, A. and Kassahun, T. (2014). Evaluation of disease incidence and severity and yield loss of finger millet varieties and mycelia growth inhibition of *P. grisea* isolates using biological antagonists and fungicides in vitro condition. *Journal of Applied Bio-sciences* 73: 5883 – 5901.

Ghimire, P., Gopal, K.C., Shrestha, S.M. and Parajuli, G. (2017). Evaluation of Fungicides and Bio-agent against Neck Blast Disease of Rice. *Journal of Plant Pathology and Microbiology* 8: 428 1-2.

Groth, D.E. and Bond, J.A. (2007). Effects of cultivars and fungicides on rice sheath blight, yield and quality. *Plant Disease* 91: 1647 – 1650.

Gururaj, B.B. (2013). Influence of weather parameters on pests and diseases of soybean. Thesis submitted to the University of Agricultural Sciences, Dharwad in partial fulfilment of the requirements for the Degree of Master of Science (Agriculture) in Agricultural Statistics 1-82 pp.

Hajime, K. (2001). Rice blast disease. *Journal of Royal Society of Chemistry* 1-3.

IRRI (International Rice Research Institute) (2002). Standard Evaluation System for Rice. International Rice Research Institute, Los Banos, Manila, Philippines. 51pp.

Jitendiya, D.O. and Chhetry, G.K.N. (2014). Effect of certain traditional cultural practices for the management of blast disease of rice in Manipur agro-climatic conditions. *Journal of Agriculture and Veterinary Science* 7(7): 01-03.

Joseph, K.M. (2014). The impact of rice blast disease, its mapping and suitability analysis for rice growing sites in the greater Mwea region. A Thesis Submitted in Partial Fulfillment for the Degree of Masters of Science in Landscape Planning and Conservation in the Jomo Kenyatta University of Agriculture and Technology pp. 4-14.

Khatib, K.J., Mohammed, B.K. and Idarous, F.M. (2013). Participatory Variety Selection of Elite Genotypes for Improving Rice Productivity in Zanzibar. Paper presented at 3rd African Rice Congress 21-24 Yaunde Cameroon October, 2013: 1 – 12.

Kihoro, J., Njoroge, J. B., Hunja, M., Elijah, A. and Daigo, M. (2013). Investigating the impact of rice blast disease on the livelihood of the local farmers in greater Mwea region of Kenya. *Springer Plus* 2(308): 1-13.

Kohli, M.M., Mehta, E.Y., Guzman, R., De viedma, L. and Cubilla, L.E. (2011). Pyricularia Blast – a Threat to Wheat Cultivation. *Journal of Genetic Plant Breed* 47(2): 130–134.

Magar, P.B., Basistha, A. and Bishnu, P. (2015). Use of chemical fungicides for the management of rice blast (*Pyricularia grisea*) disease at Jyotinagar, Chitwan, Nepal. *International Journal of Applied Science and Biotechnolology* 3(3): 474-478.

Mebratu, G.A., Thangavel, S. and Getaneh, W. (2015). Assessment of disease intensity and isolates characterization of blast disease (*Pyricularia oryzae* CAV) from

South West of Ethiopia. International *Journal of Life Sciences* 3(4): 271 – 286.

Merketa, S., Andreas, J.M., Sarah, J.G. and Mark, D.F. (2014). Robust anti-oxidant defences in the rice blast fungus Magnaporthe oryzae confer tolerance to the host oxidative burst. *New Phytologist* 201: 556-573.

Miah, G., Rafii, M.Y., Ismail, M.R., Sahebi, M., Hashemi, F.S.G., Yusuff, O. and Usman, M.G. (2017). Blast disease intimidation towards rice cultivation: a review of pathog and strategies to control. *The Journal of Animal and Plant Sciences* 27(4): 1058-1066.

Mussa, S.B. (2015). Climate change impacts and adaptation strategies of small scale Agriculture Production in Micheweni District Pemba. Unpublished Dissertation for Award of MSc. degree at Sokoine University of Agriculture, Morogoro, Tanzania. 134pp.

Nyang'au, W.O., Mati, B.M., Kalamwa, K., Wanjogu, R.K. and Kiplagat, L.K. (2014). Estimating Rice Yield under Changing Weather Conditions in Kenya Using CERES Rice Model. *International Journal of Agronomy* 8(494): 6 -12.

Ou, S.H. (1985). Rice disease 2nd edition Common Wealth Mycological Institute. Kew, England. pp. 380.

Piyasiri, M.L. (1990). Evaluation of Land Resources in Zanzibar. Food and Agriculture Organization of the United Nation (FAO) Phase I and II. Zanzibar. 219 pp.

Prabhu, M.C., Filippi, G.B., Silva, V.L. and Morais, O.P. (2009). An unprecedented outbreak of rice blast on a newly released cultivar BRS Colosso in Brazil. Advances in Genetic, Genomics and Control of Rice Blas Deseases. pp. 257-266.

Rajendra, P., Anupam, S. and Sweta, S. (2015). Influence of weather parameters on occurrence of rice blast in mid hills of Himachal Pradesh. *Himachal Journal of Agricultural Research* 41(2): 132-136.

Raveloson, H., Ratsimiala, R.I., Tharreau, D. and Sester, M. (2017). Long-term survival of blast pathogen in infected rice residues as major source of primary inoculum in high altitude upland ecology. *Plant pathology* pp. 1-9.

Rini, P., Dipankar, M. and Bhima, S.N. (2017). Effect of different meteorological parameters on the development and progression of rice leaf blast disease in western Odisha. *International Journal Plant Protection* 10(1): 52-57.

Sukanya, S.L., Yamini, D. and Fathima, S.K. (2011). Eco-friendly management of *Pyricularia oryzae* - The causal agent of blast of paddy. *Current Botany* 2(8): 46-49.

Suryadi, Y., Susilowati, D.N., Riana, E. and Mubarik, R.N. (2013). Management of rice blast disease (*Pyricularia oryzae*) using formulated bacterial consortium. Emir. *Journal of Food Agricultural* 25 (5): 349-357.

Tingting, N. (2015). Epidemiology, phytopathological and molecular differentiation and leaf infection processes of diverse strains of *Magnaporthe* spp. on wheat and rice. Dissertation to obtain the PhD. degree in the International Ph. D. Program for Agricultural Sciences in Göttingen (IPAG) at the Faculty of Agricultural Sciences, Georg-August-University Göttingen, Germany. Pp. 1-169.

Vincent, L. (2008). Rice Blast. Plant Health Australia. Pp. 1-14.

Wasihun, Y.W. and Flagote, A.B. (2016). Assessment of diseases on rice (*Oriza sativa* L.) in major growing fields of Pawe district, Northwestern Ethiopia. World Scientific News 42 (2016): 13 – 23.

William, B., Christian, C., Jim, S. and Barbara, V. (2013). Recovery plan for wheat blast caused by *Magnaporthe oryzae Triticum* pathotype. The American Phytopathological Society pp. 1-33.

CHAPTER THREE

MANUSCRIPT II

Morphology and molecular characterization of rice leaf blast pathogen (*Pyricularia oryzae Cavara*) collected in Zanzibar

[1]Ali KhatibBakar., [2]S.O.W.M. Reuben., [3]H.J.F. Lyimo

[1]**Department of Crop Science and Horticulture**, P.O, Box 97. Wete –Pemba. Email: alikhatibbakar @yahoo.com

[2]**Department of Crop Science and Horticulture,** Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Email: smreuben@yahoo.com

[3]**Department of Crop Science and Horticulture**, Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Email: flyimo_1999@yahoo.com

Target Journal and Status: International Journal of Science and Research (IJSR). Manuscript is under review process.

Abstract:

Rice (*Oryza sativa* L.) is a staple food in Zanzibar however; production does not meet the demand of over 1.3million people. Several constraints attributed to low rice production in Zanzibar island have been identified. The major and the most important constraint is rice leaf blast disease caused by the fungal pathogen *Pyricularia oryzae* Cavara. In the present study, research was conducted for diseased sample collection, isolation and characterization of isolates of rice leaf blast pathogen (*Pyricularia oryzae* Cav). Samples were collected in different locations of Zanzibar and isolated were characterized using both molecular and morphological techniques. From molecular activities, six isolates were identified in this study as ZNZ/2017/CENTRAL, ZNZ/2017/CHK_2, ZNZ/2017/MICH, ZNZ/2017/NORTH_ A2, ZNZ/2017/NORTH_ A8 and ZNZ/2017/CHK_1. Phylogenetic analysis revealed that all isolates were closely related to Japan, China and Republic of Korea isolates. This relation it may be due to importation of rice and rice planting materials. When the isolates were studied in three different solid culture media in the laboratory environment for growth rate, results revealed that oat meal agar (OMA) was considered as superior and suitable medium for mycelia growth and sporulation. It gave highest value compared to Potato dextrose agar medium (PDA) and Potato carrot agar medium (PCA) that was the least. Also isolates gave different culture characterization for colony colour, colony surface texture, colony margin, topography and colony zonation. Variations were contributed by type of media used for culturing the isolates.

Key words: Molecular, characterization, *Pyricularia oryzae,* Isolates, Conidia, Susceptible and Lesion.

3.1 INTRODUCTION

Rice is one of the most famous cereal and staple foods in Zanzibar and it is cultivated in three agro-ecosystems viz: rainfed upland, rainfed lowland and irrigated. The crop is also grown in other tropical and subtropical regions worldwide (Miah *et al.,* 2013). In Tanzania, about one third of its population consume rice as staple food and surplus is sold for raising family income (Chuwa *et al.,* 2015). However, the crop is susceptible to several diseases rice leaf blast caused by *Pyricularia oryzae* Cavara (Teleomorph *Magnaporthe oryzae* Couch) being the most limiting factor (Jonit *et al.,* 2016). Breeding resistant varieties is perhaps the cheapest approach to control rice leaf blast. However, knowledge of the structure, dynamics of pathogen population, diversity and virulence is essential for effective planning and implementation of strategies for management of the disease through breeding of durable resistant cultivars Babujee and Gnanamanickam, (2000). *Pyricularia oryzae* is specific to infect rice but due to complexity of the pathogen some strains attack and cause disease in many species of Poaceous plants and has been distributed worldwide as rice blast pathogen. It is important in all rice growing areas (Castroagudín *et al*., 2016) and therefore a major constraint to increasing rice production and high quality rice in Zanzibar (Théophile *et al.,* 2014).

The fungus has the ability to infect aerial parts of the lant at all of its growth stages from seedling to the grain filling stage when weather brings favourable conditions for blast to prevail (Gayatree *et al.,* 2017) resulting in complete death of the whole plant (Getachew *et al*., 2014). Leaf and neck blast infection causes the most destructive effect (Huili *et al.,* 2014 and Kariaga *et al*., 2016) leading to huge rice yield losses that range from 60 to 100% (Subhalakshmi and Indira, 2017) and 1-100% depending on the plant part infected, degree of varietal susceptibility and infection rating (Wasihun and Flagote, 2016); Nalley *et al*., 2016). Several weather conditions such as night temperature ranging from 20°–

26°C, relative humidity greater than 90% associated with wind run, sun shine and rainfall, leaf wetness and varietal susceptibility have been reported to influence *Pyricularia oryzae* to cause blast disease (Laxman *et al.*, 2017). Under favourable weather condition, *Pyricularia oryzae* produces conidia attached and sporulate on the surface tissue of the rice leaf and characterized by three-celled conidia which are pale brown to hyaline and pyriform (pear-like) in shape (Emmanuel *et al.,* 2013). Macroconidia produce germ tubes which develop melanized appressoria later penetrating plant cuticle and cell wall. After penetration, invasive hyphae grow biotrophically in leaf cells and later infectious growth of the pathogen results in host cell death and lesion (Huili *et al.,* 2014).

The clinical disease symptoms shown on the leaves of susceptible rice cultivar initially appear as whitish or grayish spots along the leaf margin. Later on, they become elongated and turned in to diamond shaped with pointed ends. The elder spots became necrotic in the center with brown margins, which collapse and form large lesions. As a result, leaves decrease photosynthesis hence poor food manufacturing by reducing the green leaf area surrounding the lesion region (Jamal-u-ddin *et al.,* 2013). It was also reported by Akhilesh *et al.* (2017) that rice leaf blast lesion is typically gray at the centre with a dark border and it is spindle-shaped in appearance. *Pyricularia oryzae* gives variation in growth, colour, shapes and other characteristics on different media used in culture such as Potato dextrose agar (PDA), Oat meal agar (OMA) and on Potato carrot agar (PCA). On the other hand, it was reported by Chuwa *et al.* (2015) and Deepti (2014) that molecular and morphological studies using polymerase chain reaction (PCR) is the appropriate approach and tool for identifying, characterizing and verifying rice blast pathogen *Pyricularia oryzae* in the laboratory. The later enables construction of phylogenetic tree in the detection of pathogens and distinguishing between closely related isolates. The aim of this study was to isolate, identify and do morphological and molecular characterization of *Pyricularia oryzae* Cavara from rice leaf blast samples collected from six districts of Zanzibar.

3.2 MATERIALS AND METHODS

3.2.1 Description of the study area

The study was conducted in two main islands of Zanzibar viz: Unguja Island and Pemba Island. Unguja is located between 5^o 40'and 6^o 30'South and 39^o East with total area of 1660 square kilometer (85km^2 long and 39km^2 wide) being 120 meters above sea level. However, Pemba has a total area of 988 square kilometers (67 kilometers long and 23 kilometers wide) located at 4^o 80' South and between 39^o 35' and 39^o 50' East being 95 meters above sea level. Zanzibar receives bimodal rainfall and long rains prevail during March to June. Short rains start in mid-September to November with average annual rainfall for Unguja at 1600mm while Pemba receives slightly higher rainfall of 1900mm. Mean monthly / annual temperature ranges from 26 to 32^{o}C. https://en.wikipedia.org/wiki/Zanzibar. The soils of Zanzibar are grouped in to three, namely: loamy soils (Kinongo), sand soil (Mchanga) and clayey soils (Kinamo) (Piyasiri, 1990).

3.2.1.1 Rice leaf blast samples collection

Rice leaves showing typical blast symptoms (Fig. 3.1) were collected from six rice growing districts in Zanzibar during the two main rice growing seasons of 2014/15 and 2015/2016. The districts were Micheweni, Wete and Chake Chake in Pemba and for Unguja were North A, Central and West. Rice leaf blast disease samples were collected from rice blast infected fields during the heading stage of plant growth when the blast symptom is expected to reach its maximum severity levels. In all studied areas, blast infected leaves were collected from three different rice agro-ecologies viz: rainfed upland, rainfed lowland and irrigated. The blast diseased leaf samples were collected and put in paper bags then kept in cool box protecting from wilting and transported to Sokoine University of Agriculture (SUA) in the Department of Crop Science and Horticulture laboratory for pathogen isolation using procedures designed by (Mebratu *et al.,* 2015) followed by morphology and moleculaer characterization. Before isolation processes, all samples were stored in the refrigerator at temperature of 4^0C until in use.

Figure 3.1: Rice leaf blast disease symptoms

3.2.2 Pathogen isolation

The leaf samples were cut into small pieces of 1cm long from the edge of the lesion using a pair of sterilized scissors and surface sterilized with 2% sodium hypochlorite for 2-3 minutes. Thereafter, pieces were rinsed in three chambers of sterile distilled water and transferred on to the surface of sterilized tissue paper to remove excessive water content (Deepti *et al.,* 2014; Aruna *et al.,* 2016; Akhlesh *et al.,* 2017). The cut pieces were kept in sterile petri-dishes laid with three layers of moisten filter papers and glass slide at the centre and incubated at 25- 26^0C for 48 hours (Priya *et al.,* 2013) for sporulation (Fig. 3.2).

After 48 hours of incubation mycelia growth and their morphology were examined using a stereo microscope (Wild Heer Brugg M5A) and conidia morphology of *Pyricularia oryzae* were examined and identified using a compound microscope (Leica CME). Observed conidia that showed typical morphological appearance of *Pyricularia oryzae* Cavara (Fig. 3.3) were transferred in to 90 mm petridishes containing 25 ml (PDA) potato dextrose agar medium (200 g of peeled Irish potato, 20 g glucose and 15 g agar). Petri dishes were eventually transferred to the growing chamber at 25-26^{0}C under 12 h photoperiodic condition for seven to ten days for the fungus to grow (Jamal-u-ddin *et al.*, 2013).

Figure 3.2: Incubated leaf blast infected lesion

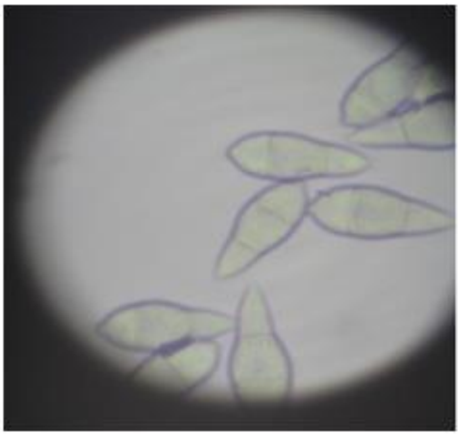


Figure 3.3: Conidia morphology of *Pyricularia oryzae* (10X)

3.2.3 DNA extraction

Fungal isolates of *Pyricularia oryzae* were cultured in 90 mm wide petri dish for 7 days on potato dextrose agar (PDA) medium and incubated at 25 - 26^{0}C. Mycelia were scraped from the surface of the medium using a cotton ear swab for each isolate. Scraped mycelia of each isolate were inoculated into the 500 ml flask containing 200 mls of autoclaved Potato dextrose broth (PDB) (200 ml of Irish potato extract and 20 g glucose) then shaked for five days at room temperature at 12 hr photoperiod using flask shaker (Wagtech

International) at 165rpm (Deepti *et al.,* 2014 and Kariga *et al.,* 2017). After five days, young mycelia were harvested from submerged culture by filtration (Ali *et al.,* 2008) whereby six layers of cotton gauze were used to squeeze wet mycelia. Squeezed mycelia were put into the 1.5ml centrifuge tubes and dried by circulated wind in the lamina flow hood chamber and stored at -20^{0}C after drying.

Thereafter, DNA of each isolate was extracted following a protocol described by Mahuku *et al.* (1998). Then 100 mg of dried mycelia, I gram of acid washed sand (for simplifying grinding) and 300 µl TES was put in to the 1.5ml centrifuge and ground with the aid of blue pellet pestles. Addition of 200 µl TES + PK 50ug/ml was made and the mixture was vortexed for 30 seconds then incubated in water bath at 65^{0}C for 30 minutes. Addition of 250 µl of 7.5 Ammonium acetate was made in the mixture and incubated in ice for 10minutes. After 10 minutes, the mixture was centrifuged at 16140rpm for 15minutes at 4^{0}C and the supernatant 400 µl was transferred to another 1.5 centrifuge tube added with equal volume of 500 µl of ice-cold isopropanol and incubated at -20^{0}C for 1-2 hrs. Tubes were centrifuged at 16140 rpm for 15minutes and supernatant was discarded and DNA pellets remained at the bottom of the tubes. The white pellet DNA was washed two times with 800 µl (70% Ethanol) and centrifuged for 1minute. After drying the pellet, it was dissolved in 100 µl warm TE buffer and stored -20^{0}C.

3.2.4 Checking good quality DNA

After DNA had been extracted, electrophoresis procedures (Fig. 3.4) were applied to observe which isolate was able to produce DNA of good quality before PCR aplification. About 3 µl of extracted DNA from each isolate and 3 µl of 6x loading dye were loaded in lanes of 2% w/v agarose gell and electrophoresed for verifying the quality of DNA extracted.

Figure 3.4: Electrophoresis procedures for observing production DNA from each isolate

3.2.5 PCR Amplification

PCR was performed in 25 µl reaction volumes using Taq 2xMaster Mix containing Taq DNA Polymerase, dNTPs, $MgCl_2$, and KCl. PCR mix consisted of 12.5 µl of 2x Master Mix, 0.5 µl primers forward, 0.5 µl primer reverse, Template 1.0 µl and 10.5 µl water. PCR amplification was performed using thermal cycler and preheated at 94°C for 4 min, followed by 30 cycles of denaturing at 94°C for 1 min, annealing at 56°C for 30 seconds, extension at 72°C for 1 min and final elongation at 72°C for 7 min and 4^0C hold (Jonit *et al.,* 2016). The PCR products were separated in agarose gel 2% and electrophoresis on 100 volts for 1hour. The gel was then soaked in ethidium bromide for DNA staining for easy visualization when exposed to UV light in the transluminator (Chuwa *et al*., 2015).

3.2.6 Molecular markers for pathogen characterization

Three pairs of molecular markers (Table 3.1) were used in this study for rice leaf blast fungal characterization whereby fifteen isolates were visualized in electrophoresis jellies from different primers. Samples were sent to Global Health Program – Mbeya Referal Hospital Tanzania for sequencing in both the forward and reverse directions using forward and reverse primers to know the typical rice blast strains which infect rice plants in Zanzibar.

Table 3.1: Three specific Primers used in the study for identification of *Pyricularia oryzae*

PRIMER	PRIMER SEQUENCE 5' -3'
ITS-1 Forward	TCGGTAGGTGAACCTGCGG
ITS-4 Reverse	TCCTCCGCTTATTGATATGC
CAL- 228 Forward	GAGTTCAAGGAGGCCTTCTCCC
CAL-737 Reverse	CATCTTTCTGGCCATCATGG
ACT - 512 Forward	ATGTGCAAGGCCGGTTTCGC
ACT -783 Reverse	TACGAGTCCTTCTGGCCCAT

3.2.7 Sequence alignment and phylogenetic analysis

The CAL gene Calmodulin sequences of *Pyricularia oryzae* isolates were edited using BioEdit version 7 to remove unknown bases and loaded for analysis through National Centre for Biotechnological Information NCBI-BLASTn programme (*https://blast.ncbi .nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch)* for comparing against corresponding sequences of other *Pyricularia oryzae* already available in GenBank for identity and similarity (Madhavan *et al.,* 2013).

3.2.8 Morphological characterization of Isolates

Three solid culture media were used for comparing the growth rate for each isolate and other culture characteristics of rice blast pathogen *Pyricularia oryzae* for specific number of days. Culture media used included (PDA) Potato dextrose agar (20 g glucose, 18 g agar Irish potato infusion to 1000 ml), (OMA) Oat meal agar ((D(+) maltose 15 g/l, agar 18 g/l and oat meal infusion to 1000 ml) and (PCA) Potato carrot agar (Potato extract 250 ml, Carrot extract 250 ml, Agar 15 g and distilled water to 1000 ml) (Mahdieh *et al.,* 2013). The mixture for all three media was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Before the media were poured in the sterilized petri dishes (approximately 25 ml in 90 mm diameter), streptomycin sulphate at the rate of 1ml per liter of medium was added as an antibiotic to inhibit bacteria growth (Jamal-u-ddin *et al.,* 2012). When the media became solid 5 mm disc of 10 days of pure preserved culture from tested six isolates of *Pyricularia oryzae* from active growing margin was inoculated in the center of the petri dishes and then incubated at 25-26^{0}C for two weeks to grow. Each treatment (media, incubation period and isolates) were replicated three times. The growth diameter for each isolate and for each medium were measured using a ruler and at 4th day, 8th days and 12th day and recorded. Other colony growth characteristics were recorded viz colour, surface texture, margin, topography and zonation (Priya *et al.,* 2013; Mebratu *et al.,* 2015 and Kariaga *et al.,* 2016).

3.3 Statistical analysis

Growth rate data of six tested isolates in different media and incubation periods were analyzed using GenStat version 16 computer statistical packages for ANOVA. Randomized complete design (CRD) with three replicates was adopted. Means were separated by Turkeys Test at ($P \leq 0.05$). The statistical model used was:

$Y_{ijkl} = \mu + S_i + M_j + D_k + SD_{(ki)} + SM_{(li)} + MD_{(kl)} + SMD_{(kli)} + \varepsilon_{ijkl}$.

Where $y_{ijkl:}$ is observation in the i^{th} strain, j^{th}medium, k^{th} day and l^{th} plot, μ = is overall mean, $S_{(i)}$ = effect of i^{th} strain, M_j = effect of j^{th} medium, D_k = effect of k^{th} day, $SD_{(ki)}$ = Interaction of k^{th}strain and i^{th}day effect, $SM_{(ij)}$ = Interaction of i^{th}strain and j^{th}medium effect, $MD_{(kl)}$ = Interaction of k^{th}medium and l^{th}day effect, $SMD_{(kli)}$ = Interaction of the three factors, ε_{ijkl} = Random error.

3.4 RESULTS

3.4.1 Molecular techniques for characterization of isolates of *Pyricularia oryzae*

Molecular characterization of rice leaf blast disease pathogen, using molecular markers has shown to be a widely used method. In the current study of *Pyricularia oryzae,* six strains of *Pyricularia oryzae* were characterized for genetic variation using three specific primers designed for *Pyricularia oryzae* forwad and reverse as indicated in (Table 3.1).

From the three specific primers used, Primers ITS 1Forwad and ITS4 reverse was able to produce strains ZNZ/2017/CHK_3, ZNZ/2017/CHK_12, ZNZ/2 017/CENTRAL, ZNZ/2017/MICH, ZNZ/2017/MICH_6, ZNZ/2017/CENTRAL_10, ZNZ/2017/MICH_12, ZNZ/2017/CENTAL_7, ZNZ/2017/CH_1, ZNZ/2017/CHK_2, ZNZ/2017/MICH_2, ZNZ/2017/NORTH_A2,ZNZ/2017/NORTH_A8, ZNZ/2017/CENTRAL_5, ZNZ/2017/ NORTH_A9 collected from Chake chake, Central, Micheweni and North A, of *Pyricularia oryzae*, the DNA's were amplified and appeared similar with polymorphic banding pattern of 500 bp (Fig. 3.5).

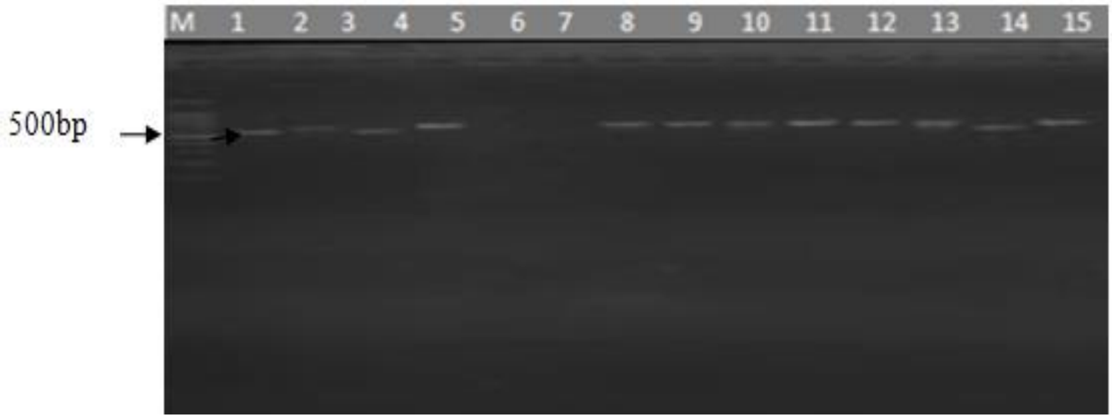


Figure 3.5: Agarose gel 2% eletrophoresis of PCR amplification products of *Pyricularia oryzae* genomic DNA using primers ITS1 and ITS4. M = Ladder (1.5kb) and number 1-15 on the jelly are the number of lanes where samples were loaded

The primers CAL- 228F and CAL-737R amplified genomic DNA of *Pyricularia oryzae* strains ZNZ/2017/CHK_3, ZNZ/2017/CCENTRAL, ZNZ/2017/MICH_6, ZNZ/2017/CHK_1, ZNZ/2017/ NORTH_A2 and ZNZ/2017/NORTH_A8 from Chake chake, Central, Micheweni and North A at 500 bp while ZNZ/2017/CHK_12, NZ/2017/CHK_2, ZNZ/2017/MICH, ZNZ/2017/CENTRAL_10, ZNZ/2017/MICH_12, ZNZ/2017/CENTAL_7, ZNZ/2017/MICH_2, ZNZ/2017/CENTRAL_5 and ZNZ/2017 /NORTH_A9 strains from Chake chake, Micheweni, Central were not amplified. On the other hand, primer ACT amplified 500 bp on ZNZ/2017/CHK_3, ZNZ/2017/ CHK_12, ZNZ/2017 /CENTRAL, ZNZ/2017/MICH, ZNZ/2017/CENTRAL _7 strains from Chake chake, Micheweni and Central while ZNZ/2017/ MICH_6, ZNZ/2017 /CENTRAL_ 10, ZNZ/ 2017/MICH_12, ZNZ/2017/CHK_1, ZNZ/2 017/CHK_2, ZNZ/2017/MICH_2, ZNZ/ 2017/NORTH_A2, ZNZ/2017/NORTH_A8, ZNZ/2017/ CENTRAL_5 and ZNZ/ 2017/NORTH_A9 from Micheweni, Central Chake chake North A were not amplified (Fig. 3.6).

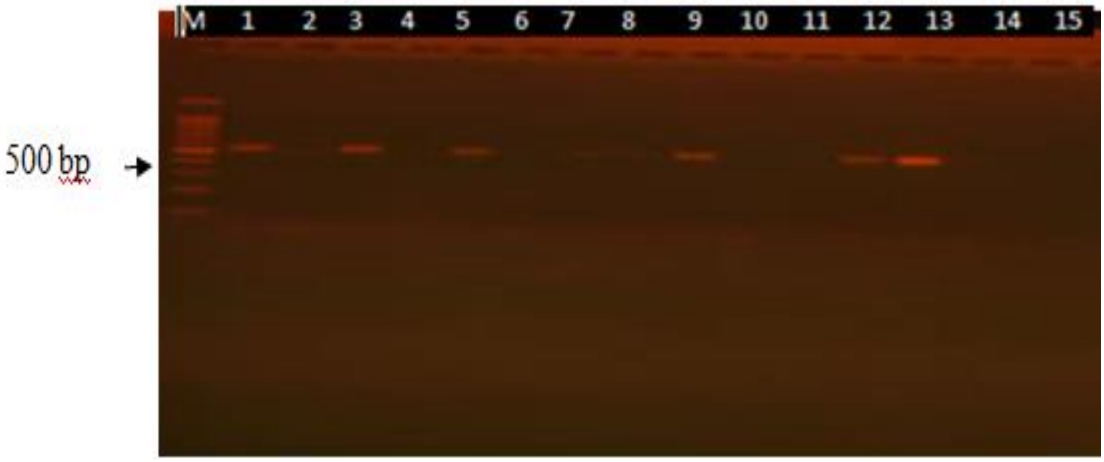


Figure 3.6: Agarose gel 2% eletrophoresis of PCR amplification products of *Pyricularia oryzae* genomic DNA using primers CAL- 228F and CAL-737R. M = Ladder (1.5kb) and number 1-15 on the jelly are the number of lanes where samples were loaded

Figure 3.7: Agarose gel 2% eletrophoresis of PCR amplification products of *Pyricularia oryzae* genomic DNA using primers ACT - 512 F and ACT - 783 R. M = Ladder (1.5kb) and number 1-15 on the jelly are the number of lanes where samples were loaded

3.4.2 Sequence alignment, phylogenetic analysis and evolutionary relationships of taxa of strains

The CAL gene Calmodulin sequences of *Pyricularia oryzae* isolates were edited using BioEdit version ver. 7 to remove unknown bases and loaded and analyzed through NCBI-BLAST search programme for comparison with the corresponding sequences of other *Pyricularia oryzae* isolates from GenBank for identification and similarity (Madhavan *et al.,* 2013). Results indicated that the sequence of isolates collected from Zanzibar had maximum identity of 98-99% with *Pyricularia oryzae* sequences presented worldwide available in NCBI database.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 1.17208837 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Tajima-Nei method (Tajima and Nei, 1984) and are in the units of the number of base substitutions per site. The analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd +3rd+

Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 587 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013). The phylogenetic analysis grouped *Pyricularia oryzae* isolate according to the similarity and close relationship. It was found that, isolates ZNZ/2017/MICH, ZNZ/2017/CHK_2, ZNZ/2017/CENTRAL and ZNZ/2017/CHK_1 from Zanzibar indicated close relationhip to the strain (Accession number KC 167651) from Republic of Korea. However, isolates ZNZ/2017/NORTH_A2 and ZNZ/2017/NORTH_A8 indicated close relationship to strain (Accession number AB274470) from Japan and Accession number KY074654 from China. (Fig. 3.8).

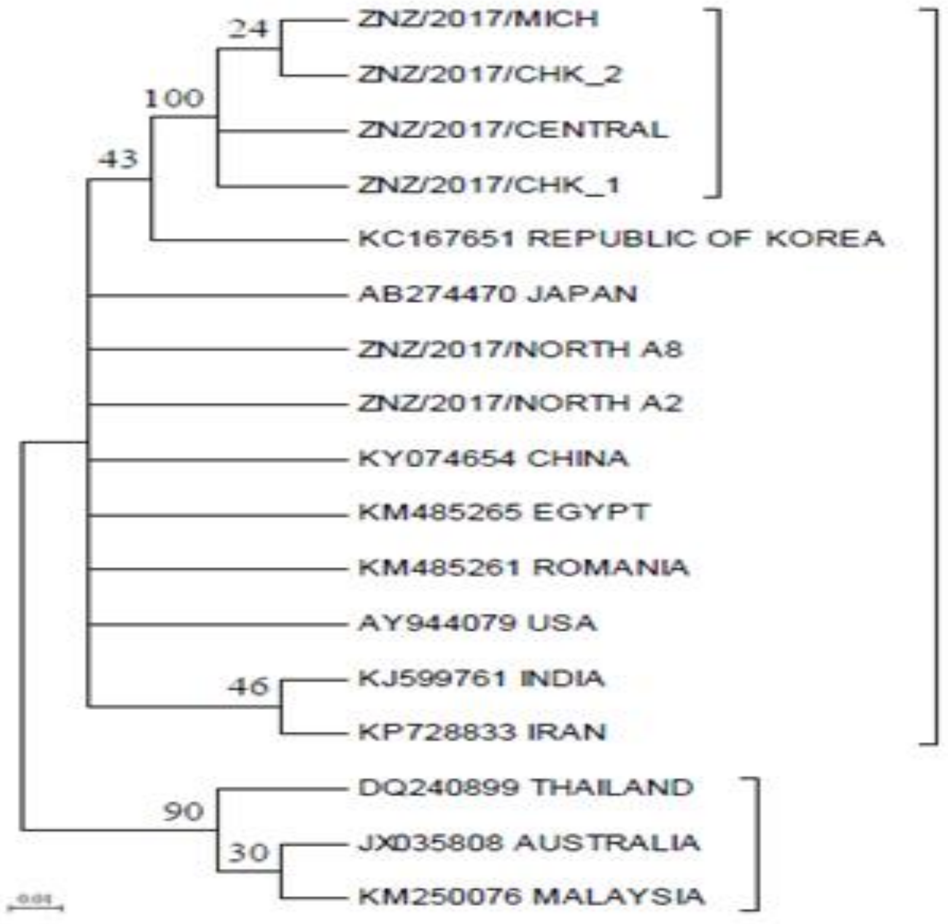


Figure 3.8: Evolutionary relationships of taxa of strains

3.4.3 Morphological culture characteristics and growth of rice leaf blast isolates (*Pyricularia oryzae*) on three different culture media

Infected leaves collected in different rice agro-ecologies were grown on three solid media viz: Oat meal agar (OMA), Potato dextrose agar (PDA) and Potato carrot agar (PCA) for charactering isolates based on culture characteristics (colony color (rear and front view), colony margin, colony surface texture, colony topography, colony zonation) and growth. Results revealed six isolate groups that showed the variable characteristics and for each medium tested. For the colony colour front view, observations were grayish white colony in the PDA, white in the OMA and whitish in PCA. However, for the rear view, PDA showed yellowish brown, pale brown for OMA and cremish brown for the PCA. On the other hand, for the colony surface texture, rough surface was recorded on the PDA, smooth on OMA and rough for PCA. Likewise, for the colony topography on PDA and PCA there was raised fluffy growth and colony flat growth on OMA. For the colony zanation on PDA there was concentric while on PCA and OMA there was no zonation (Table 3.2). Colony colour appeared rear and front view in Potato dextrose agar, Oat meal agar and Potato carrot agar (Yelowish brown, blackish brown and cremish brown).

Table 3.2: Morphological characteristics of isolates in media

Isolate	Media	Colony colour		Colony Surface texture	Colony Margin	Colony topography	Colony zonation
		Front view	Rear view				
ZNZ/2017/CENTRAL	PDA	Grayish white	Yellowish brown	Rough	Irregular	Raised fluffy growth	Concentric
	OMA	White	Pale brown	Smooth	Entire	Colony flat growth	No zonation
	PCA	Whitish	Cremish brown	rough	Irregular	Colony flat growth	No zonation
ZNZ/2017/CHK_2	PDA	Grayish white	Yellowish brown	Rough	Irregular	Raised fluffy growth	Concentric
	OMA	Blackish white	Pale brown	Smooth	Entire	Colony flat growth	No zonation
	PCA	Whitish	Cremish brown	rough	Irregular	Colony flat growth	No zonation
ZNZ/2017/MICH	PDA	Grayish white	Yellowish brown	Rough	Irregular	Raised fluffy growth	Concentric
	OMA	Blackish white	Pale brown	Smooth	Entire	Colony flat growth	No zonation
	PCA	Whitish	Cremish brown	rough	Irregular	Colony flat growth	No zonation
ZNZ/2017/NORTH_A2	PDA	Grayish white	Yellowish brown	Rough	Irregular	Raised fluffy growth	Concentric
	OMA	Blackish white	Pale brown	Smooth	Entire	Colony flat growth	No zonation
	PCA	Whitish	Cremish brown	rough	Irregular	Colony flat growth	No zonation
ZNZ/2017/NORTH_A8	PDA	Grayish white	Yellowish brown	Rough	Irregular	Raised fluffy growth	Concentric
	OMA	Grayish white	Pale brown	Smooth	Entire	Colony flat growth	No zonation
	PCA	Whitish	Creamish brown	Rough	Irregular	Colony flat growth	No zonation
ZNZ/2017/CHK_1	PDA	Grayish white	Yellowish brown	Rough	Irregular	Raised fluffy growth	Concentric
	OMA	Blackish white	Pale brown	Smooth	Entire	Colony flat growth	No zonation
	PCA	Whitish	Cremish brown	rough	Irregular	Colony flat growth	No zonation

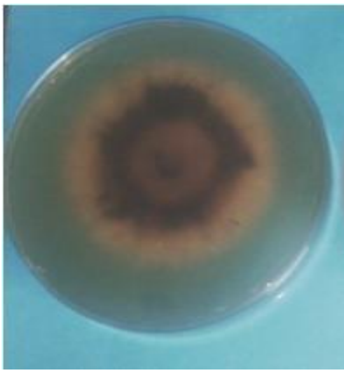


Plate 3.1: PDA: (Yelowish brown – rear view)

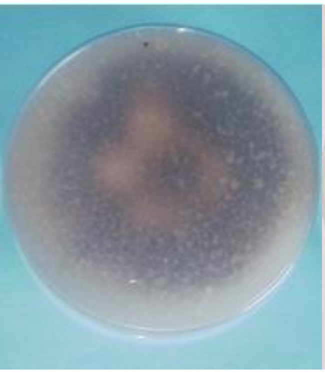


Plate 3.2: OMA: Blackish white rear view

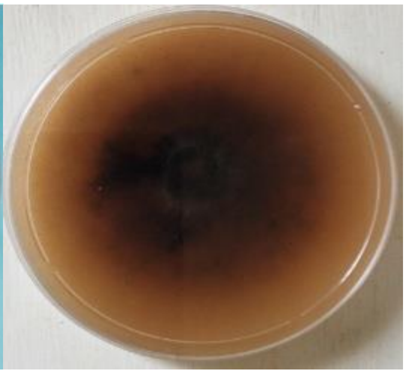


Plate 3.3: PCA: Cremish brown rear view

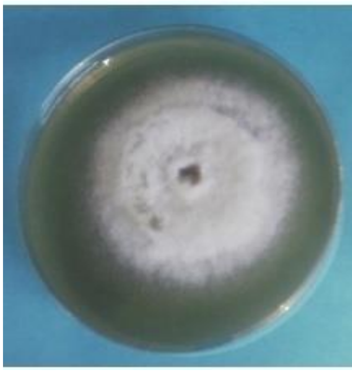


Plate 3.4: PDA: Grayish white –Front view

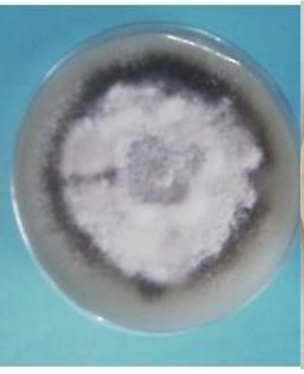


Plate 3.5: OMA: White –Front view

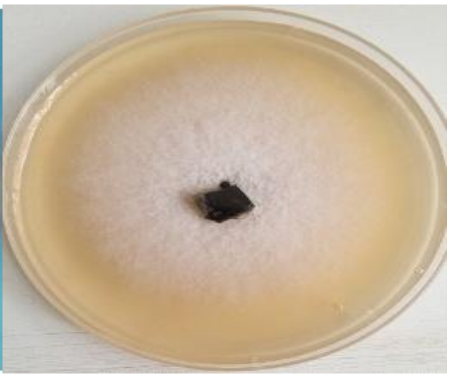


Plate 3.6: PCA: Whitish- Front view

3.5 Colony Surface Texture

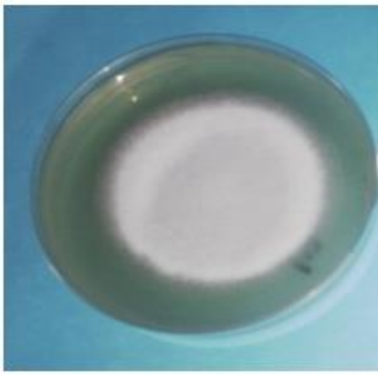


Plate 3.7: PDA Rough

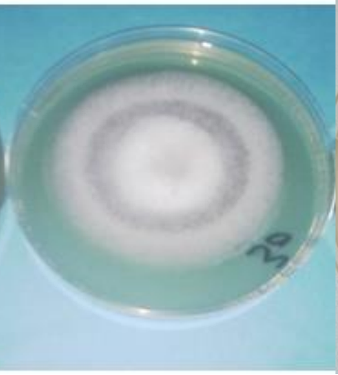


Plate 3.8: OMA Smooth

Plate 3.9: PCA Rough

3.6 Colony Margin

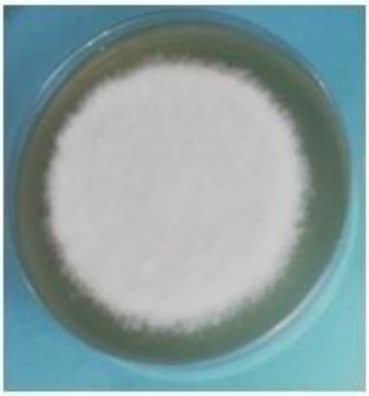


Plate 3.10: PDA: Irregular

Plate 3.11: OMA: Entire

Plate 3.12: PCA: Irregular

3.7 Colony Topography

Plate 3.13: PDA: Raised fluffy growth

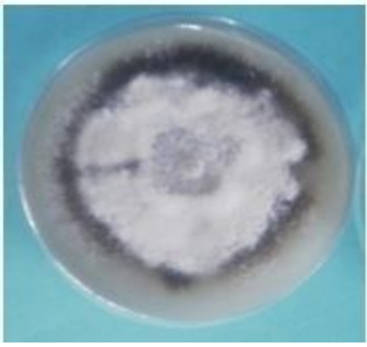


Plate 3.14: OMA: Colony flat growth

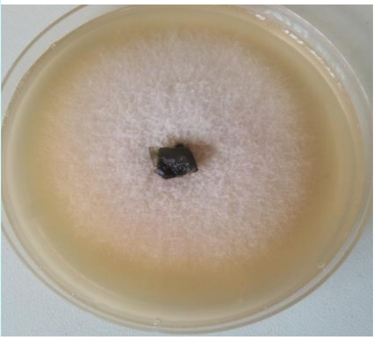


Plate 3.15: PCA Colony flat growth

3.8 Colony Zonation

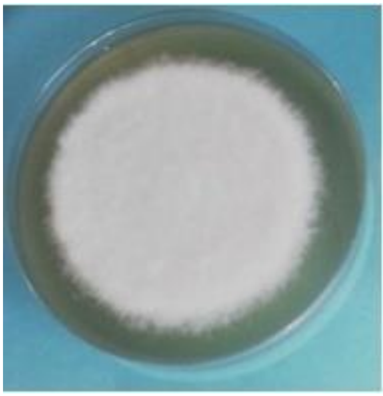


Plate 3.16: PDA No zonation

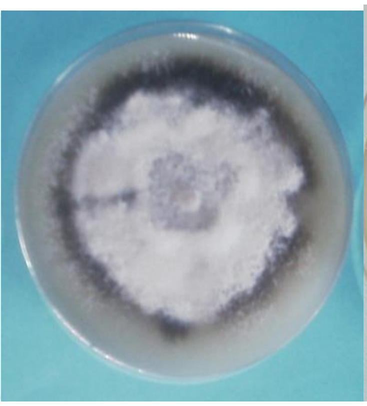


Plate 3.17: OMA No zonation

Plate 3.18: PCA: No zonation

3.9 ANOVA on Growth of Mycelia

Analysis of variance summary for the studied variables is given in Appendix 3. Significant ($P \leq 0.001$) difference was observed between three different types of media tested on the growth of mycelia, also, number of days for incubation exhibited significant ($P \leq 0.001$) effect while intraction of medium and days also showed significant ($P \leq 0.001$) effect.

3.9.1 Main effect of days on growth of mycelia

Significant difference between days and incubation was registered on mycelia growth (Table 3.3). At 4days mycelia growth is the lowest (2. 830cm) and at 12 days was the longest (7.094cm).

Table 3.3: Main effect of days on growth medium

Days	Mycelium growth (cm)
4	2.830^{a}
8	5.515^{b}
12	7.094^{c}
X	**5.146**
$SE_{\bar{x}}$ (±)	0.0573
LSD at 0.05	0.1607
Cv (%)	11.8

3.9.2 Main effect of medium on mycelia growth

Three types of medium tested in the study showed that oat meal agar medium had significantly highest growth of mycelia (5.457cm) while Potato carrot agar (PCA) registered lowest growth rate (4.580cm) Table 3.4. However, PDA (5.402 cm) and differ significantly.

Table 3.4: Main effect of medium on growth of mycelia (cm)

Medium	Mycelia growth (cm)
PCA	4.580a
PDA	5.402b
OMA	5.457b
$\bar{x}$	**5.146**
SE$_{\bar{x}}$ (±)	0.0573
LSD at 0.05	0.1607
Cv (%)	35.6

Means with different superscript letters down the column differ significantly at $P \leq 0.05$

3.9.3 Interaction effect of medium and number of days on growth of mycelia

Three media when inoculated with isolates and incubated for 4,8 and 12 days indicated that, Potato carrot agar (PCA) medium in 4 days gave significantly the lowest growth of (2.389cm) while oat meal agar medium (OMA) in 12 days had the largest growth of (7.633cm) (Table 3.5).

Table 3.5: Main effect of medium and number of days on growth of mycelia

Medium and number of days	Mycelia growth (cm)
PCA4	2.389^{a}
OMA4	2.856^{b}
PDA4	3.244^{b}
PCA8	4.856^{c}
PDA8	5.806^{d}
OMA8	5.883^{d}
PCA12	6.494^{e}
PDA12	7.156^{f}
OMA12	7.633^{g}
Mean	**5.146**
SE$_x$ (±)	0.0993
LSD at 0.05	0.2783
CV%	8.2

Means with different superscript letters down the column differ significantly at $P \leq 0.05$

However, oat meal agar medium OMA at 8 days gave of 5.833 cm mycelia growth and this did not differ significantly from Potato carrot agar (PCA) at 12 days (6.494cm). Generally, the growth rate of mycelia increased progressively with increased number of days of incubation in all types of media.

3.10 Discussion

A total of 288 rice leaf blast diseased samples were collected and transported to Sokoine University of Agriculture (SUA), Department of Crop Science and Horticulture for laboratory analysis. Isolated samples when mounted and observed under compound microscope (Leica CME) showed true mycelia growth and conidia morphology of *Pyicularia oryzae* for further investigations. Conidia did have high sporulation that were characterized by cluster of pale brown to hyaline and pyriform (pear- like) in shape, 2- 3 septate, 2-3 celled, rounded base and narrow apex. The similar result of identification of *Pyricularia oryae* conidia was confirmed and repoted by Emanuel *et al.* (2013), Nurulhidayah and Kalaivani (2014), Jamal-u-ddin *et al.* (2015), Kariaga *et al.* (2016) and Ritu Raj (2017). On PCR amplification the three primers used was successfully amplified gene regions of *Pyricularia oryzae* collected in Zanzibar.

During PCR analysis, ITS primers (Internal Transcribed Spacer) amplified many isolates compared to other two primers used. A similar result was reported by Chuwa (2015) and Fernaz *et al.* (2016) when investigated the Pathogenic Variation and Molecular Characterization of *Pyricularia oryzae*, Causal Agent of Rice Blast Disease in Tanzania. It is a powerful method and highly variable sequences of great importance in distinguishing fungal species identification and epidemiology. ITS primers are highly specific to *Pyricularia oryzae* pathogen.

The ITS, CAL and ACT primers were successfully amplified with 500 bp long, respectively. Isolates loaded on BLASTn programme resulted to 98-99% homology to *Pyricularia oryzae* in the GenBank indicated that all isolates used in this study were identical to those of *Pyricularia oryzae* in the Gen Bank, that did not show any differences in banding patterns as it was reported by Chuwa (2015). The banding patterns of all six

Pyricularia oryzae strains collected in Zanzibar showed similarity. They were not genetically significantly different. Phylogenic results revealed that the isolates collected from Zanzibar, were ZNZ/2017/CENTRAL, ZNZ/2017/CHK_2, ZNZ/2017/CHK_1, ZNZ/2017/NORTH_2, ZNZ/2017/MICH and ZNZ/2017/NORTH_ A8. They were closely related with the sequence originated from Republic of Korea, (Accession No. KC167651), Japan (Accession No. 274470) and China (Accession number KY 074654). A possible reason for this closest relation of isolates could be the importation of milled and rice planting materials from China, Japan and Korea in Zanzibar as reported by Aisha *et al.* (2015).

Living organisims including fungi requires food and energy from the substrate for its growth and reproduction upon which they live in nature. It is necessary to furnish those essential elements and compounds in the growth medium. Not all growth media are in good qually for fungi to grow. Some media make fungus to grow very fast and other media make fungi to grow slowly due to nutritional stutus of the media. Thus, a combination of incubation day and type of media gives rapid growth rate of fangal mycelia and sporulation. Generally, the growth rate of mycelia increased progressively with increased number of days of incubati and type of media (Varsha *et al.*, 2015). From this study revealed that Oat meal agar (OMA) believed to be the best media and suitable for mycelia growth and for conidial production of the fungus *Pyricularia oryzae* especially when exposed at 12 hours in light and 12 hours in darkness reported by Akhilesh *et al.* (2017). The maximum growth diameter of mycelium of *Pyricularia oryzae* was supported by Oat meal agar. The result found herein similar to the studies reported by Jamal-u-ddin *et al.* (2013), Chandrakanth *et al.* (2014), Mebratu *et al.* (2015), Gohel and Chauhan (2015) and Kalavati *et al.* (2016).

The colony and morphological characteristics of the *Pyricularia oryza*e are the important basic factors for identification and observing different forms such as colony colour, surface texture, margin, zonation and colony topography. The present study results are in concurrence with the findings of Shanmugapackiam *et al.* (2019) who reported that the colony colour of the *Pyricularia oryzae* varies from greyish to whitish colour. This implies that type of media did have the greater influenche in culturing fungi.

3.11 Conclusion

The study revealed that there some evidence that many rice growing areas in Zanzibar were invaded with rice blast (*Pyricularia oryzae)* and plants suffered to disease. However, on the basis of the present study, the population of rice blast strains collected from different areas of Zanzibar were analyzed and found genetically homogeneous due to the molecular analysis showed no differences in banding patterns between strains. In cluster analysis, the strains were grouped into two main groups showing. Strains existing in Zanzibar are close related the trains of the republic of Korea and Japan. Combination of media type and number of days incubated, resulting fast radial growth rate of *Pyricularia oryzae* whereby Oat meal agar (OMA) is considered as a superior medium for growing rice blast pathogen. Different types of media used for rice blast culturing has the attendance to bring variation of fungal morphological charactericts as basic factor for identification.

3.12 Recommendations

i. Recommended use of molecular techniques is more accurate method and widely adopted to determine the genetic characteristic of organism; such as sequencing of different regions of DNA which is considered as alternatives to morphological methods of identification of *Pyricularia oryze.*

ii. In the laboratory, when culturing rice blast pathogen (*Pyricularia oryzae*) using Oat meal agar medium (OMA) is recomended as the suitable medium for fast growing and for high spore production of blast pathogen.

iii. Incubation period of blast pathogen ranging from 4th to 8th days in suitable period for the fangus to grow fast. Above that period the growth rate is declined.

iv. Combination of number of days and suitable type of medium (Oat meal agar) give heavy mycelium amount during incubation period.

REFERENCES

Aisha, N., Shah, M.D., Padder, B.A., Farahnaz, R., Ahanger, F.A. and Mushtaq, A. (2015). Molecular Characterization and Phylogenetic Analysis of *Magnaporthae oryzae* B.C. Couch Causing Blast Disease of Rice (*Oryza sativa*) in Kashmir. *SKUAST Journal of Research* 17(1): 15-22.

Akhilesh, K.K., Neha, S., Mukesh, K. S., Roshan, K., Kushram, T., Kumar, T.K. and Sanath, V.B.K. (2017). Growth of rice blast fungus *Pyricularia oryzae* (Cavara) on different solid and liquid media. *International Journal of Current Microbiology and Applied Sciences* 6: 1154-1160.

Akhlesh, K.K., Neha, S. and Sanath, V.B.K. (2017). Survey, Collection, Isolation and Identification of Isolates of *Pyricularia oryzae* causing rice blast in Southern Karnataka by using host differential lines. *International Journal of Agricultural Science and Research (IJASR)* 7(3): 285-294.

Ali, H.B., Kamel, A.A., Mohammed, A.M. and Abdulaziz, A.A. (2008). In-house Protocol for Isolation of Restrictable and Amplifiable Genomic DNA from Plants, Fungi and Bacteria. *Genes, Genomes and Genomics* 2(1): 77-80.

Aruna, J., Vijay, K.S., Rambabu, R., Ramesh, S., Yashaswini, C., Bhaskar, B., Madhavi, K.R., Balachndran, S.M., Ravindrababu, V. and Prasad, M.S. (2016). Morphological characterization of five different isolates of *pyricularia oryzae* causing rice blast disease. *Progressive Research – An International Journal* 11: 3377-3380.

Babujee, L. and Gnanamanickam, S.S. (2000). Molecular tools for characterization of rice blast pathogen (*Magnaporthe grisea*) population and molecular marker- assisted breeding for disease resistance. *Current Science* 78(3): 248-257.

Castroagudín., V.L., Moreira, S.I., Pereira, D.A.S., Moreira, S.S., Brunner, P.C., Macie, J.L.N., Crous, P.W., McDonald, B.A., Alves, E. and Ceresini, P.C. (2016). *Pyricularia graminis-tritici*, a new *Pyricularia* species causing wheat blast. *Persoonia* 37: 99 –216.

Chandrakanth, R., Jagadeesh, D. and Devaki, N.S. (2014). Black light mediated growth and sporulation of *magnaporthe oryzae*. *International Journal of Agricultural Science and Research (IJASR)* 4(4): 25-30.

Chuwa, C.J., Robert, B.M. and Mnyuku, S.O.W.R. (2015). Pathogenic Variation and Molecular Characterization of *Pyricularia oryzae*, Causal Agent of Rice Blast

Disease in Tanzania. *International Journal of Science and Research* 4(11): 131-1139.

Deepti, S., Shamim, M.D., Deepak, K., Pramila, P., Khan, N.A. and Singh, K.N. (2014). Morphological and Molecular Characterization of *Pyriculariaoryzae* causing blast disease in rice (*Oryza sativa*) from North India. *International Journal of Scientific and Research Publications* 4 (7): 1- 9.

Emmanuel, E.G., Cynthia, C.D. and Joselito, D.D. (2013). Inhibitory activity of *Chaetomium globosum* Kunze extract against Philippine strain of *Pyricularia oryzae* Cavara. *Journal of Agricultural Technology* 9(2): 333-348.

Farnaz, A., Jugah, K., Abbas, N., Seyed-Reza, H., Ganesan, V. and Siti-Khadijah, R. (2016). Characterisation of *Magnaporthe oryzae* Isolates from Rice in Peninsular Malaysia. *Journal of Genetic Plant Breeding* 54(4): 145-156.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783-791.

Gayatree, P., Chinmayee, S., Manoj, K.Y., Aravindan, S., Ngangkham, U., Raghu, S., Prabhukarthikeyan, S.R., Srikanta, L., Jitendra, K.T., Sonali, K. and Mayabini, J. (2017). Morphological and molecular characterization of *Magnaporthe oryzae* from *Chhattisgarh Oryza. Journal Association of Rice Research Workers* 54(3): 330-336.

Getachew, G., Tesfaye, A. and Kassahun, T. (2014). Morphological, physiological and biochemical studies on Pyricularia grisea isolates causing blast disease on finger millet in Ethiopia. *Journal of Applied Biosciences* 74: 6059– 6071.

Gohel, N.M. and Chauhan, H.L. (2015). Nutritional and Physiological Studies on the Growth and Sporulation of *Pyricularia oryzae* Cavara Isolated from Rice. *Trends in Biosciences* 8(22): 6381-6387.

Huili, Z., Zhongshou, W., Chenfang, W., Yang, L. and Jin-Rong, X. (2014). Germination and infectivity of microconidia in the rice blast fungus Magnaporthe oryzae. Macmillan. pp. 1-9. [Publishers Limited [www.nature.com/nature communicationns]. Site visited on 23/4/2018.

Jamal-u-ddin, H., Mubeen, L.A., Mumtaz, A.P., Ali, K.M. and Serwar, S.G. (2012). In-vitro evaluation of fungicides, plant extracts and bio-controlagents against rice blast pathogen *Magnaporthe oryzae* couch. *Pakistani Journal Botany* 44(5): 1775-1778.

Jamal-U-ddin, H., Abdul, M. L., Muhammad, A. K., Muhammad, A. R. and Ghulam, S. S. (2013). Influence of abiotic factors on the vegetative growth and sporulation of *Magnaporthe oryzae* couch. *Pakistan Journal of Phytopathology* 25(01): 65-70.

Jamal u-ddin, H., Mubeen, L.A., Sultan, M., Gulam, S.S. and Aslam, M.R. (2015). Impact of nutritional sources on vegetative growth and conidial formation of *Magnaporthe oryzae* Couch. *Journal of European Academic Research*

3(3): 2764-2773.

Jonit, N.Q., Low, Y.C. and Tan, G.H. (2016). *Xanthomonas oryzae* pv. *oryzae,* Biochemical Tests, Rice (*Oryza sativa*), Bacterial Leaf Blight (BLB) Disease, Sekinchan. *Journal of Applied and Environmental Microbiology* 4(3): 63-69.

Kalavati, T., Prasannakumar, M.K., Jyothi, V., Chandrashekar, S.C., Bhagyashree, M., Raviteaz, M. and Amrutha, N. (2016). Cultural and morphological studies on Ponnampet leaf and neck blast isolates of *Magnaporthe grisea* (Herbert) barr on rice (*Oryza sativa* L.) *Journal of Applied and Natural Science* 8(2): 604 – 608.

Kariaga, M.G., Wakhungu, J. and Hassan, K.W. (2016). Identificationof Rice Blast (Pyricularia Oryzae Cav.) Races from Kenyan Rice Growing Regions Using Culture and Classical Charaterization, *Journal of Research in Agriculture and Animal Science* 4: 16-24.

Kariaga, M.G., Wakhungu, J., Were, H., Muoma, D., Onamu, R. and Wekesa, C. (2017). Rice Blast *Pyricularia oryzae* CAV. Isolates in Kenya Characterized Sequenced and Registered. *International Journal of Life Sciences Research* 5(3): 23-30.

Laxman, S.R., Taru, S., Puchakayala, M. and Parimal, S. (2017). Effect of Temperature on Growth and Sporulation of Rice Leaf Blast Pathogen Magnaporthe oryzae. *International Journal of Current Microbiology and Applied Sciences* 6(3): 394-401.

Madhavan, S., Malathi, S. Rabindran, R., Paranidharan, V. and Velazhahan, R. (2013). Molecular characterization of *Mangnoporthegrisea*isolatedfrom buffel grass (*Cenchrusciliaris*) in Tamil Nadu, India. *Annual Plant Protection Science* 22 (1): 122-126.

Mahuku, G.S. (1998). Asimple Extraction method suitable for PCR Based analysis of Plant, Fungi and Bacteria DNA. *Plant molecular biology reporter* 22: 71-81.

Mebratu, G.A., Thangavel, S. and Getaneh, W. (2015). Assessment of disease intensity and isolates characterization of blast disease (*Pyriculariaoryzae* CAV) from South West of Ethiopia. *International Journal of Life Sciences* 3(4): 271 - 286.

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 Biological Report 48pp.

Nalley, L., Tsiboe, F., Durand-Morat, A., Shew, A. and Thoma, G. (2016). Economic and Environmental Impact of Rice Blast Pathogen (*Magnaporthe oryzae*). Alleviation in the United States. *PLoS ONE* 11(12): 1-15.

Nurulhidayah, M.M. and Kalaivani, N. (2014). Morphological and Molecular Characterization of Mognaporthe oryzae (Fungus) from infected rice leaf plant. American Institute of Pysics Conference proceedings 1614. pp.756-760.

Piyasiri, M.L.H. (1990). Land Evaluation and land Suitability classiftcation - Unguja and Pemba islands) Food and Agriculture Organization of the United Nations (FAO) Technical Cooperation Programme pp. 1-156.

Priya, V., Saveetha, K., Sankaralingam, A., Rabindran, R. and Robin, S. (2013). Variability in *Pyriculariaoryzae*from different rice growing regions of Tamil Nadu, India. *African Journal of Microbiology Research* 7 (26): 3379-3388.

Ritu, R. (2017). Perpetuation and management of *Pyricularia grisea* cav. causing blast disease of basmati rice. Dissertation Submitted to the Punjab Agricultural University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology. Department of Plant Pathology pp. 1-134.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.

Shanmugapackiam, S., Irulandi, S., Murali, S.P., Nagaraja, A. and Raguchander, T. (2019). Cultural, Morphological, Physiological and Biochemical Characteristics of Magnaporthe grisea Isolates of Finger Millet. *International Journal of Current Microbiology and Applied Sciences* 8 (08): 1830-1845.

Subhalakshmi, T. and Indira, D.S. (2017). Blast of Rice in Manipur and its Bio-control by *Pseudomonas fluorescens* and *Trichoderma sp. International Journal of Current Microbiology and Applied Sciences* 6: 1619-1634.

Tajima, F. and Nei, M. (1984). Estimation of evolutionary distance between nucleotide sequences. *Molecular Biology and Evolution* 1: 269-285.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.

Théophile, O.D.J.O., Akiko, K., Takuhito, N.O.D.A., Bonaventure, C.A., Yacoub, S., Takashi, K., Seiji, Y. and Yoshimichi, F. (2014). Pathogenicity Analysis of Blast (*Pyricularia oryzae* Cavara) Isolates from West Africa. *Journal of Agricultural Research Question* 48 (4): 403 – 412.

Varsha, G., Mahadevu, P. and Prudhvi, R.V. (2016). Study of suitable culture media and other abiotic factors for the growth and sporulation of Magnaporthe oryzae Economic Enviromelat and Conservation 22 (2): 297-301.

Wasihun, Y.W. and Flagote, A.B. (2016). Assessment of diseases on rice (*Oriza sativa* L.) in major growing fields of Pawe district, Northwestern Ethiopia. World Scientific News 42: 13-23.

CHAPTER FOUR

Manuscript III

Identification of resistant genotypes to rice leaf blast disease caused by *pyricularia oryzae* using rice blast differential lines and traditional varieties in Zanzibar

[1]Ali Khatib Bakar., [2]S.O.W.M. Reuben., [3]H.J.F. Lyimo

[1]**Department of Crop Science and Horticulture**, P.o. Box 97. Wete Pemba. Email: alikhatibbakar@yahoo.com

[2]**Department of Crop Science and Horticulture,** Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Email: smreuben@yahoo.com

[3]**Department of Crop Science and Horticulture**, Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Email: flyimo_1999@yahoo.com

Target Journal and Status: International Journal of Science and Research (IJSR). Manuscript is under review process.

Abstract

Rice leaf blast disease caused by fungal pathogen *Pyricularia oryzae* Cavara is an economically important disease distributed in rice growing areas of the world. In Zanzibar, rice leaf blast is considered a major disease, causing severe yield losses due to unavailability of rice genotypes with high resistance potential. Nineteen rice blast differential genotypes including one susceptible check (BKN Supa) and ten traditional varieties preferred by farmers in Zanzibar were inoculated with strain of *Pyricularia oryzae* and evaluated pathogenicity (virulence potential) to blast in the screen house. Complete Randomized Design (CRD) with three replicates was applied. Phenotypic disease score data for susceptible (S) or resistant (R) reaction of the tested genotypes to the *Pyricularia oryzae* was assessed through visual observation by examining the leaves for blast infection symptoms using standard scale of 0-9 developed by IRRI, Area under disease progress (AUDPC) were developed based on six scores at interval of ten days viz; 15, 25, 35, 45, 55 and 65days. Yield and growth yield parameters were assessed. A total of 13 rice blast differential genotypes were resistant to the disease and were recommended for breeding purposes for the benefit of Zanzibar rice farmers. Four varieties were moderately resistant and three was highly susceptible including one susceptible check which had the highest area under disease progress. From ten traditional varieties tested, none was found resistant. Three varieties were found moderately susceptible and seven was susceptible to disease.

Key words: Differential genotypes, Severity, AUDP, Inoculation, and Pathotypes

4.1 INTRODUCTION

Several cereal crops including maize, sorghum, millet and rice (*Oryza sativa* L.) are cultivated in Zanzibar, among them rice is a major cereal crop that is cultivated and consumed as a staple food that saves 3.1million people of Zanzibar (Nalley *et al.,* 2016, Gayatree *et al.,* 2017; Zelalem *et al.,* 2017). In Zanzibar, demand of rice is increasing as the population increases annually while production is low hence; there is high deficit of rice to meet population demand. Low yields are due to biotic and abiotic factors including rice leaf blast disease caused by *Pyricularia oryzae* Cavara which causes huge yield losses annually (Dagnachew *et al.,* 2014). As a consequence, Zanzibar imports 80% of required milled rice from Tanzania mainland and overseas. This disease impairs rice production in many rice growing countries of the world (Vasudevan *et al.,* 2016; Sabin *et al.,* 2016; Halima *et al.,* 2017).

Infection symptoms caused by the fungal pathogen *Pyricularia oryzae* vary depending on the environmental conditions of the areas, plant age, cultivar resistance and susceptibility. For resistant cultivars the early stage lesions often remain small in size ranging from1-2 mm and brownish to dark brown in color. For susceptible cultivars in rice fields disease is recognized by broadly spindle shaped spots, with the pale ashy center and brownish red margins seen on the leaf. With severe infection, several such spots coalesce and the lamina is destroyed (Akhilesh *et al.,* 2017). Lesions may initially appear gray-green and water-soaked with a darker green border and expand rapidly to several centimeters in length.

On susceptible cultivars, older lesions often become light tan in color with necrotic borders. The fungus tends to adapt to adverse widely environmental conditions depending on favourable meteorological factors. It attacks all above ground parts of a rice plant including the leaf blade, collar region, nodes, calm and grain in all rainfed upland rice, lowland and irrigated ecologies (Aruna *et al.,* 2016). The most commonly affected organs are leaves and panicles (necks). Severely infected rice leaves by blast result to impaired photosynthesis as the leave fails to manufacture its food leading to death. Infected panicles cause economic yield losses from unfilled grains depending on the rice cultivar, plant infected organs and degree of severity. The most important source of inoculum of *Pyricularia oryzae* is crop residues including seeds and straws where pathogen survives over seasons, Raveloson *et al.* (2017) reported that the presence of rice infected residues in the field after harvest had positive effects on the onset of blast epidemics.

Several management practices for controlling rice leaf blast disease have been employed with little success because the most common means of blast transmission is by seeds (seed born) (Hubert *et al.,* 2015). The most viable management strategies of rice leaf blast control is the use of resistant cultivars which has been found cheapest, economic and most effective ways in practice (Aram *et al.,*2013; Fukuta *et al.,* 2014; Miah *et al.,* 2017). Breeding rice cultivars for blast disease resistance should be an economic approach in disease management. However, control is not always durable because of resistance breakdown as a result of continuous evolvement of new races. Thus, released resistant cultivars often show high levels of susceptibility within a short period after have been released (Khan *et al.,* 2016). It has been reported by Fukuta *et al.* (2014) that the International Collaborative Research aims at developing a differential system that can identify the pathogenicity of blast fungi and genotypes containing resistant genes in rice varieties based on the gene-for-gene theory.

Fungicides application to rice blast infected field has been used as an effective approach in disease control while type of fungicide used may vary from area to area due to availability and degree of efficiency. It is advisable to rotate the type of fungicides used to prevent the fungus from developing resistance to limited types of fungicides. In the study of Vinod *et al.* (2017) application of fungicide Tricyclazole 75% WP for blast control showed the best results as it gave minimum disease incidence in both leaf and neck blast at 16.3 and 21.22 % respectively. Lack of effective rice blast resistant varieties is a major cause of spreading disease consequently notoriously low yields. No research has been done previously to study this disease in Zanzibar. Several elite varieties have been introduced in Zanzibar but none of them have reported reduced disease to a significant level. Therefore, the main objective of this study is to establish rice genotypes with effective rice blast resistance genes and environmental conditions for increased rice productivity in Zanzibar.

4.2 MATERIALS AND METHODS

4.2.1 Description of the study area

The experiment was conducted in the screen house of the Department of Crop Science and Horticulture at Sokoine University of Agriculture (SUA) in Morogoro region located at latitude 6^{o} 50” S and longitude 37^{o} 11’ 09’’ E with an elevation of 929 meters above sea level. It experienced a bimodal rain pattern with a dry period between June and November. Mean annual rain fall is 800 mm and temperature ranges from 24 to 34^{0}C.

4.2.2 Plant materials used in the study

Conducting an experiment, single conidial isolates of *Pyricularia oryzae* isolated from Zanzibar was established and evaluation according to the degree of virulence. The virulence frequency of the selected isolate was tested under screen house conditions at the Department of Crop Science and Horticulture at Sokoine University of Agriculture (SUA), Morogoro, Tanzania. In the experiment 19 rice blast differential genotypes (Table 4.1) and 10 traditional varieties (Table 4.2) preferred by farmers in Zanzibar including BKN supa which is susceptible to rice leaf blast disease in Zanzibar were utilized. The test material was planted in plastic trays (20 x 18 x 15cm) containing approximately 8 kg of dry soil mixed with 2.7g of Triple Supper Phosphate fertilizer as a basal and moistened before seed was sown (Anne *et al.,* 2002; Sanusan *et al.,* 2010; Sarker *et al.,* 2017).

Table 4.1: Nineteen rice blast differential genotypes used in the study

No.	Entry no.	Designation	Target gene	Source	Reference
1	IR85417	IRBLb-IT13[CO]	Pib	IRRI	Dakawa Res. Centre
2	LTH	Lijiangxintuanheigu		IRRI	Dakawa Res. Centre
3	IR85425	IRBLta2-Re[CO]	Pita-2	IRRI	Dakawa Res. Centre
4	IRBL7	IRBLkp-K60	Pik-p	IRRI	Dakawa Res. Centre
5	IRBL10	IRBLz5-Ca	Piz5	IRRI	Dakawa Res. Centre
6	CO39	CO39		IRRI	Dakawa Res. Centre
7	IRBL27	IRBLta2-Pi	v bfchfdj	IRRI	Dakawa Res. Centre
8	IRBL30	IRBL11-Zh	Pi11(t)	IRRI	Dakawa Res. Centre
9	IRBLZ-FU	IRBLZ-FU	Piz	IRRI	Africa Rice SUA
10	IRBL1-CL	IRBL1-CL	Pi1	IRRI	Africa Rice SUA
11	MOROBEREKAN	MOROBEREKAN	Pi5(t),Pi7	IRRI	Dakawa Res. Centre
12	IRBL20-IR-24	IRBL20-IR-24	Pi20	IRRI	Africa Rice SUA
13	IRBL23	IRBL12-M	Pi12(t)	IRRI	Dakawa Res. Centre
14	IR93324	IRBLta-Me[CO]	Pita	IRRI	Dakawa Res. Centre
15	IR85429	IRBLzt-IR56[CO]	Piz-t	IRRI	Dakawa Res. Centre
16	IRBL8	IRBLkh-K3	Pik-h	IRRI	Dakawa Res. Centre
17	IRBL2	IRBLa-C	Pia	IRRI	Dakawa Res. Centre
18	IR85422	IRBLkp-K60[CO]	Pik-p	IRRI	Dakawa Res. Centre
19	IR85414	IRBL7-M[CO]	Pi7(t)	IRRI	Dakawa Res. Centre

Table 4.2: Traditional rice varieties collected from farmers and used in the research study

No	Rice genotype	Sources of seed	Resistant gene	Time harvested and collected
1	Dula,	Farmers stock	Lucking	2017
2	Baramata,	Farmers stock	Lucking	2017
3	TOX	Farmers stock	Lucking	2017
4	Alibadru,	Farmers stock	Lucking	2017
5	Kikuba	Farmers stock	Lucking	2017
6	Supa BC,	Farmers stock	Lucking	2017
7	Kijino	Farmers stock	Lucking	2017
8	Ringa,	Farmers stock	Lucking	2017
9	Kibeu	Farmers stock	Lucking	2017
10	Supa	Farmers stock	Lucking	2017

Before seeds were sown floating technique was conducted for obtaining good quality seeds for sowing with the ability to prevent seed rotting, dumping off and elimination of seed borne disease. Seeds were put in the bucket of water 20liter capacity and seeds dropped in. Floated seeds were rejected and sunken were used for sowing. Pots were kept on the metal bench and arranged in a complete randomized design (CRD) and replicated three times. Each replication for rice blast differential genotypes and traditional varieties consisted of 30 pots thus made 90 pots for three replications. In each pot three seeds were sown and seedlings were thinned to one in each pot at two weeks after sowing (Khatib, 2016). Preparation of fungal spore suspension and inoculation. The fungus *Pyricularia oryzae* was isolated from collected infected rice leaves in Zanzibar, put in filter paper and stored aseptically at -20^{o}C (Khan *et al.*, 2016). It was grown on Oat agar medium (OMA) (Rolled Oat 30g, (D (+) maltose 15g/l and agar 18g/l), thereafter sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes (Jamal-U-ddin *et al.*, 2013). About 25 ml of medium was poured in the 9cm diameter petri-dishes and 5mm disc of *Pyricularia oryzae* was inoculated and incubated at 25-26^{O}C for 15days for sporulation. Cultures were maintained in 12 hr light and 12 hr dark alternatively (Gaitonde *et al*., 2016). For heavy sporulation, mycelia were scraped with a sterilized cotton ear swab then covered with the two layers of cheese clothes then exposed to continuous near-ultraviolet light at 25-26^{O}C for 5 -7days (Takahashi *et al.*, 2009 and Khan *et al.*, 2014).

After 15 days of incubation at 25-26^{O}C, spore suspension was prepared as it was reported by Vinayak *et al.* (2018) and Vineela *et al.* (2015) whereby in each each petri- dish, 20ml of distilled water were added to make a spore suspension. Spores were werecounted using hermacytometer and adjusted to a concentration of 1×10^5 spores /ml added with Tween-20 (0.02%) for enhancing the adherence of conidia to rice leaves.

4.2.3 Seedlings inoculation

Inoculation was done in the evening by spraying method using a hand sprayer when young rice seedlings had reached a 3-4 leaf stage (Chuwa *et al.*, 2015). The rice genotype BKN Super, used as control was sprayed with sterile water. Inoculated plants in the screen house were covered with nylon material (Fig.4.1) for 48h to maintain 95-100% humidity for influencing pathogenicity (Hajano *et al.*, 2011). Young seedlings were daily monitored for every 4h in the day time and sprinkled with clean water for maintaining humidity and facilitating blast infection and to observe the presence of infection symptoms caused by *Pyricularia oryzae.*

Figure 4.1: Inoculated rice seedlings in the screen house while covered with nylon material to maintain 95-100% relative humidity

4.2.4 Crop managerial practices

Standard crop managerial practices such as over head irrigation, weeding and fertilization was adopted and applied timely for crop growth optimization and observation for different characteristics of rice blast disease symptoms (Pandey, 2016).

Hand watering cane was used as overhead irrigation to maintain the soil in moist condition throughout the growing period except when the crop showed symptoms of reaching physiological maturity. Top dressing fertilizer in the form of UREA at 2.9g per pot was applied in two splits at tillering stage initiation and second split at blooming to panicle initiation stage. Weed control measures in the rice pots was done by hand pulling whenever weed emerged (Dibyendu *et al.*, 2017). The yield and yield components provides real indications for what may have caused a high or low yield of the studied crop. For each genotype, panicles were harvested and all spikelets were removed and dried on the sun for 4-5 days. By using grain moisture meter full filled grains were measured to 14% moisture content. Thereafter, grains harvested from each genotype were weighed using weighing balance in gram (g) and recorded.

4.2.5 Disease Assessment

Rice leaf blast disease symptoms for some genotypes, appeared earlier after two weeks of (Figure 2a and 2b). Phenotypic disease score data for susceptible (S) or Resistant (R) reaction o f the tested genotypes to the *Pyricularia oryzae* was assessed through visual observation by examining the leaves for blast infection symptoms as reported by Daniel *et al.* (2016); Mohmmod *et al.* (2018). Leaf blast reaction was assessed six times by repetition by the interval of 10 to 60 days from the date of inoculation giving 6 readings at 15, 25, 35, 45, 55 and 65days taking into consideration only two types of reaction of the host, compatible or susceptible according to Prabhu *et al.* (2002). The procedure follow standard evaluation system of 0-9 scale developed by IRRI (1996). Regular daily monitoring of the crop in the screen house was done until harvest. Rice leaf blast disease severity was calculated using the formula described by Khan *et al.* (2016); Rini *et al.* (2017), Teshome and Tegegn (2017).

$$\text{Percentage disease severity} = \frac{\text{Sum of Numerical Ratings}}{\text{Number of Plants Scored x Maximum Score on Scale}} \quad \text{x 1 00}$$

Rice leaf blast quantified disease progress was calculated using the area under disease development progress (AUDPC) after Simko and Piepho (2012), Teshome and Tegegn (2017). Area Under disease progress (AUDPC) was calculated by addition of two data set of severity infection percentage recorded from the first reading to the second reading on alternate days and divided by two to find the average. Thereafter the mid-value obtained was multiplied by the time interval as shown below:

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Where, yi = initial infection percentage (disease score)

Yi+1= progressive infection percentage

Ti+1-ti= time interval between the readings

Based on severity levels 0-15% leaf infection were considered as resistant (R), 15.1-30% infection as moderately resistant (MR), 30.1–50% infection as moderately susceptible (MS) and 50.1-100% infection as susceptible (S) Puri *et al*. (2006) and Patrizia *et al.* (2015).

At harvest when all studied rice genotypes reached physiological maturity, measurements of yield and yield components which are the basic phenotypic characteristics were applied including plant height (cm) which is related to the productivity and growth rate of a plant. Plant height of each line was measured using tape measure from base of the plants to tip of the grains as described by Puri *et al.* (2006). Other measurements recorded was performed including number of grains per panicle, number of panicles per plant, number of productive and unproductive tillers per plant, number of tillers in each pot was counted when the plant reached maximum tiller formation stage (Bekele and Getahun, 2016) while plant height measurement was done at harvest.

Panicle length (cm), one thousand grain weight (g) adjusted to 14% moisture content, number of secondary branches per panicle (Doni *et al.*, 2015). Percent spikelet sterility caused by rice blast disease for each variety was determined using the formula described by Hubert (2017).

$$\text{Spikelet sterility \%} = \frac{\text{Number of unfilled grains}}{\text{Number of filled grains + number of unfilled grains}} \times 100$$

4.3 Data collection and analysis

Data for severity were transformed using arcsine transformation to make them normally distributed using the formula: Y = arcsine $\sqrt{P}$ = $\sin^{-1}$ where: Y= the result of the transformation. Data were analysed using GENSTAT 16 edition computer statistical package for ANOVA to determine significant differences between the studied 19 rice blast differential genotypes and 10 traditional varieties compared with most susceptible to blast. Comparison between means was done using Turkey Multiple Range Test (TMRT).

4.4 RESULTS

Rice leaf blast disease infection was varied between varieties. Some of the varieties reacted resistant and some varieties reacted susceptible to the disease. During the experimental duration temperature in the screen house was ranged from 21.5 to 38.4^{o}C with the average of 27.4 ^{o}C. Relative humidity ranged from 31.626 to 100RH with the average of 71.11RH. Infection began on bottom leaves and progressing to the flag leaves (Fig. 4.2 and Fig. 4.3).

Figure 4.2: Rice leaf blast disease symptoms of susceptible genotype

Figure 4.3 Rice leaf blast resistant

4.4.1 Combined ANOVA mean squares for rice blast differential genotypes and traditional varieties

ANOVA Summary for differential genotypes (mean squares). The findings of analysis of variance are shown in Appendix 4. All the studied variables indicated significant genotypic effects except tiller ability (%). Appendix 5 indicates ANOVA results for traditional varieties. All the variables except 1000 seed weight (g) and tillering ability indicated significant genotypic effect and spikelet sterility (%).

4.4.2 Severity analysis of differential genotypes

Differential genotypes number 1to 8 do not differ significantly and 9 to 12 do not differ significantly as well as number 13 and 14. However, between number 1to 8, 9 to 12 and 13 to 14 showed significant different. Traditional varieties indicated no significant different for number 1 and 2. Also number 3 to 9 are statistical similar but differed significantly to number 1 and 2 is shown in Table 4.3 and 4.4.

Table 4.3: List of genotypes that did not differ significantly from the lowest severity value

No.	Differential genotypes	Severity value (%)
1	IRBL12-M	11.72abc
2	IRBLkp-K60[CO]	11.72abc
3	IRBLta-Me[CO]	11.72abc
4	IRBLta2-Re[CO]	13.57abc
5	IRBLb-IT13[CO]	13.57abc
6	IRBLzt-IR56[CO]	13.57abc
7	IRBLa-C	14.18abc
8	IRBLta2-Pi	14.48abc
9	IRBLz-Ca	14.80abcd
10	IRBL11-Zh	15.42abcd
11	IRBLkh-K3	15.42abcd
12	IRBL1-CL	20.97abcd
13	LTH	93.19f
14	CO39	95.05f

Table 4.4: List of traditional varieties that did not differ significantly from the lowest severity value

No.	Traditional varieties	Severity value (%)
1	TOX	36.39^{a}
2	Dula	36.40^{a}
3	Alibadru	38.25^{ab}
4	Supa	55.54^{ab}
5	Kikuba	61.08^{ab}
6	Ringa	61.70^{ab}
7	Kibeu	66.0.^{4ab}
8	Baramata	66.65^{ab}
9	Supa BC	74.05^{ab}

4.4.3 Mean values of rice blast differential genotypes on rice leaf disease progress (AUDP) in screen house condition and their reactions

Among the nineteen rice blast differential genotypes evaluated for pathogenicity study, six observations were made at an interval of ten days. Results summarized in Table 4.5. There was a significantly ($P \leq 0.001$) difference between differential genotypes for area under disease progress (AUDPC). The highest area under disease progress was recorded to CO39 (13283), LTH (1295.5) followed by BKN Supa (121.1) which they all reacted susceptible (S) to the disease. Moroberekan had the lowest value (271.5) followed by IRBLta-Re [CO] (398.1) both reacted resistant (R). Thirteen differentia genotypes reacted resistant (R), four genotypes reacted moderate resistant (MR) and three genotypes reacted susceptible (S) to disease.

Table 4.5: Mean values of rice blast differential genotypes on rice leaf disease progress (AUDP) in screen house condition and their reactions

Genotypes	Genes	Total severity %	AUDPC I	AUDPC II	AUDPC III	AUDPC IV	AUDPC V	AUDPC VI	TOTAL AUDPC	DISEASE REACTION
IRBL1-CL	Pi1	20.97^{bc}	0.00^{a}	0.00^{a}	78.20^{ab}	119.4^{bc}	143.5cd	170.7^{f}	511.8^{bcde}	MR
IRBL11-Zh	Pi11(t)	15.42^{ab}	0.00^{a}	0.00^{a}	52.13^{ab}	100.0^{bc}	127.8^{bcd}	142.2^{cdef}	422.2^{abcd}	R
IRBL12-M	Pi12 (t)	11.72^{a}	0.00^{a}	0.00^{a}	78.20^{ab}	110.9^{bc}	110.9^{abc}	113.6^{abc}	413.6^{abcd}	R
IRBL20-IR24	Pi 20	24.67^{c}	0.00^{a}	26.07^{a}	97.57^{ab}	143.5^{c}	157.9^{d}	157.9^{def}	582.9^{de}	MR
IRBL7-M[CO]	Pi7 (t)	24.05^{c}	0.00^{a}	26.07^{a}	89.10^{ab}	136.3^{bcde}	157.9d	170.7^{f}	580.1^{cde}	MR
IRBLa-C	Pia	14.18^{ab}	0.00^{a}	26.07^{a}	89.10^{ab}	110.9^{abcde}	110.9^{abc}	110.9^{a}	447.9^{abcd}	R
IRBLb-IT13[CO]	Pib	13.57^{ab}	0.00^{a}	0.00^{a}	26.07^{a}	89.1^{ab}	119.4^{bcd}	143.5^{cdef}	378.0^{ab}	R
IRBLkh-K3	Pik-h	15.42^{ab}	0.00^{a}	26.07^{ab}	36.97^{a}	97.6^{ab}	135.0^{bcd}	142.2^{acdef}	437.9^{abcd}	R
IRBLkp-K60	Pik-p	11.10^{a}	0.00^{a}	26.07^{ab}	36.97^{a}	63.0^{ab}	100.0^{ab}	119.4^{abc}	345.4^{ab}	R
IRBLkp-K60[CO]	Pik-p	11.72^{a}	0.00^{a}	0.00^{a}	52.13^{ab}	100.0^{abc}	110.9abc	110.9^{ab}	373.9^{ab}	R
IRBLta-Me [CO]	Pita	11.72^{a}	0.00^{a}	0.00^{a}	26.07^{a}	63.0^{ab}	108.5^{abc}	127.8^{abcd}	325.4^{a}	R
IRBLta2-Pi	vbfchfdj	14.18^{ab}	0.00^{a}	0.00^{a}	52.13^{ab}	100.0^{bc}	119.4^{abc}	135.0^{abcde}	406.5^{abc}	R
IRBLta2-Re[CO]	Pita-2	13.57^{ab}	0.00^{a}	0.00^{a}	52.13^{ab}	100.0^{bc}	119.4abc	126.6^{abc}	398.1ab	R
IRBLZ-FU	Piz	27.75^{c}	0.00^{a}	78.20^{b}	119.37^{b}	143.5^{c}	157.9^{d}	164.3^{ef}	663.3^{c}	MR
IRBLz5-Ca	Piz5	14.80^{ab}	0.00^{a}	0.00^{a}	78.20^{ab}	110.9^{bc}	119.4^{abc}	126.6^{abc}	435.0^{abcd}	R
IRBLzt-IR56[CO]	Piz-t	13.57^{ab}	0.00^{a}	0.00^{a}	26.07^{a}	89.1^{abc}	119.4^{abc}	143.5^{cdef}	378.0^{ab}	R
Moroberekan	Pi5(t),Pi7	8.63a	0.00^{a}	0.00^{a}	26.07^{a}	37.0^{a}	89.1^{a}	119.4^{abc}	271.5^{a}	R
BKN Supa	-	78.37^{d}	89.10^{b}	157.10^{c}	199.70^{c}	230.9^{d}	259.2^{e}	281.1^{g}	1217.1^{f}	S
LTH	-	93.19^{e}	89.10^{b}	164.30^{c}	216.10^{c}	226.0^{d}	293.8^{f}	306.1^{g}	1295.4^{f}	S

CO39	-	95.05^{e}	110.90^{c}	182.95^{c}	220.97^{c}	259.4^{d}	256.3e	297.8g	1328.3f	S
Mean ($\bar{x}$)		**26.68**	**14.46**	**35.60**	**82.70**	**121.50**	**145.8**	**160.50**	**561.00**	
SE (±)		**2.464**	**3.447**	**313.44**	**21.34**	**17.60**	**10.63**	**9.32**	**52.70**	
LSD at 0.05		**7.042**	**9.852**	**38.41**	**60.99**	**50.30**	**30.38**	**26.65**	**150.60**	
CV (%)		**16.0**	**41.3**	**63.3**	**44.7**	**25.1**	**12.6**	**10.1**	**16.3**	
P-Value		**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	

Means with different superscript letters down the column differ significantly at P≤0.05

4.4.4 Mean value of rice leaf blast differential genotypes on yield and growth yield parameters in screen house condition

Significant differences at (P ≤ 0.001) based on plant height among the rice blast differential genotypes were observed as indicated in Table 4.6. Genotypes CO39 and IRBL ta-2Re [CO] recorded the shortest plants (70.3cm) and were statisticaly similar while IRBLa-C recorded highest plant height (134.0cm) followed by IRBLz5-Ca (127cm) and BKN Supa (control) (126.7cm). Tillering showed no significant (P≥0.05) difference between genotype ranging from 64.37 – 90.00% whereby BKN Supa (control) registered lowest (64.3%) followed by IRBLz5-Ca (66.14%) and the highest values were for IRBLta2-Re[CO], CO39, IRBL11-Zh, IRBL7-M[CO] and Moroberekan which were similar (90.00%). On other hand, significant (P ≤ 0.001) variation was observed in panicle length among the studied genotypes. The records for panicle length ranged from 23.42 for IRBLta2-Pi to 28.99cm for Moroberekan. Significant effects of genotypes were observed on spikelet sterility ranging from 15.58 for IRBL1-CL to 36.11for IRBLb-IT13 [CO]. Moroberekan registered significantly highest value (30.85g) of 1000seed weight and lowest (15.66g) on IRBLkp-K60 when dried and adjusted to 13% grain moisture content. On number of secondary branches IRBLta2-pi registered lowest (7.33) and highest number was registered for Moroberekan (16.67) (Table 8). Significantly (P ≤ 0.001) variation was observed in panicle yield among differential genotype. The highest panicle yield value was recorded (12.73 g) from Moroberekan and the lowest (4.73 g) for IRBL11Zh.

Table 4.6: Mean value of rice leaf blast differential genotypes on yield and growth yield parameters in screen house condition

Genotypes	Plant height (cm)	Tillering ability (%)	Panicle length (cm)	Number secondary branches	Spikelet sterility (%)	1000 seeds weight (g)	Panicle yield (g)
IRBLkp-K60	89.0^{abc}	84.41^{a}	26.75^{bcd}	13.33^{def}	30.64^{defg}	15.66^{a}	5.300abcd
IRBL1-CL	106.7^{cdef}	79.23^{a}	28.98^{f}	11.67^{bcdef}	15.58^{a}	19.56^{b}	8.667fg
IRBLta2-Re[CO]	70.3^{a}	90.00^{a}	27.14^{bcdef}	13.67^{defg}	25.60^{abcdefg}	19.57^{b}	7.400bcdefs
IRBLb-IT13[CO]	76.3^{ab}	71.20^{a}	27.93^{cdef}	14.67^{fg}	36.11^{g}	19.74^{b}	6.667bcdefs
IRBLkh-K3	112.7^{defg}	82.60^{a}	27.00^{bcde}	8.67^{ab}	21.24^{abcde}	20.36^{bc}	7.133bcdfs
IRBL20-IR24	97.3^{bcde}	80.43^{a}	28.25^{def}	13.33^{def}	31.84^{efg}	20.72^{bc}	6.633bcdefs
CO39	73.0^{a}	90.00^{a}	28.22^{def}	14.00^{efg}	31.57^{efg}	21.13^{cd}	7.93cdefs
BKN Supa	126.7^{fg}	64.37^{a}	27.14^{bcdef}	13.00^{cdef}	32.90f^{g}	22.05^{de}	5.433abcde
IRBLZ-FU	85.3^{abc}	69.23^{a}	26.47^{bcd}	14.00^{efg}	32.50^{fg}	22.39^{ef}	8.50efg
IRBLzt-IR56[CO]	128.3^{fg}	82.85^{a}	28.69^{ef}	11.00^{bcde}	22.50^{abcdef}	22.96^{efg}	8.23defg
IRBL11-Zh	106.7^{cdef}	90.00^{a}	26.60^{cd}	11.33^{bcde}	19.12^{abc}	23.07^{efg}	4.73ab
IRBLkp-K60[CO]	91.3^{abcd}	70.65^{a}	25.54^{b}	10.67^{bcd}	29.14^{cdefg}	23.17^{efg}	8.967g
IRBL7-M[CO]	80.3^{ab}	90.00^{a}	26.97^{bcde}	12.00^{cdef}	22.86^{abcdef}	23.50^{fg}	6.70bcdefs
IRBLta-Me[CO]	125.3^{fg}	69.23^{a}	27.26^{bcdef}	8.67^{abcd}	16.10^{ab}	24.02^{g}	7.467bcdefg
IRBLz5-Ca	127.0^{fg}	66.14^{a}	27.91^{cdef}	10.00^{abc}	25.94^{abcdefg}	24.18^{g}	6.900bcdefs
IRBLa-C	134.0^{g}	67.18^{a}	28.27^{def}	10.67^{bcd}	25.85^{abcdefg}	25.57^{h}	6.067abcdefs
LTH	116.0^{efg}	72.33^{a}	27.26^{bcdef}	8.67^{ab}	27.04^{bcdefg}	26.02^{hi}	4.867abc
IRBL12-M	78.0ab	80.63^{a}	26.13^{ab}	12.67^{cdef}	25.12^{abcdefg}	27.10^{i}	5.73abcdef
IRBLta2-Pi	97.7^{bcde}	78.25^{a}	23.42^{a}	7.33^{a}	23.58^{abcdef}	29.73^{j}	5.067a
Moroberekan	125.3^{fg}	90.00^{a}	28.99^{f}	16.67^{g}	20.29^{abcd}	30.85^{j}	12.73h
Mean ($\bar{x}$)	**102.4**	**78.40**	**27.25**	**11.80**	**25.78**	**23.068**	**6.96**
SE (±)	**6.82**	**8.28**	**0.553**	**0.963**	**3.276**	**0.3912**	**0.587**
LSD at 0.05	**19.50**	**23.66**	**1.580**	**2.753**	**9.362**	**1.1181**	**1.677**
CV(%)	**11.5**	**18.3**	**3.5**	**14.1**	**22.0**	**2.9**	14.6
P-value	**0.001**	**0.315**	**0.001**	**0.001**	**0.001**	**0.001**	0.001

Means with different superscript letters down the column differ significantly at $P \leq 0.05$

4.4.5 Mean values of rice traditional varieties on rice leaf disease progress in screen house condition

Significant ($P \leq 0.05$) differences between genotypes was observed on disease severity that varied from 36.39 for TOX to 77.76% for Kijino (Table 4.7). The highest disease severity was recorded at booting stages. Kijino had significantly the highest disease severity (77.76%) followed by Supa BC (74.05%) and Baramata (66.65%) which were statistically identical while the lowest disease severity was registered on TOX (36.39%) followed by Dula (36.40%) and Alibadru (38.25%). The area under disease progress curve (AUDPC) recorded at an interval of ten days in screen-house conditions increased gradually over time and assessed to the maximum severity time. Total area under disease progress curve

(TAUDPC) ranged between 438.4 – 1080.3 based on the calculated two data points of disease severity. The highest total disease progress curve was registered (1080.3) for Kibeu followed by (1031.0) forn Baramata. Alibadru (438.4) had the lowest (Table 4.7).

Table 4.7: Mean value of rice traditional varieties on rice leaf disease progress (AUDPC) in screen house condition.

Genotype	Disease severity (%)	AUDPC I	AUDPC II	AUDPC III	AUDPC IV	AUDPC V	AUDPC VI	TOTAL AUDPC	REACTION
Alibadru	38.25^{a}	0.00^{a}	29.75^{a}	68.9^{a}	89.4^{a}	112.5^{a}	137.8^{a}	438.4^{a}	**MS**
Supa	55.54^{ab}	0.00^{a}	54.72^{a}	103.3^{a}	142.3^{a}	168.1^{a}	186.4^{a}	654.8^{a}	**S**
Dula	36.40^{a}	26.06^{a}	63.02^{a}	108.4^{a}	141.4^{a}	182.0^{a}	221.0^{a}	741.9^{a}	**MS**
TOX	36.39^{a}	26.06a	89.08^{a}	119.4^{a}	149.9^{a}	182.0^{a}	203.2^{a}	769.6^{a}	**MS**
Kikuba	61.08^{ab}	39.57^{a}	88.77^{a}	127.5^{a}	145.2^{a}	166.8^{a}	182.1^{a}	749.9^{a}	**S**
Ringa	61.70^{ab}	52.11^{a}	108.44^{a}	157.1^{a}	209.7^{a}	240.1^{a}	263.5^{a}	1030.8^{a}	**S**
Kijino	77.76^{b}	55.81^{a}	110.34^{a}	136.4^{a}	163.3^{a}	182.5^{a}	191.7^{a}	840.1^{a}	**S**
Kibeu	66.04^{b}	63.02^{a}	122.04^{a}	174.8^{a}	215.5^{a}	245.6^{a}	259.4^{a}	1080.3^{a}	**S**
Supa BC	74.05^{b}	73.93^{a}	121.74^{a}	146.6^{a}	167.6^{a}	178.0^{a}	178.9^{a}	866.7^{a}	**S**
Baramata	66.65b	78.17^{a}	142.20^{a}	186.1^{a}	214.8^{a}	235.6a	174.2^{a}	1031.0^{a}	**S**
Mean ($\bar{x}$)	**57.4**	**25.03**	**93.00**	**133.00**	**164.**	**189.00**	**200.00**	**820.00**	
SE (±)	**7.99**	**25.03**	**33.7**	**39.10**	**46.0**	**51.1**	**59.4**	**234.8**	
LSD at 0.05	**23.58**	**73.82**	**99.6**	**115.5**	**135.6**	**150.8**	**175.3**	**692.7**	
CV (%)	**24.1**	**104.4**	**62.8**	**51.0**	**48.6**	**46.8**	**51.5**	**49.6**	
P-value	**0.007**	**0.316**	**0.421**	**0.615**	**0.655**	**0.751**	**0.905**	**0.705**	

Means with different superscript letters down the column differ significantly at $P \leq 0.05$

4.4.6 Mean values of rice leaf blast Traditional varieties on yield and growth yield parameters in screen house condition

Plant height significantly ($P \leq 0.001$) varied among the traditional varieties tested ranging between 41.83 cm for TOX to 103.40cm for Supa (Table 4.8). TOX had manimum plant height (41.83 cm) and recorded maximum weight (23.65 g) in 1000 seeds weight and maximum tillering ability (77.36%) while maximum plant height was recorded (103.40 cm) in Supa. Significant ($P \leq 0.001$) variation was found in panicle length and in number of secondary branches while genotype Supa BC recorded maximum branches. It was observed that among traditional varieties studied, spikelet sterility registered lowest for Ringa (27.72%) followed by Kibeu (28.95%) and maximum was recorded in Alibadru (38.10%) followed by Dula (35.27%) and the later did not differ from Kikuba, Kijino, Baramata, Supa BC, Supa and TOX (Table 4.8).

Table 4.8: Mean values of rice leaf blast Traditional varieties on yield and growth yield parameters in screen house condition

Genotype	Plant height (cm)	Tillering ability (%)	Panicle length (cm)	Number secondary branches (no)	Spikelet sterility (%)	1000 seeds weight (g)	Panicle yield
Alibadru	58.10^{b}	54.58^{a}	19.70^{a}	10.22^{ab}	38.10^{a}	18.92^{a}	5.767a
Dula	74.20^{c}	69.39^{a}	$24.^{42bcd}$	11.33^{abc}	35.27^{a}	19.49^{a}	5.460a
Ringa	92.57^{def}	55.17^{a}	23.50^{abcd}	10.77^{ab}	27.72^{a}	19.82^{a}	6.837ab
Kikuba	88.73^{de}	57.14^{a}	23.59^{bcd}	10.55^{abc}	32.43^{a}	19.93^{a}	6.530ab
Kijino	99.10^{ef}	69.23^{a}	23.45^{abcd}	9.77^{a}	29.82^{a}	19.96^{a}	6.850ab
Baramata	88.80^{de}	54.89^{a}	24.96^{cd}	12.55^{abc}	32.58^{a}	20.66^{a}	7.020ab
Supa BC	94.03^{def}	58.10^{a}	26.72^{d}	14.33^{c}	30.89^{a}	20.66^{a}	7.013ab
Supa	103.40^{f}	54.06^{a}	25.72^{d}	13.11^{bc}	32.78^{a}	21.08^{a}	7.013ab
Kibeu	82.27^{cd}	54.22^{a}	21.48^{abc}	9.66^{a}	28.95^{a}	21.52^{a}	7.210ab
TOX	41.83^{a}	77.36	21.03^{ab}	10.55^{ab}	31.18^{a}	23.65^{a}	8.103b
Mean ($\bar{x}$)	**82.30**	**60.4**	**23.46**	**11.37**	**31.97**	**20.57**	**6.80**
SE (±)	**2.731**	**6.61**	**0.760**	**0.951**	**2.467**	**0.958**	**0.461**
LSD at 0.05	**8.056**	**19.49**	**2.242**	**2.806**	**7.278**	**2.825**	**1.361**
CV(%)	**5.7**	**18.9**	**5.6**	**14.5**	**13.4**	**8.1**	**11.7**
P-value	**0.001**	**0.179**	**0.001**	**0.037**	**0.208**	**0.107**	**0.033**

Means with different superscript letters down the column differ significantly at $P \leq 0.05$

4.5 Discussion

Rice leaf blast disease caused by a fungus *Pyricularia oryzae* is one of the major diseases of rice in Zanzibar and it is a leading rice production constraint causing high yield losses every season (Jae *et al.,* 2014). The disease is widespread in all rice growing ecologies as most of the available varieties are susceptible to the disease. Use of rice blast resistant disease varieties would offer a better management of disease compared to other control measures (Miah *et al.,* 2017). A set of nineteen rice blast differential lines with one most susceptible rice variety (BKN Supa used as control) and ten traditional varieties inoculated with *Pyricularia oryzae*, showed that disease severity levels varied among the lines ranging from 8.63- 95.05% for rice blast differential lines and for traditional varieties from 36.39 – 77.76%. Selected lines have been advocated for use in identification of rice leaf blast resistance for Zanzibar. With this variation, it is possible to select resistant genotypes for use in breeding or for production.

Disease reaction of inoculated strain of *Pyricularia oryzae* against international differentials revealed thirteen lines viz: IRBL11-Zh *(Pi11(t),* IRBL12-M *(Pi12(t)),* IRBLa-C *(Pia),* IRBLb-IT13[CO] *(Pib),* IRBLkh-K3 *(Pik-h),* IRBLkp-K60 *(Pik-p),* IRBLkp-K60[CO] *(Pik-p),* IRBLta-Me[CO] *(Pita),* IRBLta2-Pi *(vbfchfdj),* IRBLta2-Re[CO] *(Pita-2),* IRBLz5-Ca *(Piz5),* IRBLzt-IR56[CO] *(Piz-t)* and Moroberekan (*Pi5(t), Pi7*) contained potential resistant genes for resistance breeding program. They exhibited incompatibility with inoculated strain in the screen house as indicating that, the accessions have genes confering resistance.

This result corresponded to those of Aram *et al.* (2013), Salomon *et al.* (2014) and Chuwa *et al.* (2015). The inoculated *Pyricularia oryzae* found narrow virulent to four international differentials and said moderate resistant lines viz: IRBL1-CL (*Pi1*), IRBL20-

IR24 (*Pi20*), IRBL7-M [CO] (*Pi7*(*t*) and IRBLZ-FU (*Piz*), while three susceptible varieties were BKN Supa (local control), LTH and CO39. Some resistant genotypes lose their resistance over time during cultivation and become susceptible to blast fungus as it was reported by (Selisana *et al.*, 2017) that the effectiveness of rice blast R genes varies significantly because of different races composition from region to region. Jayawardana *et al*. (2014) reported that most of the rice blast differential genotypes have retained their resistance over the years while some of them which were resistant to blast fungus have lost their resistance. Use of rice blast resistant varieties could overcome rice production constraints. Wang *et al.* (2013) reported that use of host resistance has proven to be the most effective strategy and economical method for controlling rice leaf blast disease. Based on findings blast resistant genes *(Pi11(t), (Pi12(t)), (Pia), (Pib), (Pik-h), (Pik-p), (Pik-p), (Pita), (vbfchfdj), (Pita-2), (Piz5), (Piz-t) and (Pi5(t), Pi7)* were confirmed the most effective resistance genes and effective strategy required to rice breeders for improving blast resistance in Zanzibar to achieve longer lasting resistant genotypes as suggested by Shamshad *et al.* (2019) that effective resistance genes should be recommended to rice breeders for improving blast resistance in this region.

Due to frequency variability of pathotypes, Zanzibar island need some rice genotypes with durable resistance from breeders which is influenced by epidemiological factors as well as interaction between host and pathogen (Siti and Latiffa 2019). The AUDPC value recorded in six classes (15, 25, 35, 45, 55 and 65 days) at an interval of 10 days differed significantly with genotypes. Based on the percentage disease severity, disease symptoms initiation was earlier for some susceptible genotypes and for resistant genotypes, disease initiation was delayed. This implied that with delayed rice leaf blast disease initiation, slower rates of disease development occurred as supported by Mukherjee *et al.* (2009). The lowest total AUDPC was observed on Moroberekan (271.5) whereas highest was observed on CO39 (1328.3) followed by LTH (1295.4) and BKN Supa (control) (1217.1) which imply that lowest total value of area under disease progress curve denoted the degree of resistance and higher total value denoted susceptibility. Thus, genetic variability exists among the genotypes on resistance to the blast. Three categories were listed based on the total area under disease progress curve which are resistant, moderate resistant and susceptible (Table 4.5).

On ten traditional varieties studied, the area under disease progress curve (AUDPC) revealed that among ten traditional varieties studied no one was found resistant. Most of them were susceptible to *Pyricularia oryzae* and few were laid on moderate resistant category. Occurrence of leaf blast disease symptoms was initiated and occurred at the young stage of rice plant growth hence caused high damage on the leaves gradually and interfered plant physiological growth. Diseased weakened plants were due to lack of application of gene-for-gene” resistance against blast disease as reported by Nancy (1983) reported that a number of plant genes have been characterized that confer resistant gene on the plant as a mechanism for preventing disease attack. The moderate rice resistant varieties can be used in breeding programs for the purposes of producing high yielding and better quality genotypes for release as cultivars to rice growers. Application of AUDPC techniques is considered as the best option to estimate a variety as resistant or susceptible overtime.

On growth and yield parameters for differential genotypes there were significant differences for all traits due to genotypes that were genetically different. Measured plant growth and yield parameters rice is among the important growth traits that determines or modifies yield. These traits varied among genotypes due to differences in their genetic makeup (Roy *et al.*, 2014). A genotype Moroberekan exhibited maximum tillering ability, panicle length, number of secondary branches, 1000seed weight in the present study which can be used as a donor of genes for many useful agronomical traits Girish *et al.* (2006); Pyeong-Sook *et al*. (2015). Traditional rice varieties in Zanzibar might be improved using natural selection by evaluating their merits on drought resistance, salt tolerance, soil fertility, lodging, tolerance to insect pests and diseases including rice blast (*Pyricularia oryzae)*. Rice breeders may have pin pointing traditional rice varieties important elements of crop genetic resources for their diversity and wide adaptability hence important for incorporation in to breeding programme to get new varieties (Vinayak *et al.*, 2018).

4.6 Conclusion

From this study, 19 rice blast differential genotypes, one local check, and ten traditional varieties were evaluated in the screen house condition for pathosystem. In ANOVA results showed that there were significantly $P < 0.001$ difference between all studied variables. On rice blast differential genotypes tested only 13 genotypes their genes were found incompatible (R) to the inoculated *Pyricularia oryzae*, 4 were moderate resistant (MR) and three were susceptible (S). Highest total disease severity was registered 95.06% for CO39 followed by It was found LTH (97.19) and (78.3%) for BKN Supa used as control and the lowest was recorded for Moroberekan (8.63). For traditional varieties none of them were found resistant but the lowest total disease severity was registered for TOX (36.39%) and highest was for Kijino (77.76). Lowest total area under development progress curve for deferential genotypes was recorded for Moroberekan (271.5) and highest was for CO39 (1328.3). Thus, for traditional varieties Kibeu was the highest 1080.3 and Alibadru (438.4) was the lowest.

4.7 Recommendations

1. Due to significant variations of pathotype reactions, genotypes hold genes viz: *(Pi11(t), (Pi12(t)), (Pia), (Pib), (Pik-h), (Pik-p), (Pik-p), (Pita), (vbfchfdj), (Pita-2), (Piz5), (Piz-t) and (Pi5(t),Pi7)* were confirmed the most effective resistance genes that breeders can utilize for improving blast resistance in Zanzibar to achieve longer lasting resistant genotypes

2. Tox as a cultivated rice variety in Zanzibar could be introgressed with exotic genes to improve resistant meanwhile maintaining its characteristic of high panicle yield.

REFERENCES

Akhilesh, K. K., Neha, S. and Sanath-kumar, V. B. (2017). Survey, Collection, Isolation and Identification of Isolates of *Pyricularia oryzae* causing rice blast in Southern Karnataka by using host differential lines. *International Journal of Agricultural Science and Research* (IJASR) 7(3): 285-294.

Aram, P., Nadali, B., Nadali, B. and Gorbanali, N. (2013). Identification of rice genotypes resistant to panicle blast. *International Journal of Agriculture and Crop Sciences* 5 (12): 1346-1350.

Aruna, J., Vijay, K.S., Rambabu, R., Ramesh, S., Yashaswini, C., Bhaskar, B., Madhavi, K.R., Balachndran, S.M., Ravindrababu, V. and Prasad, M.S. (2016). Morphological characterization of five different isolates of *pyricularia oryzae* causing rice blast disease. *Progressive Research – An International Journal* 11(VI): 3377-3380.

Bekele, A.F. and Getahun, D.W. (2016). Influence of Phosphorous and Nitrogen Fertilizer Rate on Grain Yield of Rice at Kamashi Zone of Benshal-gul Gumuz Region, Ethiopia. *Journal of World Economic Research* 5(2): 8-14.

Chuwa, J.C., Robert, B.M. and Mnyuku, S.O.W.R. (2015a). Assessment of Grain Yield Losses Caused by Rice Blast Disease in Major Rice Growing Areas in Tanzania. *International Journal of Science and Research* (IJSR) 4 (10): 2211-2218.

Chuwa, J.C., Robert, B.M. and Mnyuku, S.O.W.R. (2015b). Pathogenic Variation and Molecular Characterization of *Pyricularia oryzae*, Causal Agent of Rice Blast Disease in Tanzania. *International Journal of Science and Research* 4(11): 1131-1139.

Dagnachew, L., Santie, de V., Masresha, F., Teshome, B., Tesfaye, A., Geleta, G., Getachew, G. and Kassahun, T. (2014). Pathogenicity and yield loss assessment caused by *Magnaporthe oryzae* isolates in cultivated and wild relatives of finger millet (*Eleusine coracana*). *Indian Journal of Agricultural Research* 48 (4): 258 – 268.

Daniel, P.A., Rafael, S.T., Bruno, S.L., Renata, D.F.L., Fabyano, F.S., Cosme, D.C., Carlos, N. and Derly, J.H.S. (2016). Artificial neural network for prediction of the area under the disease progress curve of tomato late blight. *Scientia Agricola* 74(1): 51-59.

Dibyendu, M., Anannya, G., Debashis, R., Adyant, K., Diana, S., Soumen, B., Ratikanta, G., Pintoo, B. and Anurup, M. (2017). Yield loss assessment of rice (O*ryza Sativa* L.) due to different biotic stresses under system of rice intensification (SRI). *Journal of Entomology and Zoology Studies 5*(4): 1974-1980.

Doni, F., Norela, S., Anizan, I., Wan, N.W., Che, R.C., Abidah, A. and Wan, M.W. (2015). Impact of System of Rice Intensification (SRI) on Paddy Field Ecosystem: Case Study in Ledang, Johore, Malaysia. *Journal of Pure and Applied Microbiology* 9(2): 927-933.

Fukuta, Y., Koga, I., Ung, T., Sathya, K., Kawasaki-Tanaka, A., Koide, Y., Kobayashi, N., Obara, M., Yadana, H. and Hayashi, N. (2014). Pathogenicity of Rice Blast (*Pyricularia oryzae* Cavara) Isolates from Cambodia. *Japan Agricultural Research Quarterly* 48(2): 155 – 166. [http://www.jircas.affrc.go.jp]. site visited on 20/6/2018.

Gaitonde, V., Prudhvi, R.V. and Vijay, N. (2016). Molecular Characterization and virulence pattern studiesof rice (*Oryza Sativa*) blast (*Magnaporthe Oryzae*) disease. *International Journal of Agriculture, Environment and Biotechnology* 9(2): 145-151.

Gayatree, P., Chinmayee, S., Manoj, K.Y., Aravindan, S., Ngangkham, U., Raghu, S., Prabhukarthikeyan, S.R., Srikanta, L., Jitendra, K.T., Sonali, K. and Mayabini, J. (2017). Morphological and molecular characterization of *Magnaporthe oryzae* from Chhattisgarh. *Oryza* 54(3): 330-336.

Girish, T.N., Gireesha, T.M., Vaishali, M.G., Hanamareddy, B.G. and Shailaja, H. (2006). Response of a new IR50/Moroberekan recombinant inbred population of rice (*Oryza sativa* L.) from an indica japonica cross for growth and yield traits under aerobic conditions. *Euphytica* 152: 149–161.

Hajano, J., Mumtaz, A.P., Qayoom, A.R. and Mubeen, A.L. (2011). Rice blast-mycoflora, symptomatology and pathogenicity. *International Journal of Animals Veterinary Medicine Sciences* 5(1): 53-63.

Halima, Q., Awais, R. and Muhammad, A. (2017). Evaluation of Rice Germplasm for Resistance against *Pyricularia oryzae* the Cause of Rice Leaf Blast. *Asian Research Journal of Agriculture* 4(3):1-6 [https://sahainan.wordpress.com /2015/05/25/seed-floating-test/]. Site visited on 3/6/2018.

Hubert, J., Mabagala, R.B. and Mamiro, D.P. (2015). Efficacy of Selected Plant Extracts against Pyricularia grisea, Causal Agent of Rice Blast Disease. American *Journal of Plant Sciences* 6: 602-611.

IRRI (1996). International Rice Research Institute Standard Evaluation System for Rice. Inger. Genetic resources center, IRRI, Manila, Philippines. 67pp.

Jamal-U-ddin, H., Abdul, M.L., Muhammad, A.K., Muhammad, A. R. and Ghulam, S. S. (2013). Influence of abiotic factors on the vegetative growth and sporulation of *Magnaporthe oryzae* couch. *Journal of Phytopathology* 25(01): 65-70.

Jayawardana, W.A.D., Jayasekera, G.A.U., Wijesundera, R.L.C., Dissanayake, D.M.N., Sooriyapathirana, S.D.S.S., Weebadde, C.K., Perera, K.L.N.S., Gunapala, K.R.D. and Hettige, P. (2014). Evaluation of DNA Markers Linked to Blast Resistant Genes, *Pikh*, *Pit (p)* and *Pita*, for Parental Selection in Sri Lankan Rice Breeding. *Journal of Tropical Agricultural Research* 26(1): 82 – 93.

Hubert, J. (2017). Pathogenic variation, diagnosis and reaction of elite rice genotypes to rice yellow mottle virus in Tanzania. A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy of the Sokoine University of Agriculture, Morogoro Tanzania pp. 1-233.

Khan M.A.I., Partha, P.S., Rejwan, B., Enamul, K., Abul, K.C., Yoshimichi, F., Ansar, A. and Mohammad, A. (2014). Phenotypic screening and molecular analysis of blast resistance in fragrant rice for marker assisted selection. *Comptes Rendus Biologies* 337: 318-324.

Khan, M.A.I., Ali, M.A., Monsur, M.A., Kawasaki-Tanaka, A., Hayashi, N., Yanahigara, S., Obara, M., Mia, M.A.T., Latif, M.A. and Fukuta, Y. (2016). Diversity and distribution of rice blast (*Pyricularia oryzae*) races in Bangladesh. *Plant disease* 100: 2025- 2033.

Khatib, B.H. (2016). Performance of new released upland Nerica rice varieties under different combined rates of nitrogen and phosphorus fertilizers in Zanzibar. A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science in crop science of Sokoine University of Agriculture. Morogoro, Tanzania pp. 1-106.

Miah, G., Rafii, M.Y., Ismail, M.R., Sahebi, M., Hashemi, F.S.G., Yusuff, O. and Usman, M.G. (2017). Blast disease intimidation towards rice cultivation: A review of pathogen and strategies to control. The Journal of Animal and Plant Sciences 27(4): 1058-1066.

Mohammod, H., Ansar, A., Mohammad, D.H. and Abu, T.M. (2018). Detection of Blast Resistant Gene in Rice by Host-pathogen Interaction and DNA-Marker. University. *Journal of Agricultural Research* 6(1): 23-30.

Mukherjee, A. K., Mohapatra, N.K. and Nayak, P. (2009). Estimation of area under the disease progress curves in a rice-blast pathosystem from two data points. European *Journal of Plant Pathology*. pp. 1-7.

Nalley, L., Tsiboe, F., Durand-Morat, A., Shew, A. and Thoma, G. (2016). Economic and Environmental Impact of Rice Blast Pathogen (*Magnaporthe oryzae*) Alleviation in the United States. *PLoS ONE* 11(12): 1-15.

Nancy, A.E. (1983). Plant Disease Susceptibility Genes?. *The Plant Cell*. 14: 19-86.

Pandey, S. (2016). Screening of Rice Cultivars for Quantification of Apparent Infection Rate of Leaf Blast. *Middle East Journal of Agriculture Research* 5(4): 430-434.

Patrizia, T., Gabrele, M. and Luigi, T. (2015). Resistance to neck blast caused by *Pyricularia oryzae* inItalian rice cultivars. *European Journal of Plant Pathology* 142: 49–59.

Prabhu, A.S., Filippi, M.C. and Araújo, L.G. (2002). Pathotype diversity of *Pyricularia grisea* from improved upland rice cultivars in experimental plots. *Fitopatologia Brasileira* 27(5): 468-473.

Puri, K.D., Shrestha, S.M. and Joshi, K.D. (2006). Reaction of different rice lines against leaf and neck blast under field condition of Chitwan valley. *Journal of Institute of Agriculture and Animal Science* 27: 37- 44.

Pyeong-Sook, K., Dong-Min, K., Ju-Wang, K., Hyun-Sook, L. and Sang-Nag, A. (2015). QTL Mapping of Rice Root Traits at Different NH4+ Levels in Hydroponic Condition. *Plant Breeding Biotechnology* 3(3): 244-252.

Raveloson, H., Ratsimiala, R.I., Tharreau, D. and Sester, M. (2017). Long-term survival of Blast Pathogen in Infected Rice Residues as major source of primary inoculum in high altitude upland ecology. *Journal of Plant pathology Plant Pathology* 67: 610–618.

Rini, P., Dipankar, M. and Bhima, S.N. (2017). Effect of different meteorological parameters on the development and progression of rice leaf blast disease in western Odisha. *International Journal Plant Protection* 10(1): 52-57.

Roy, S.K., Ali, M.Y., Jahan, M.S., Saha, U.K., Ahmad-Hamdani, M.S., Hasan, M.M., and Amirul, A. (2014). Evaluation of growth and yield attributing characteristics of indigenous Boro rice varieties. *Life Science Journal* 11(4): 122 – 126.

Sabin, K., Subedi, B., Bhandari, A., Giri, D.R., Shrestha, B., Neupane, P., Shrestha, S. M. and Gaire, S.P. (2016). Screening of Different Rice Genotypes against (Pyricularia grisea) Sacc. In Natural Epidemic Condition at Seedling Stage in Chitwan, Nepal. *Advances in Crop Science and Technology* 4(231): 1-6.

Salomon, K.A., Djdy, K.A., Gisele, Y.H., Symphorien, A., Ibnou, D., Yacouba, S. and Yowovi, M.D.G. (2014). Cultural and Pathological studies of Pyricularia oryzae Isolates at Aboney Calavi in Benin. *Journal of Plant Pathology* 13(1): 44-49.

Sanusan, S., Polthanee, A., Audebert, A., Seripong, S. and Mouret, J.C. (2010). Growth and Yield of Rice (*Oryza sativa* L.) as affected by cultivars, Seeding Depth and Water Deficits at Vegetative stage. *Asian Journal of Plant Science* 9(1): 36-43.

Sarker, U.K., Uddin, M.R., Sarkar, M.A.R., Salam, M.A. and Hasan, A.K. (2017). Physiological parameters and yield differ in rice (*Oryza sativa* L.) cultivars with variable water management systems. *Archives of Agriculture and Environmental Science* 2(4): 247-256.

Selisana, S.M., Yanoria, M.J., Quime, C.B., Chaipanya, G., Lu Opulencia, R., Wang, G.L., Mitchell, T., Correll, J.N., Talbot, N.J., Leung, H. and Zhou, B. (2017). Avirulence (AVR) Gene-Based Diagnosis Complements Existing Pathogen Surveillance Tools for Effective Deployment of Resistance(R) Gene Against Rice Blast Disease. *Phytopathology* 107(6): 711-720.

Shamshad, A., Jahangir, I., Dipankar, M., Mandal, N.P., Chandeshwar, P. and Mukund, V. (2019). Identification of Broad Spectrum Blast Resistance Genes for North-

East and Eastern India using Standard International Blast Differential. *International Journal of Current Microbiology and Applied Sciences* (8)04: 2639-2648.

Simko, I. and Piepho, H.P. (2012). The area under the disease progress stairs: Calculation, advantage and application. *Phytopathology* 102(4): 381- 389.

Siti, N.M. and Latiffah, Z. (2019). Pathotype Identification of Rice Blast Pathogen, *Pyricularia oryzae* Using Differential Varieties in Peninsular Malaysia. *Tropical Life Sciences Research* 30(2): 181–190.

Takahashi, W., Miura, Y. and Sasaki, T.A. (2009). A novel inoculation method for evaluation of grey leaf spot resistance in Italian ryegrass. *Journal of Plant Pathology* 91 (1): 171-176.

Teshome, E. and Tegegn, A. (2017). Comparative Study of Powdery Mildew (*Erysiphe polygoni*) Disease Severity and its effect on yield and yield components of field Pea (*Pisum sativum* L.) in the Southeastern Oromia, Ethiopia. *Journal of Plant Pathology* 8(5): 1-5.

Vasudevan, K., Casiana, M.V.C., Wilhelm, G. and Navreet, K.B. (2016). Large scale gerplasm screening for identification of novel rice blast resistance resources. *Original Research Article* 5(505): 1-4.

Vinayak, T., Mahendra, R., Ramachandra, A., Krupa, K.N., Ningaraj, D., Deepak, C.A. and Harini, K.K.M. (2018). Screening of traditional rice varieties (TRVs) for blast resistance. *Journal of Pharmacognosy and Phytochemistry* 7(1): 1384-1388.

Vineela, C., Surya, B. and David, J.M. (2015). *In-vitro* vs *in-vivo* Inoculation: Screening for Resistance of Australian Rice Genotypes against Blast Fungus. *Rice Science* 22(3): 132-137.

Vinod, K.N., Prasant, P.S. and Dushyant, K.K. (2017). Efficacy of Fungicides and Bio-Agents against *Pyricularia gresia* in Paddy and Yield Gap Analysis Thought Frontline Demonstration. International *Journal of Current Microbiology and Applied Sciences* 6(4): 2338-2346 [http://www.ijcmas.com]. site visited on 21/12/2017.

Wang, J.C., Jia, Y., Wen, J.W., Liu, W.P., Liu, X.M., Li, L., Jiang, Z.Y., Zhang, J.H, Guo, X.L. and Ren, J.P. (2013). Identification of rice blast resistance genes using international monogenic differentials. *Journal of Crop Protection* 45(2013): 109-116.

Zelalem, Z., Paul, G., Jimmy, L. and Richard, E. (2017). Reaction of introduced Korean rice genotypes for resistance to rice blast in Uganda. *Journal of Plant Breeding and Crop Science* 9(7): 98-105.

CHAPTER FIVE

MANUSCRIPT IV

Assessment of rice yield losses caused by rice leaf blast disease (*Pyricularia oryzae Cavara*) in Zanzibar

[1]Ali Khatib Bakar., [2]S.O.W.M. Reuben., [3]H.J.F. Lyimo

[1]**Department of Crop Science and Horticulture**, P. O. Box 97, Wete Pemba. Email: alikhatibbakar@yahoo.com

[2]**Department of Crop Science and Horticulture,** Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Email: smreuben@yahoo.com

[3]**Department of Crop Science and Horticulture**, Sokoine University of Agriculture, P. O. Box 3005 , Morogoro, Email: flyimo_1999@yahoo.com

Target Journal and Status: International Journal of Science and Research (IJSR). Manuscript is under review process.

Abstract

Rice leaf blast disease caused by *Pyricularia oryae* Cav, is one of the most devastating diseases of rice in most rice growing areas of the world. Use of chemical fungicide is an important tool to control rice blast disease. In this study the field experiment was conducted during two main cropping seasons of 2016-2017 and 2017-2018 at Kizimbani Agricultural Research Institute (KARI) in Unguja in West district and at Matangatuani Agricultural Research Station in Pemba in Micheweni district. A susceptible rice blast variety in Zanzibar BKN Supa was planted in Randomized Complete Block Design with three replications to assess disease infection and yield loss caused by *Pyricularia oryzae* There were two treatments viz; one set of protected plots and second set of unprotected following pair plot technique design. Fungicide Megasin – M70% WP was sprayed on the protected plots during the experiment period and unprotected plots left natural as control. SPSS statistical package IBM Version 21 was used to analyse data using t-test to compare two sample variable means. In Pemba, during 2016-2017 cropping season, percentage yield loss (63.789%/ha) deferred with the percentage yield loss (49.548%/ha) registered in 2017-2018. In Unguja, 2016-2017 cropping season yield loss (84.621% /ha) and 2017-2018 was (55.906%/ha) was registered. There was significant variations of percentage yield loss between seasons. The results implies that for both islands of Unguja and Pemba in the first seasons 2016 – 2017 rice yield loss was higher due to the environment conditions favoured to disease while in the second seasons 2017 – 2018 yield loss was low, this observation was associated with the environmenal conditions less favoured to disease. Futher more in comparison of percentage rice yield loss registered in Pemba from 2016-2018 was registered low at 56.671% and Unguja from 2016 to 2018 registered higher at 70.670%. The result was may be associated with several factors including inoculum pressure, weather conditions, efficacy of the fungicide applied etc.

Key words: Zanzibar, *Pyricularia oryzae,* yield loss, severity and BKNSupa

5.1 INTRODUCTION

In Zanzibar island, rice (*Oryza sativa* L) is one of the major food crops that constitute a staple diet to serve 1.3 million people and has tremendous economic importance. Rice is cultivated all over the world however it is predominant in Asian countries (Rijal *et al.,* 2017). The crop is commonly affected by biotic and abiotic factors but the most important is rice leaf blast disease. The disease is incited by ascoomycete fungus *Pyricularia oryzae* Cavara [synonym *Pyricularia. grisea* Sacc, teleomorph *Magnaporthe grisea* (Hebert) as reported in many countries worldwide where rice is grown (Katsantonis *et al*., 2017). Rice leaf blast disease has continued to be a major production constraint in Zanzibar capable of causing infection at any stage of crop growth on vegetative parts (Surender *et al.,* 2017).

However, the losses due to the disease have not been determined in Zanzibar. The disease is widespread in all rice growing ecologies as most of the available varieties are susceptible to the disease. In Zanzibar, rice yield per unit area is very low 0.5 - 1.0 ton/ha for upland rice and Irrigation is 2.1 tons/ha. Potential yield of rainfed upland is 5 tons/ha and 8 tons/ha for irrigated (Khatib *et al*., 2009; Sekiya *et al.,* 2013 and Khatib, 2016). Reducing rice leaf blast disease losses is an important approach to increased productivity (Dibyendu *et al.,* 2017).

The blast symptoms mostly occur on leaf, nodal, neck, panicle and on seeds which cause heavy yield losses ranging from 80-100% and sometimes total crop can be destructed when favourable disease conditions (Prem *et al.,* 2015; Raboin *et al*., 2016, Ghimire *et al*., 2017 and Wasimfiroz *et al.,* 2018). When *Pyricularia oryzae* affecting leaves, it causes diamond-shaped white to gray lesions with dark green to brown borders which is surrounded by a yellowish halo. When plants reach heading stage, the pathogen invades the panicles and infects spikelet, the rachis branch and neck of panicle (neck blast). Neck blast is usually the most destructive damage which can lead to zero yields (Takashi *et al.*, 2018).

Santosh *et al.* (2018) reported that when *Pyricularia oryzae* infected rice and cause neck rot or panicle blast disease, the host plant will be either killed or hinder seed development due to large amount of unfilled grains. However, severe epiphytic conditions may result between 70–90% losses in field condition when predisposition factors such as relative humidity ranged from 70 -89%, presence of dew on plant, excessive nitrogen fertilizer dose, drought weather condition and high mean temperature values ranging from 26-31^{O}C (Vinayak *et al.*, 2018). It was reported by Ashkani *et al.* (2015) that, in large rice producing areas under blast favourable conditions blast yield losses ranged from 30-50%. It has been estimated that more than 50% of rice harvests may be lost in a field infected by the blast disease each year, which is enough to feed an estimated 60 million people. Effective use of fungicides offers an alternative management of rice leaf blast disease in the nursery and in the field (Uda *et al.*, 2018).

The viable approaches on rice leaf blast disease control was based on planting of resistant cultivars, application of fungicides which has been widely practiced in many rice growing areas in the world and improving cultural practices and other field managerial practices. Efforts have been made by rice researchers by developing and identifying rice genotypes for improving rice production with substantial costs reduction in disease management. Use of resistant cultivars is the only practical approach to control the disease where beneficiaries cannot afford the cost of fungicides. It has been reported that, some varieties carried at least single blast resistant gene and some with multi genes which are specific to the type of pathogen races (Akanke *et al.*, 2018). The objective of this study was to evaluate rice yield losses caused by rice leaf blast disease (*Pyricularia oryzae* Cavara) in Zanzibar so as to give information on economic losses from the pathogen.

5.2 MATERIALS AND METHODS

5.2.1 Description of the study area

Zanzibar is the union of two sister islands viz; Unguja and Pemba surrounded with numerous small islands and islets. The experiment was conducted in the field at two locations viz: Unguja and Pemba Islands in two cropping seasons of 2016/2017 and 2017/2018 during the long rainfall (masika season) which normally start from March to May and short rains starting from September to November. In Unguja the experiment was set up at Kizimbani Agricultural Research Station in West district, situated between 6^o S' latitude and 39^o 16'E longitude and 220 meters above seas level (m.a.s.l). In Pemba, the field experiment was set up at Matangatuani Agriculture research station in Micheweni district, Nothern region situated at latitude 05° 09'S latitude and 39°49'E longitude. Both districts are characterized by bimodal rainfall distribution. In Micheweni, temperatures range from 21- 34^OC and mean annual rain fall is about 1860mm.

5.3 Methodology

5.3.1 Experimental design and field layout

The field experiment was laid out in randomized complete block design with three replications divided into two separate sets. One set of the plots was kept protected from rice leaf blast disease infection by regular application of fungicide (Megasin – M70% WP) of 40-50g/15liter of water and the other set was unprotected (control), exposed to natural infection throughout the crop growth in the field. Yield loss due to rice leaf blast disease infection was calculated following paired plot technique design. Control plots receiving no fungicide spray were used for treatment comparison in the experiment.

Each replicate was 10 m x 4 m = 40 m^2 separated from each other by 2.0 m and each sub plot was 4 m x 4 m = 16m^2 separated by 2.0 m. Total experimental area was16m x 10m = 160 m^2. Sprayed fungicide contained 700 g per kilogram Thiophanet- methyl, a wide spectrum, systemic fungicide having combined curative and preventive properties with no phytotoxic effect on crop plant.

5.3.2 Seeds preparation for sowing and crop management

The seed of BKN Supa rice variety which is very susceptible to blast disease in Zanzibar and preferred by most farmers were used in this experiment to evaluate yield losses under field conditions. Before sowing, seeds were water soaked in the gunny bag and incubated for 24hr and new water was changed after 12 hrs and then incubated for 48 hr in the dark to hasten early and easy germination (Pramesh *et al.,* 2016; Pramesh *et al.,* 2017). The land was ploughed by hand hoe followed by two harrowings after two weeks for easy soil leveling and maintaining even water and nutrient distribution. Phosphate fertilizer (TSP) and nitrogen (UREA) fertilizers were applied in the soil at the rate of 80 kg N + 50 kg P /ha. Phosphate fertilizer was applied in single split before sowing and nitrogen fertilizer was applied in two splits, one split during tillering initiation and the second during booting stage and seed rate was estimated at 50 – 60 kg per ha. (Khatib, 2010). Three to four seeds were sown using dibbling method at a spacing of 20 cm x 20 cm and each plot had 400 plants after they had been thinned to one per hill at 14 – 18 days after sowing. Weeding was done to minimize weed infestation by hand pulling of germinated weed flora twice during the experimental period. The first practice was done when plants had attained 3-5 leaves at three weeks and the second weeding was carried out when seedlings reached tillering initiation. Some weeds such as Goat weed (*Ageratum conyzoides*) and narrow leaved weed such as Nut grass (*Cyperus rotundus*) dominated the plots. The field plots were maintained in moist condition by extra irrigation except at active tillering, panicle initiation and flowering stages which are the important rice physiological stages which do not demand excessive moisture content (Dibyendu *et al.,* 2017). The fungicide Megasin – M70% WP was sprayed to the rice plants to control the disease four times starting at tiller initiation, two weeks from first disease symptoms appearance, at 15 days after the second spray and the last spray when the crop reached 15% panicle emergence **(**Gaikwad and Balgude (2016) and Wasimfiroz *et al.*,2018). Selected fungicide was applied using 15liters capacity knapsack sprayer that was started immediately after development of the first observable rice leaf blast disease symptoms.

5.3.3 Disease assessment and evaluation

The experimental plots were scouted regularly for disease onset observation. Disease incidence and severity in both sets of plot viz; protected and unprotected plots were assessed. Disease severity assessment, ten hills from each plot were selected randomly and tagged. Thereafter, disease grading was recorded at 15, 25, 35, 45, 55 and 65 days after planting from three upper most diseased leaves of each tagged hill, as to identify the pattern of the dynamics of the epidemics **(**Sester *et al.,* 2014, Sandeep, 2016). Disease grading was done following the systematic evaluation system of 0-9 designed by Internationl Rice Research Institute (IRRI 2002) as follow:

No lesion observed (Highly Resistant).

i. Small brown specks of pin point size (or) larger brown specks without sporulating centre (Resistant).

ii. Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. (Moderately Resistant).

iii. Lesion type is the same as in scale 2, but significant numbers of lesions are on the upper leaves (Moderately Resistant).

iv. Typical susceptible blast lesions of 3 mm or longer, infecting less than 4% of leaf area (Moderately Susceptible).
v. Typical blast lesions infecting 4-10% of the leaf area (Moderately Susceptible).
vi. Typical blast lesions infecting 11-25% of the leaf area (Susceptible).
vii. Typical blast lesions infecting 26-50% of the leaf area (Susceptible).
viii. Typical blast lesions infecting 51-75% of the leaf area many leaves are dead (Highly Susceptible).
ix. Typical blast lesions infecting more than 75% leaf area affected (Highly Susceptible).
    a. Percentage disease severity and disease incidence for the recorded infection scores were then calculated using the formula as described by Wasihun and Flagote, (2016) and Yashaswini *et al.* (2017) as described as:

$$\text{Percentage disease severity (\%)} = \frac{\text{Sum of scores}}{\text{Number of observations x highest number in rating scale}} \text{ x } 100$$

$$\text{Percentage disease incidence (\%)} = \frac{\text{Number of infected plant units}}{\text{Total number of units assessed}} \text{ x } 100$$

When the crop reached physiological maturity, agronomic growth traits were evaluated including percentage (%) tillering ability, panicle length (cm), plant height (cm), percentage (%) spikelet sterility, number of secondary branches (no) and 1000seed weight (g) following method of Octaviano *et al.* (2018). Grain yield estimation were recorded after harvested from each plot marking 3m x 2m (6m^2) section as described by Ganesh *et* *al.* (2012) and Pramesh *et al.* (2016). All tillers within the wooden frame (6m^2 quadrant) were cut by sharp knife at underground level (biological yield) dried on the sun shine and measured in weight (kg) in order to determine biomass then harvest index (HI). Dried grains at 14% moisture content were used to determine the percentage grain yield loss caused by the fungus *Pyricularia oryzae* using the formula described by Chuwa *et al.* (2016), Hossain *et al.* (2016), Chakraborty *et al.* (2017) and (Teshome and Tegegn, 2017) as follows:

$$YL = \frac{(Y_1 - Y_2)}{Y_1} \text{ x } 100$$

Where:

YL% = Percentage yield loss.

Y_1 = Mean grain yield on the protected plot (Plot with maximum protection).

Y_2 = Mean grain yield on the unprotected plot (i.e. unsprayed plot).

Harvested yield data and yield attributing characters of rice were subjected to statistical analysis and significance was tested using the "t-test" as:

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{S^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \sim t_{n_1 + n_2 - 2} \, d.f$$

Where,

X_1 = Average yield in treated plot (Protected).

X_2 = Average yield in untreated plot (Unprotected).

S^2 = Pooled variance.

n_1 = Sample size for Protected plots.

n_2 = Sample size for Unprotected plots.

‘t’ = Calculated value.

5.4 Statistical data analysis

Data collected was for disease severity, disease incidence, plant height, number of tillers, panicle length, number of secondary branches, spikelet sterility, tiller ability, 1000 seed weight, grain yield, biomass and harvest index. The statistical analysis of the recorded data was done by t- Test: two-sample assuming equal variance. Data were analyzed using SPSS Statistical package version 21 IBM.

5.4.1 Correlations among disease incidence, severity, yield and yield components

Disease incidence, severity, yield and yield components were correlated to observe the relationships between studied variables. The relationships among agronomic traits were obtained using Pearson correlation coefficient and probability levels of Independence using SPSS. During correlation analysis, variables that were computed were yield loss (%), incidence (%), severity (%), plant height (cm), tillering abiliy (%), panicle length (cm), spikelet sterility (%), panicle number (no), 1000 seed weight (g), number of secondary branches (no), harvest index (%) and yield (kg).

5.5 Results

Observation of crop in the field showed that plots of unprotected set found highly infected to disease. Plants were found stunted in growth and reduction of plant population due to severe disease infection. Plots of protected set were not found infected, their plants founded healthy, vigourous taller and completed plant population (Fig. 5.1 and 5. 2).

Figure 5.1: Unprotected plot with fungicide

Figure 5.2: Protected plot with fungicide

5.5.1 Estimation of rice yield and yield growth components losses of protected against unprotected plots from fungicide in Pemba during 2016-2017 cropping season

Pemba Island during 2016-2017 cropping season grain yields (ton per hector) on protected plots had a 4.877 t /ha, while unprotected plots gave 1.766 t/ha giving a loss of 63.789%. The difference between the two means was significant ($p\leq 0.001$). The protected plots consistently gave consistently significant higher means (Table 5.1), for the rest of variables viz; plant height, tillering ability, panicle length, spikelet sterility, number of panicles, number of secondary branches, harvest index and 1000seed weight.

Table 5.1: Estimation of rice yield and yield growth components losses of protected against unprotected plots from fungicide in Pemba during 2016-2017 cropping season

	Rice yield attributes							
Treatment	Grain yield (ton per ha)	Plant height (cm)	Tillering ability (%)	Panicle length (cm	Spikelet sterility (%)	No. of panicle /6m^2	No. of sec. branches	Harvest Index (%)
Protected	4.877	121.880	74.197	24.627	36.434	1016.000	15.866	49.640
Unprotecte	1.766	98.167	55.969	16.013	16.475	941.000	9.666	30.166
Mean	**3.111**	**23.713**	**18.228**	**8.6133**	**19.959**	**75.000**	**6.200**	**19.473**
Yield Loss%	**63.789**	**19.456**	**24.567**	**34.978**	**54.781**	**7.382**	**39.077**	**39.230**
Df	2	29	29	14	14	2	14	2
t	47.418	9.825	7.617	6.990	18.469	7.087	11.196	11.859
Std. deviation	0.114	13.220	13.107	4.772	4.185	18.330	2.144	2.844
Std. Error	0.065	2.413	2.393	1.232	1.080	10.583	0.553	1.642
P value	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.007

Table 5.2: Comparison of protected and unprotected plots of rice yield and growth components in Pemba during 2017-2018 cropping season

Treatment	Grain yield (ton per ha)	Plant height (cm)	Tillering ability (%)	Panicle length (cm)	Spikelet sterility (%)	No. of panicle /$6m^2$	No. of sec. branches	Harvest Index (%)	1000seed weight (g)
Protected	4.866	107.490	75.597	23.346	34.603	1231.000	12.733	48.443	25.433
Unprotected	2.455	88.840	62.480	19.880	15.006	870.333	7.933	34.196	21.068
Mean	**2.411**	**18.650**	**113.117**	**3.466**	**19.596**	**360.666**	**4.800**	**14.200**	**4.366**
Yield Loss %	**49.548**	**17.350**	**17.331**	**14.846**	**56.634**	**29.299**	**37.697**	**29.400**	**17.163**
Df	2	29	29	14	14	2	14	2	2
T	9.227	5.553	3.473	4.071	7.069	2.903	8.064	11.106	3.757
Std. deviation	0.452	18.395	20.688	3.298	10.736	124.259	2.305	2.221	2.013
Std. Error	0.261	3.358	3.777	0.851	2.772	124.259	0.595	1.282	1.162
P value	0.012	0.000	0.002	0.001	0.000	0.101	0.000	0.008	0.064

5.5.2 Estimation of rice yield and yield growth components losses of protected against unprotected plots from fungicide in Unguja during 2016-2017 cropping season

Yield estimation in Unguja Island was significant during 2016-2017 cropping season, whereby grain yields (ton per hector) on protected plots recorded 4.838 t /ha, while unprotected plots recorded 0.744 t/ha giving a loss of 84.621%. The two different treatment means gave significant ($p = 0.005$). On protected plots yield growth components variables viz; plant height, tillering ability, panicle length, spikelet sterility, number of panicles, number of secondary branches, harvest index and 1000seed weight stand consistently showed higher means values (Table 5.3).

Table 5.3: Estimation of rice yield and yield growth components losses of protected against unprotected plots from fungicide in Unguja during 2016-2017 cropping season

	Rice yield attributes								
Treatment	Grain yield (ton per ha)	Plant height (cm)	Tillering ability (%)	Panicle length (cm	Spike let sterility (%)	No. of panicle /6m^2	No. of sec. branches	Harvest Index (%)	1000seed weight (g)
Protected	4.838	104.550	81.210	25.400	14.775	1017.000	14.733	50.370	22.966
Unprotected	0.744	62.653	52.718	16.013	32.846	610.000	9.733	21.976	17.283
Mean	**4.094**	**41.896**	**28.491**	**9.386**	**18.071**	**407.000**	**5.000**	**28.393**	**5.683**
Yield Loss%	**84.621**	**40.074**	**35.084**	**36.957**	**55.017**	**40.020**	**33.937**	**56.371**	**24.745**
Df	2	29	29	14	14	2	14	2	2
t	13.727	10.838	11.986	9.937	15.038	19.229	7.264	8.977	29.568
Std. deviation	0.516	21.268	13.020	3.658	4.654	36.660	2.672	5.478	0.332
Std. Error	0.298	3.883	2.377	0.944	1.201	21.166	0.690	3.162	0.192
P value	0.005	0.000	0.000	0.000	0.000	0.003	0.000	0.012	0.001

5.5.3 Estimation of rice yield and yield growth components losses of protected against unprotected plots from fungicide in Unguja during 2017-2018 cropping season

In Unguja 2017-2018 cropping season there were significant variations between two traetments (protected and unprotected) means. Protected plots regisreed higher mean yield value of 4.572 tons/ha while unprotected plots were registered 2.016 tons/ha. The different means gave the yield loss of 55.906%. Other tested variables showed higher mean values on protected plots (Table 5.4).

Table 5.4: Estimation of rice yield and yield growth components losses of protected against unprotected plots from fungicide in Unguja during 2017-2018 cropping season

	Rice yield attributes								
Treatment	Grain yield (ton per ha)	Plant height (cm)	Tillering ability (%)	Panicle length (cm	Spikelet sterility (%)	No. of panicle /6m^2	No. of sec. branches	Harvest Index (%)	1000seed weight (g)
Protected	4.572	121.700	78.179	23.453	12.604	1234.000	12.533	47.606	27.206
Unprotected	2.016	89.536	57.978	17.213	35.174	853.666	9.133	36.196	17.846
Means	**2.555**	**32.163**	**20.201**	**6.240**	**22.569**	**380.333**	**3.400**	**11.410**	**9.360**
Yield Loss%	**55.906**	**26.429**	**25.839**	**26.606**	**64.167**	**30.821**	**27.128**	**23.968**	**34.404**
Df	2	29	29	14	14	2	14	2	2
t	4.556	11.770	7.965	6.748	8.527	13.865	4.841	5.896	5.159
Std. deviation	0.971	14.967	13.892	3.581	10.250	47.511	2.720	3.352	3.142
Std. Error	0.566	2.732	2.536	0.924	2.646	27.430	0.702	1.935	1.814
P value	0.045	0.000	0.000	0.000	0.000	0.005	0.000	0.028	0.036

5.5.4 Comparative losses due to rice leaf blast disease on the basis of rice yield and yield growth attributes on rice in protected against unprotected plots in Pemba 2016-18

During two years of the study 2016-2018 cropping seasons in Pemba Island grain yields harvested (ton per hector) on protected plots ws 4.872t /ha and 2.111 t/ha was harvested from unprotected plots. The two different means were highly significant ($p \leq 0.001$) provided a yield loss of 56.671%. Mean values of other tested variables recorded higher on protected plots compared to unprotected plots (Table 5.5).

Table 5.5: Comparative losses due to rice leaf blast disease on the basis of rice yield and yield growth attributes on rice in protected against unprotected plots in Pemba 2016-18

	Rice yield attributes								
Treatment	Grain yield (ton per ha)	Plant height (cm)	Tillering ability (%)	Panicle length (cm	Spikelet sterility (%)	No. of panicle /$6m^2$	No. of sec. branches	Harvest Index (%)	1000seed weight (g)
Protected	4.872	114.685	74.897	23.986	15.741	1123.500	14.300	49.041	24.883
Unprotected	2.111	93.503	59.225	17.946	35.519	905.666	8.800	32.181	20.716
Means	**2.761**	**21.181**	**15.672**	**6.040**	**19.778**	**217.883**	**5.500**	**16.860**	**4.166**
Yield Loss%	**56.671**	**18.469**	**20.925**	**25.181**	**55.683**	**28.290**	**38.462**	**34.379**	**16.746**
Df	5	59	59	29	29	5	29	5	5
t	13.978	10.200	6.992	6.883	13.526	2.569	13.094	11.279	6.738
Std. deviation	0.483	16.085	17.363	4.806	8.008	207.712	2.300	3.661	1.514
Std. Error	0.197	2.076	2.241	0.877	1.462	84.798	0.420	1.494	0.618
P value	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.001

5.5.5 Comparative losses due to rice leaf blast disease on the basis of rice yield and yield growth attributes on rice in protected against unprotected plots in Unguja 2016-18

The estimated grain yield loss of 70.670% was registerd in Unguja during 2016-2018 cropping seasons. The two different means between protected and unprotected plots was highly significant ($p \leq 0.001$). Mean values of other tested variables recorded higher on protected plots compared to unprotected plots including plant height, tillering ability, panicle length, spikelet sterility, number of panicles, number of secondary branches, harvest index and 1000 seed weight stand consistently showed higher means values (Table 5.6).

Table 5.6: Comparative losses due to rice leaf blast disease on the basis of rice yield and yield growth attributes on rice in protected against unprotected plots in Unguja 2016-18

Treatment	Grain yield (ton per ha)	Plant height (cm)	Tillering ability (%)	Panicle length (cm)	Spikelet sterility (%)	No. of panicle /6m^2	No. of sec. branches	Harvest Index (%)	1000seed weight (g)
Protected	4.705	113.125	79.694	24.426	13.690	1125.500	13.633	49.988	25.086
Unprotected	1.380	76.095	53.348	16.613	34.010	731.833	9.433	29.086	17.565
Means	**3.324**	**37.030**	**24.346**	**7.813**	**20.320**	**393.666**	**4.200**	**19.901**	**7.521**
Yield Loss %	**70.670**	**32.734**	**33.059**	**31.984**	**59.747**	**34.977**	**30.808**	**41.814**	**29.891**
Df	5	59	59	29	29	5	29	5	5
T	7.452	15.191	13.482	10.972	13.657	23.711	8.299	4.803	6.494
Std. deviation	1.092	18.882	13.987	3.900	8.149	40.667	2.771	10.150	2.837
Std. Error	0.446	2.437	1.805	0.712	1.487	16.602	0.506	4.143	1.158
P value	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.001

5.5.6 Percent disease severity over times for Pemba and Unguja islnd during 2016-2018 cropping seasons

Percentage disease severity in two islands of Pemba and Unguja during 2016- 2018 showed significant. There was a progressive increment of percent disease severity with time exceptional decline at 15 days followed by a steady increase there after. (Table 5.7).

Table 5.7: Percent disease severity over times for Pemba and Unguja islnd during 2016-2018 cropping seasons

	Percent disease severity in Pemba in 2016-18						Percent disease severity in Unguja in 2016-18					
Treatments	15 days	25 days	35 days	45 days	55 days	65 days	15 days	25 Days	35 days	45 days	55 days	65 days
Protected	0.000	0.000	5.214	6.517	6.517	6.517	0.000	0.000	2.607	3.910	5.214	5.214
Unprotected	13.160	14.762	20.015	21.870	22.625	23.118	13.085	15.933	20.542	22.658	23.998	24.095
Mean	**13.160**	**14.762**	**14.801**	**15.353**	**16.107**	**16.600**	**13.085**	**15.933**	**17.935**	**18.748**	**18.784**	**18.881**
df	5	5	5	5	5	5	5	5	5	5	5	5
t	5.000	5.000	8.378	10.312	15.683	10.597	4.028	4.980	9.992	10.801	10.709	11.451
Std deviation	6.447	7.232	4.327	3.647	2.515	3.836	7.614	7.837	4.427	4.116	4.283	4.038.
Std error	2.632	2.952	1.766	1.488	1.027	1.566	3.108	3.199	1.807	1.680	1.748	1.648
*p*value	0.004	0.004	0.000	0.000	0.000	0.000	0.008	0.004	0.000	0.000	0.000	0000

5.5.7 Percent disease incidence over times for Pemba and Unguja island during 2016-2018 cropping seasons

Percentage disease incidence in both islands (Pemba and Unguja) showed significant during the crop duration of 2016- 2018. The percentage disease incidence was significantly lower at all times in protected plots while on unprotected plots percentage disease incidence revealed higher. (Table 5.8).

5.8: Percent disease incidence over times for Pemba and Unguja islnd during 2016-2018 cropping seasons

	Percent disease incidence in Pemba in 2016-18						Percent disease incidence in Unguja in 2016-18					
Treatments	15 days	25 days	35 days	45 days	55 days	65 days	15 days	25 Days	35 days	45 days	55 days	65 days
Unprotected	25.821	40.786	52.837	66.364	74.647	81.421	28.887	41.298	54.583	67.169	75.623	83.290
Protected	0.000	0.000	2.219	4.297	5.548	5.608	0.000	0.000	2.613	5.094	6.222	6.222
Mean	**25.821**	**40.786**	**50.618**	**62.067**	**69.099**	**75.813**	**28.887**	**41.298**	**51.970**	**62.074**	**69.401**	**77.068**
df	5	5	5	5	5	5	5	5	5	5	5	5
t	4.882	14.078	13.347	16.039	20.447	28.177	14.951	31.063	20.876	20.232	18.840	29.633
Std deviation	12.956	7.096	9.289	9.479	8.277	6.590	4.732	3.256	6.097	7.515	9.023	6.428
Std error	5.289	2.897	3.792	3.869	3.379	2.690	1.932	1.329	2.489	3.068	3.683	2.624
*p*value	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

5.5.8 Correlation coefficients among studied variables for Pemba during the experimental period

Results on correlation analysis of variables are shown in Table 5.9. Significant positive correlations were observed between yield loss with plant height, panicle length, secondary branches, harvest index and yield; plant height with tiller ability, panicle length, 1000seed weight, secondary branches, harvest index and yield; tiller ability with panicle length, panicle number, 1000 seed weight, secondary branches, harvest index and yield; panicle length with panicle number, 1000 seed weight, secondary branches, harvest index and yield; spikelet sterility with incidence and severity; panicle number with 1000 seed weight and yield; 1000 seed weight secondary branches, harvest index and yield; secondary branches with harvest index and yield; incidence with severity. On the other hand, significant negative correlations were observed between yield loss with spikelet sterility,incidence and severity; plant height with spikelet sterility, incidence and severity; tiller ability with spikelet sterility, incidence and severity; panicle length with spikelet sterility, incidence and severity; spikelet sterility with panicle number, 1000 seed weight, secondary branches, harvest index and yield; panicle number with incidence and severity; 1000 seed weight with incidence and severity; incidence with harvest index and yield; severity with harvest index with yied and harvest index with yield (Table 5.9). Multiple Correlation Coefficients of yield and yield components for Pemba during the experimental period.

Table 5.9: Correlation coefficients among studied variables for Pemba during the experimental period

	Yield loss (%)	Plant Height (cm)	Tiller Ability (%)	Panicle Length (cm)	Spikelet Sterility (%)	Panicle Number (no)	1000 Seedwt (g)	Seconday Branch (no)	Incidence (%)	Severity (%)	Harvest Index (%)	yield (kg)
Yield loss%	1											
Plant Height (cm	.872*	1										
Tillerability(%)	.673	.646*	1									
Panicle length(cm)	.911*	.644*	.820**	1								
Spikelet sterility%	-.879*	-.674*	-.876**	-.852**	1							
Paniclenumber(no)	.425	.327	.704*	.613*	-.667*	1						
1000seedwt(g)	.524	.619*	.799**	.736**	-.803**	.897**	1					
Secondarybranch(no)	.834*	.946**	.730**	.755**	-.809**	.482	.722**	1				
Incidence(%)	-.732	-.834**	-.793**	-.756**	.833**	-.758**	-.927**	-.887**	1			
Severity(%)	-.811	-.796**	-.855**	-.856**	.946**	-.734**	-.892**	-.916**	.948**	1		
HarvestIndex(%)	.862*	.728**	.877**	.931**	-.960**	.734**	.874**	.856**	-.906**	-.978**	1	
Yield (kg)	.820$^{*\,b}$	.704*	.903**	.907**	-.963**	.763**	.891**	.838**	-.909**	-.979**	-.979**	1

**. Correlation is significant at the 0.01 level (2-tailed)

*. Correlation is significant at the 0.05 level (2-tailed)

5.5.9 Correlation coefficients among studied variables for Unguja during the experimental period

Results on correlation analysis are shown in Table 10. Significant positive correlations were observed between plant height with tiller ability, panicle length, panicle number, 1000 seed weight, secondary branches, harvest index and yield; tiller ability with panicle length, panicle number, 1000 seed weight, secondary branches, harvest index and yield; panicle length with panicle number, 1000 seed weight, secondary branches, harvest index and yield; spikelet sterility with severity and severity; panicle number with 1000 seed weight, harvest index and yield; 1000 seed weight with harvest index and yield; secondary branches with harvest index and yield; incidence with severity and harvest index with yield. Signicant negative were observed between yield loss with spikelet sterility, incidence and severity; plant height with spikelet sterility, incidence and severity; tiller ability with spikelet sterility, incidence and severity; panicle length with spikelet sterility, incidence and severity; spikelet sterility with panicle number, 1000 seed weight, secondary branches, harvest index and yield; panicle number with 1000 seed weight, incidence and severity; 1000 seed weight with incidence and severity; secondary branch with incidence and severity; incidence with harvest index and yield; severity with harvest index and yield (Table 5.10).

Table 5.10: Correlation coefficients among studied variables for Unguja during the experimental period

	Yield loss %	Plant Height (cm)	Tiller Ability (%)	Panicle Length (cm)	Spikelet sterility%	Panicle Number (no)	1000 Seedwt (g)	Seconday Branch (no)	Incidence (%)	Severity (%)	Harvest Index (%)	yield (kg)
Yield loss%	1											
Plant Height (cm	.576	1										
Tiller ability (%)	.753	.886**	1									
Panicle length(cm)	.687	.807**	.935**	1								
Spikeletsterility%	-.738	-.765**	-.899**	-.911**	1							
Panicle number (no)	.403	.931**	.778**	.679*	-.645*	1						
1000 seedwt(g)	.643	.866**	.681*	.647*	-.601*	.796**	1					
Secondaybranch(no)	.362	.615*	.812**	.834**	-.727**	.516	.351	1				
Incidence (%)	-.684	-.853**	-.970**	-.959**	.962**	-.725**	-.659*	-.838**	1			
Severity (%)	-.607	-.902**	-.956**	-.956**	.913**	-.773**	-.725**	-.835**	.982**	1		
Harvest Index (%)	.572	.902**	.936**	.897**	-.806**	.820**	.738**	.764**	-.893**	-.915**	1	
Yield (kg)	.580	.876**	.938**	.929**	-.855**	.770**	.768**	.797**	-.928**	-.940**	.969**	1

**. Correlation is significant at the 0.01 level (2-tailed)
*. Correlation is significant at the 0.05 level (2-tailed)

5.6 Discussion

Fungicide has been widely used to control a number of pathogen causing disease on rice. *Pyricularia oryzae* have been reported to cause blast disease on rice with estimated losses up to 100%. In this study efficacy of Megasin – M70% WP fungicide in controlling rice blast disease for rice yield loss assessment were investigated along with its effects under field condition. Its application effect not only controlled the disease but also resulted in improving higher yield that was harvested from protected plots as compared to unprotected plot. Similar, results were reported by (Sandeep (2016) and Qudsia *et al.* (2017) that fungicides show effective management of the disease over untreated check.

In accordance to yield losses, during 2016-2017 cropping season in Pemba, percentage yield loss deferred with the percentage yield loss registered in 2017-2018. In Unguja, 2016-2017 and 2017-2018 there were significant difference in percentage yield losses. The results imply that for both islands Unguja and Pemba in the first seasons 2016 – 2017 rice yield loss was higher due to the environment conditions favoured to disease while in the second seasons 2017-2018 yield loss was low, this observation was associated with the environmenal conditions less favoured to disease. Futher more in comparison of percentage rice yield loss registered in Pemba from 2016-2018 was low and Unguja from 2016 to 2018 registered higher. The results were may be associated with several factors including inoculum pressure, weather conditions, efficacy of the fungicide applied etc. as reported by Wasihun and Flagote (2016), Udhayakumar e*t al.* (2019) that higher yield loss is depending on cultivar susceptibility, environmental conditions and management system, which causes yield losses up to 100%. On the other hand, a set of protected plots revealed that yield attributes such as plant height (cm), tillering ability (%), panicle length (cm), panicles (no.) 1000 seed weight (g) secondary branches (no.) harvest index (%) and yield (kg) did had higher mean values on protected plots compared to a set of unprotected plots.

Contrary rice leaf blast disease incidence and severity their mean values had declined on protected plots while on unprotected was significantly higher as it was reported by Sandeep (2016) and Ganesh *et al.* (2012) that fungicide proved to be affective in the management of rice blast disease.

Variations in percentage disease index values, in protected plots over the unprotected infection from protected plots started at 35 day from planting date and continue gradually but with little rate up to crop maturity. In unprotected plots, rice blast disease symptoms started at 15 days after planting and continued with higher rate. The probable reason is the efficient of applied Fungicide in control disease as a protective measure. Likewise, disease incidence in protected plots disease symptoms was realized at 35 days after planting while in unprotected plots disease symptoms appeared at 15 days after planting and continue with higher rate. Similar findings were reported by Dibyendu *et al.* (2017).

In context of correlation coefficient in Pemba island observed that plant height was positive correlated with yield loss ($r = 0.872$). This indicated that the taller genotypes possessed a high probability of reducing rice yield loss from abiotic factors such as flood and other small vermins compared to dwarf genotypes. Panicle length, number of secondary branches, harvest index and yield showed significant positive relationship with yield loss at .911, .834, .862 and .820 respectively. Long panicle with fully filled spikelet increased yield and reduce yield loss.

On the other hand, high number of secondary branches of the rice panicles influence higher yield, but little number of secondary branches on the panicle contribute yield loss due to pathogen infection or other genotypic effect. Low harvest index may caused by yield loss due to several biotic and abiotic factors while higher harvest index may imply less yield loss. In addition, Unguja island all tested yield attributes were positive and negative significant correlated except for yield loss which its values showed moderate correlated but not significant.

5.7 Conclussion

The study revealed that comparative assessment for efficacy of fungicide Megasin – M70% WP helped us to understand the extent of rice yield losses from fully protected plots and unprotected plots in field conditions. Mean values of tested variables were registered higher compared with the means values registered from unprotected plots. During first cropping season 2016-2017 in Pemba island rice yield loss was registered 63.789 % and in the second cropping season 2017-2018 was 49.548%. Unguja in 2016-2017 yield loss was 84.621% and in the second cropping season 2017-2018 was 55.906%. In addition, in two cropping seasons from 2016 to 2018, Pemba Island registered 56.671% and for Unguja registered 70.670%. Disease incidence and severity values observed higher in protected plots and in unprotected plots registered lower. Fungicide is an effective measure to manage rice leaf blast disease, therefore, fungicide can be used as an alternative to resistant cultivar to control the rice blast disease in the field.

5.8 Recommendations

i. Applications of fungicide give protection from blast infection which gives low disease intensity.
ii. Rice seeds should be treated with fungicide or hot water before sowing to destroy rice blast pathogen life cycle.
iii. Rice seedlings must be spreyed with suitable fungicide soon after first disease symptoms appeared.

References

Akanke, I.B., Abalo, I.K., Hafiz, A.S., Habib, R., Moussa, S., Adolphe, A. and Clement, A. (2018). Screening of Rice Accessions Resistant to Blast in Benin. *Journal of Diseases and Medicinal Plants* 4 (3): 69-79.

Ashkani, S., Rafii, M.Y., Shabanimofrad, M., Miah, G.G., Sahebi, M. and Azizi, P. (2015). Molecular Breeding Strategy and Challenges towards Improvement of Blast Disease Resistance in Rice Crop. Front. *Plant Science* 6(1): 1-14.

Chakraborty, S., Hazarika, M.K., Khobragade, C.B., Paul, S.K. and Bora, J. (2017). Study on in-bed drying system for the drying of paddy in Assam. *International Food Research Journal* 24(3): 1345-1348.

Chuwa, C.J. (2016). Rice blast disease caused by *Pyricularia oryzae*: Epidemiology, characterization and yield loss in major rice growing areas of Tanzania. A thesis submitted in fulfilment of the requirements for the degree of doctor of philosophy in crop science Sokoine University of Agriculture. Morogoro, Tanzania. pp. 1-114.

Dibyendu, M., Anannya, G., Debashis, R., Adyant, K., Diana, S., Soumen, B., Ratikanta, G., Pintoo, B. and Anurup, M. (2017). Yield loss assessment of rice (O*ryza Sativa* L.) due to different biotic stresses under system of rice intensification (SRI). *Journal of Entomology and Zoology Studies* 5(4): 1974-1980.

Gaikwad, A.P. and Balgude, Y.S. (2016). Metominostrobin, a Novel Strobilurin Fungicide for Managing Rice Blast. *Journal of Rice Research* 9(1): 50-52.

Ganesh, N.R.., Gangadhara, N.B., Basavaraja N.T. and Krishna. N.R. (2012). Fungicidal management of leaf blast disease in rice. *Global Journal of Bio-science and Biotechnology* 1(1): 18-21.

Ghimire, P., Gopal, K.C., Shrestha, S.M. and Parajuli, G. (2017). Evaluation of Fungicides and Bio-agent against Neck Blast Disease of Rice. *Journal of Plant Pathology and Microbiology* 8(12): 1-2.

Hossain, M.A., Awal, M.A., Ali, M.R. and Alam, M.M. (2016). Use of moisture meter on the post-harvest loss reduction of rice. *Progressive Agriculture* 27 (4): 511-516.

IRRI (2002). Standard Evaluation System for Rice. International Rice Research Institute Philippines. pp. 54.

Katsantonis, D., Kadoglidou, K., Dramalis, C. and Puigdollers, P. (2017). Rice Blast Forecasting Models and their Practical Value: A Review. *Phytopathologia Mediterranea* 56 (2): 187–216.

Khatib, B. H. (2010). Perfomance of new released upland nerica rice varieties under different combined rates of nitrogen and phosphorus fertilizers in Zanzibar. A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science in crop science of Sokoine University of Agriculture. Morogoro, Tanzania.

Khatib, J.K. and Makame, S.M. (2009). Evaluation of rice mutant lines for improving small holder crop productivity in Zanzibar. Paper presented in Ist Annual Agriculture Review Workshop, Zanzibar, 30-31 July, 2009.

Octaviano, I.Y., Kris, A., Sofie, L., Alexandre, D., Wouter, V., Drissa, S., Patrick, V.D. and Geert, H. (2018). Combining High Yields and Blast Resistance in Rice (*Oryza* spp): A Screening under Upland and Lowland Conditions in Benin. *Sustainability* 10 (2500): 1-16.

Pramesh, D., Maruti, K., Muniraju, M., Mallikarjun, K., Guruprasad, G.S., Mahantashivayogayya, K., Reddy, B.G.M., Gowdar, S.B. and Chethana, B.S. (2016). Bio-efficacy of a Combination Fungicide against Blast and Sheath Blight Diseases of Paddy. *Journal of Experimental Agriculture International* 14(4): 1-8.

Pramesh, D., Saddamhusen, A., Muniraju, K.M. and Kirana, M.K. (2017). A Combination Fungicide for the Management of Sheath Blight, Sheath Rot and Stem Rot Diseases of Paddy. *International Journal of Current Microbiology and Applied Sciences* 6 (9): 3500-3509.

Prem, B.M., Basistha, A. and Bishnu, P. (2015). Use of chemical fungicides for the management of rice blast (*Pyricularia grisea*) disease at Jyotinagar, Chitwan, Nepal. *International Journal of Applied Science and Biotechnology* 3(3): 474-478.

Qudsia, H., Akhter, M., Riaz, A., Haider, Z. and Mahmood, A. (2017). Comparative Efficacy of Different Chemical Treatments for Paddy Blast, Brown Leaf

Spot and Bacterial Leaf Blight Diseases in Rice (*Oryza Sativa* L.). Application of Microbiol Open Access 3: 138.

Raboin, L.M., Elsa, B., Didier, T., Alain R., Julien, F. Brigitte, C. and Nourollah, A. (2016). Association mapping of resistance to rice blast in upland field conditions. *Rice* 9(59): 1-12.

Rijal, T.R., Govinda, B.H., Purushhotam, J. and Keshab, B. K. (2017). Identification of Resistant Genotypes on Rice against Blast Disease under Field Condition at Rampur, Chitwan. *International Journal of Applied Science Biotechnology.* 5(4): 505-510.

Sandeep, P. (2016). Effect of fungicides on leaf blast and grain yield of rice in Kymore region of Madhya pradesh in India. *Bangladesh Journal of Botany* 45(2): 353-359.

Santosh, S., Sunil, Z., Huma, N., Pushpendra, S. S. and Hadi, H. K. (2018). Efficacy of certain botanicals in the management of blast of paddy (*Oryza sativa* L.) caused by *Pyricularia oryzae.* J*ournal of Pharmacognosy and Phytochemistry* 7(1): 2014-2016.

Sekiya, N., Khatib, J. Kh., Subira, M. M., Motonori, T., Nobuaki, O. and Hideki A. (2013). Performance of a Number of NERICA Cultivars in Zanzibar, Tanzania: Yield, Yield Components and Grain Quality. *Plant Production Science* 16(2): 141 – 153.

Sester, M., Raveloson, H., Tharreau, D. and Dusserre, J. (2014). Conservation Agriculture Cropping System to Limit Blast Disease in Upland Rainfed Rice. *Plant Pathology* (63): 373–381.

Surender, K., Abhilasha, A. L., Narender, K., Shweta, J., Harish, K., Amit, K. and Mohit, K. (2017). Effect of bio control agents and botanicals against Blast of Paddy caused by *Pyricularia oryzae. International Journal of Chemical Studies* 5(1): 314-318.

Takashi, K., Masashi, S., Eiji, K., Kiyoshi, I., Shu, H. and Yoichi, T. (2018). Assessment of rice panicle blast disease using airborne hyperspectral imagery. *The open Agriculture Journal* (10): 28-34.

Teshome, E. and Tegegn, A. (2017). Comparative Study of Powdery Mildew (*Erysiphe polygoni*) Disease Severity and Its Effect on Yield and Yield Components of Field Pea (*Pisum sativum* L.) in the Southeastern Oromia, Ethiopia. *Journal of Plant Pathology and Microbiology* 8(5): 1-5.

Uda, M.N.A., Harzana, S.N., Shamiera, S.N., Nur, H. I., Maisara, A. M., Akhir, M.K.R. H., Salimi, M.N., Nuradibah, M.A., Uda, H. and Subash, C.B.G. (2018). Antimicrobial Activity of Plant Extracts from Aloe Vera, Citrus Hystrix, Sabah Snake Grass and Zingiber Officinale against *Pyricularia Oryzae* that causes Rice Blast Disease in Paddy Plants. IOP Conference Series: *Materials Science and Engineering* 318(2018): 1-10.

Udhayakumar, R., Muthukumar, A. and Kanagarajan, R. (2019). Evaluation of filia 52.5 (Tricyclazole 34.2% + propiconazole 10.7%) against rice blast disease. *Journal of Pharmacognosy and Phytochemistry* 8(3): 396-399.

Vinayak, T., Mahendra, R., Ramachandra, A., Krupa, K.N., Ningaraj, D., Deepak, C.A. and Harini, K.K.M. (2018). Screening of traditional rice varieties (TRVs) for blast resistance. *Journal of Pharmacognosy and Phytochemistry* 7(1): 1384-1388.

Wasihun, Y.W. and Flagote, A.B. (2016). Assessment of diseases on rice (*Oriza sativa* L.) in major growing fields of Pawe district, Northwestern Ethiopia. *World Scientific News* 42 (2016): 13-23.

Wasimfiroz, M., Hosagoudar, G.N., Narayanaswamy, H. and Balanagouda, P. (2018). Management of leaf blast and neck blast of rice caused by *Pyricularia oryzae* under field condition. *Journal of Pharmacognosy and Phytochemistry* 7(2): 968- 971.

Yashaswini, C., Narayan, R.P., Pushpavati, B., Srinivasa, R. and Seshu, M.M. (2017). Prevalence of Rice blast (*Magnaporthe oryzae*) incidence in South India. *Bulletin of Environment, Pharmacology and Life Sciences* 6(1): 370-373.

CHAPTER SIX

6.0 GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 General Conclusion

Rice leaf blast disease caused by fungus *Pyricularia oryzae,* is the most important diseases in Zanzibar that causing great yield losses every rice cropping season. The disease is a major production constraint in all rice agro-ecologies including rainfed low land, rainfed up land and irrigated. The present study investigated that, in Pemba island, disease incidence, severity and prevalence were significantly lower than in Unguja. This was associated by majority of farmers practices cultural practices and culturalin combination with fungicides in desease management. In the six districts surveyed, significant disease variation occurred on disease incidence, severity, cultural and cultural in combination with fungicide whereby chake chake district had the lowest and North A district had highest. Furthermore, disease Maximum incidence and severity were exhibited in rainfed upland ecology and lowest in irrigated agro-ecology.

During pathogen isolation and results of molecular characterization, six strains were identified viz: ZNZ/2017/CENTRAL, ZNZ/2017/CHK_2, ZNZ/2017/CHK_1, ZNZ/2017/NO RTH_2, ZNZ/2017/MICH and ZNZ/2017/NORTH_ A8. However, pylogenetic analysis revealed that identified strains found genetically homogeneous due to the molecular analysis showed no differences in banding patterns between strains. Strains were close related to the trains of the republic of Korea and Japan. In morphological characterization, signified that combination of media type and number of days incubated, resulting fast radial growth rate of *Pyricularia oryzae* whereby Oat meal agar (OMA) is considered as a superior medium for growing rice blast pathogen. From 19 rice blast differential genotypes, one local check and ten traditional varieties evaluated in the screen house condition for pathosystem. In ANOVA results showed that there were significantly P < 0.001 difference between all studied variables. On rice blast differential genotypes tested only 13 genotypes their genes were found incompatible (R) to the inoculated *Pyricularia oryzae*, viz; IRBL11-Zh *(Pi11(t),* IRBL12-M *(Pi12(t)),* IRBLa-C *(Pia),* IRBLb-IT13[CO] *(Pib),* IRBLkh-K3 *(Pik-h),* IRBLkp-K60 *(Pik-p),* IRBLkp-K60[CO] *(Pik-p),* IRBLta-Me[CO] *(Pita),* IRBLta2-Pi *(vbfchfdj),* IRBLta2-Re[CO] *(Pita-2),* IRBLz5-Ca *(Piz5),* IRBLzt-IR56[CO] *(Piz-t)* and Moroberekan *(Pi5(t), Pi7)* 4 were moderate resistant (MR) viz; IRBL1-CL *(Pi1),* IRBL20-IR24 *(Pi20),* IRBL7-M [CO] *(Pi7(t),* and IRBLZ-FU *(Piz)* and three were susceptible (S) viz; BKN Supa (local control), LTH and CO39. Highest total disease severity was registered 95.06% for CO39 followed by It was found LTH (97.19) and (78.3%) for BKN Supa used as control and the lowest was recorded for Moroberekan (8.63. For traditional varieties none of them were found resistant but the lowest total disease severity was registered for TOX (36.39%) and highest was for Kijino (77.76). Lowest total area under development progress curve for deferential genotypes was recorded for Moroberekan (271.5) and highest was for CO39 (1328.3). Thus, for traditional varieties Kibeu was the highest 1080.3 and Alibadru (438.4) was the lowest. There was no traditional variety identified resistant to the disease.

Assessment of rice grain yield loss in Zanzibar using fungicide Megasin – M70% WP to control disease, results deferred from fully protected plots and unprotected plots during experimental durations. Mean values of tested variables were registered higher compared with the means values registered from unprotected plots. During first cropping season 2016-2017 in Pemba island rice yield loss was registered 63.789 % and in the second cropping season 2017-2018 was 49.548%. Unguja in 2016-2017 yield loss was 84.621% and in the second cropping season 2017-2018 was 55.906%. In addition, in two cropping seasons from 2016 to 2018, Pemba Island registered 56.671% and for Unguja registered 70.670%. Disease incidence and severity values observed higher in fully protected plots than in unprotected plots. Fungicide is an effective measure to manage rice leaf blast disease, so it can be used as an alternative to resistant cultivar to control the rice blast disease in the field.

6.2 Recommendations

i. Farmers should be encouraged to adopt cultural practices and combination with fungicides for reduction of rice leaf blast infection rate particularly under irrigated ecologies of Zanzibar.

ii. Rice farmers in Zanzibar island should encouraged to participate more in rice irrigation farming. It is more efficient, economically production and efficient in their resource allocation than those in rain fed production.

iii. Recommended use of molecular techniques is more accurate method and widely adopted to determine the genetic characteristic of organism; such as sequencing of different regions of DNA which is considered as alternatives to morphological methods of identification of *Pyricularia oryze.*

iv. In the laboratory, when culturing rice blast pathogen (*Pyricularia oryzae*) using Oat meal agar medium (OMA) is recommended as the suitable medium for fast growing and for high spore production of blast pathogen.

v. Due to significant variations of pathotype reactions, genotypes hold genes viz: *(Pi11(t), (Pi12(t)), (Pia), (Pib), (Pik-h), (Pik-p), (Pik-p), (Pita), (vbfchfdj), (Pita-*

2), (Piz5), (Piz-t) and (Pi5(t), Pi7) were confirmed the most effective resistance genes that breeders can utilize for improving blast resistance in Zanzibar to achieve longer lasting resistant genotypes.

vi. Tox as a traditional cultivated rice variety in Zanzibar; it could be introgressed with exotic genes to improve resistant, meanwhile maintaining its characteristic of high panicle yield.

vii. Applications of fungicide give protection from blast infection which gives low disease intensity, hence rice seeds should be treated with fungicide or hot water before sowing to destroy rice blast pathogen life cycle. However, rice seedlings should be sprayed with suitable fungicide soon after first disease symptoms appeared.

APPENDICES

Appendix 1: SOCIAL GUESTIONS

Rice farmer information during………………………. cropping season

1. Name of the island**...**

2. Name of the district ………………………………………………………………

3. Location name………………………………………………………………..........

4. Rice agro-ecology……………………………………………………………………

6. Which rice variety do you grow…………………………………………….............

6. Do you know rice blast disease? (See photo)

(a) Yes

(b)No

7. What is the local name of this disease at your home place?..

8. Have you been observed symptoms of rice leaf blast in your farm?

(a) Yes

(b) No

9. Do you think this disease is a problem in your farm in relation to yield?

(a)Yes

(b) NO

10. What control measures you take to eradicate the disease?

(a) Application of cultural practice (mentioned) ☐

(b) Application of chemical fungicide only ☐

(c) Application of both; cultural practice combined with chemical fungicide ☐

Appendix 2: ANOVA summary for the variables (Mean squares)

Source of variation	Degree of freedom	Disease incidence (%)	Disease severity (0-9)	Degree of freedom	Disease prevalence (%)	Disease awareness (%)	Cultural Control (%)	Cultural &chemic control (%)
Island	1	67957.5***	42.25***	1	0.91**	1320.84***	818.91**	778.5*
District (Island)	4	6512.1***	43.82***	4	0.15^{ns}	100.88^{ns}	365.79**	349.4*
Ecology	2	25897.6***	398.47***	2	0.11$^{\mathbf{ns}}$	321.45**	1347.90***	1524.4***
Year	1	23193.3***	9.50^{ns}	1	0.02^{ns}	50.69^{ns}	190.16^{ns}	176.0^{ns}
EXI	2	1823.6**	4.13^{ns}	2	0.11^{ns}	116.32^{ns}	59.91^{ns}	60.5^{ns}
YXI	1	46307.1***	1.36^{ns}	1	0.02^{ns}	257.82^{ns}	819.30**	955.3**
EXY	2	2421.6**	29.34***	2	0.34^{ns}	71.76^{ns}	119.45^{ns}	112.2^{ns}
IXEXY	2	1643.7*	1643.70^{ns}	2	0.34^{ns}	11.55^{ns}	173.95^{ns}	116.2^{ns}
Error	560	405.4	401.0	20	0.16^{ns}	73.38	99.11	102.7
Total	575			35				

* Significant at $P \leq 0.05$
* * Significant at $P \leq 0.01$
***Significant at $P \leq 0.001$
Ns not significant at $P \geq 0.05$

Appendix 3: ANOVA Summary for the studied variables (Mean squares given)

Source of variation	df	Growth of mycelium (ms)
Strain	5	0.3506
Medium	2	13.0467***
Days	2	251.0469***
Strain. Day	10	0.1418^{ns}
Strain. Medium	10	0.2321^{ns}
Medium. Day	4	1.0191***
Strain. Medium. Day	20	0.1754^{ns}
Residual	108	0.1774^{ns}
Total	161	

*** Significant at P≤ 0.001, Ns not significant at P ≥ 0.05

Appendix 4: ANOVA Summary for differential genotypes for the studied variables

Source of variances	Df	1000seed Wt (g)	Spikelet Sterility (%)	Tiller Ability (%)	Plant Height (cm)	No.sec. branch	Panicle Length (cm)	Panicle yield (g)	Severity (%)
Genotype	19	39.050***	98.94***	244.1	1348.3***	17.067***	5.0975***	2254.39***	12.291^{xxx}
Residual	40	0.459	32.19	205.60	139.70	2.783	0.917	12.291	18.21
Total	59								

Appendix 5: ANOVA Summary for differential genotypes

Source of variences	df	1000 seed Wt (g)	Spikelet sterility%	Tiller Ability (%)	Plant Height (cm)	No. sec. branch	Panicle cm	Panicle Length yield (g)	Severity (%)
Traditional Varieties	9	5.292	27.75	210.80	1114.32***	7.002*	14.294***	1.689^{xxx}	712.2^{*xx}
Residual	20	2.751	18.26	131.0	12.37	27.14	1.734	0.6377	191.60
Total	29								

Appendix 6: Disease incidence collection data sheet during survey

FN	1																															
QN	**1**			**2**			**3**			**4**			**5**			**6**			**7**			**8**			**9**			**10**				
VR	TP	IP	%	TP	IP	%	TP	IP	%	TP	IP	%	TP	IP	%	TP	IP	%	TP	IP	%	TP	IP	%	TP	IP	%	TP	IP	%		
DV																																

TP = Total number of plants in the quadrant, IP = Number of infected plants in the quadrant, % = Percentage of disease incidence, FN = Field number, QN.= Quadrant number, VR = Variables, DV=Disease value

Appendix 7: Disease severity collection data sheet from the field

FIELD NUMBER	**1**												
QUADRANT NO.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**TOTAL SCORE**	**AVE. SCORES**	**SEVER.%**
DISEASE SCORE													