EFFECTS OF RICE YELLOW MOTTLE VIRUS DISEASE ON PERFORMANCE OF RICE GENOTYPES

KENNEDY ELISHA, JOMANGA

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

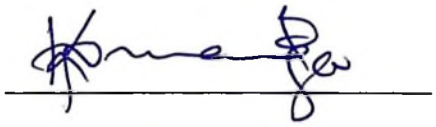
2014

ABSTRACT

Screen house experiments were conducted at Sokoine University of Agriculture during the season of 2011/12, to investigate the reaction and effect of Rice Yellow Mottle Virus (RYMV) disease on rice genotypes. Virulence among the three RYMV strains S4, 5 and 6 were tested. Disease reaction was observed on 1-9 scale. Results of pathogenicity test indicated that strain S5 was the most virulent strain followed by S6 and S4. Kalalu (resistant variety) was similarly affected by S5 as susceptible varieties. In experiment two and three, 52 rice genotypes were inoculated with RYMV strains S4 and S5, in order to identify rice genotypes resistant to RYMV disease. Results based on symptoms and grain weight indicated that eleven (11) rice genotypes were resistant/tolerance to RYMV disease. Grain weight reduction ranged from 1.01 to 43.69 %, while disease severity score of 1 to 5 were observed. These eleven rice genotypes were IR 73886-9-2-4-1-1, IR 71605-2-1-5-3-4, IR 69705-1-1-1-4-2, IR 71028-3-1-2-5-1, IR 71029-3-1-5-5, Matata G1, IRR 134, Matata G2, IR 69704-4-4-4-8-1-1-1, Adday Sel and IR 71027-43-3-2-B-3. Thirty seven (37) rice genotypes were susceptible, including SARO-5 and Supa (susceptible controls). The decrease in plant height and grain weight due to RYMV disease ranged from 12.01 % to 63.66 % and 48 % to 86 % respectively. The effect of RYMV disease on number of tillers per plant, showed both increase and decrease in tillers produced among inoculated genotypes. Therefore, future field research should focus on testing identified resistant rice genotypes against RYMV strain S5.

DECLARATION

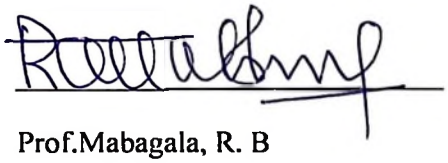
I, **KENNEDY ELISHA JOMANGA** do declare to the Senate of Sokoine University of Agriculture that, the work presented is my original work and has not been or concurrently submitted for higher degree or award in any other university.



22/9/2014

Kennedy Elisha Jomanga — **Date**

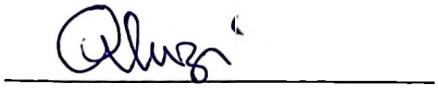
(MSc. Crop Science Candidate)



22/09/2014

Prof.Mabagala, R. B — **Date**

(Supervisor I)



22/09/2014

Dr. Ashura-Luzi, Kihupi — **Date**

(Supervisor II)

COPYRIGHT

ACKNOWLEDGEMENTS

I am greatly thankful to the ALMIGHTY GOD who gave me the mental and physical strength to start and end this research in good health, peace and harmony. I wish to express my sincere gratitude to the Belgium Technical Corperation for offering me scholarship to pursue MSc degree in Crop Science (Crop protection).

Special acknowledgment goes to Prof. Robert B. Mabagala and Dr. Ashura Luzi-Kihupi, for their advice, support, comments and encouragements.

I owe many thanks to Prof. Rweyemamu, the Head of department, Department of Crop Science, and all academic staffs in the department for their support, critics and ecouragement during the course of my work, which enabled this work to be completed.

I wish to express my profound gratitude to Dr. Mpunami from ARI-Mikocheni for moral and material support during the research work. The Africa Rice Center office at SUA facilitated by Dr. Ashura Luzi-Kihupi., Dr. Negusse, Z. and Mr. Ndomondo for providing me different materials including rice genotypes and Rice Yellow Mottle Virus inocula.

Lastly I owe many thanks to Mr. Sylvester, Bonge and Juma for their endless and tireless support throughout the research period.

DEDICATION

To my late Grandfather Festus Cheyo Jomanga, may the **Lord** rest his soul in peace. To my lovely family members for their support during the preparation of this dissertation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

+SSRNA	Positive sense single strains RNA virus
CV (%)	Coefficient of variation
D'se	Disease
Df	Degree of freedom
DMRT	Duncan Multiple Range Test
dsDNA	Double strand Deoxyribo Nucleic Acid
FAO	Food and Agriculture Organization
Fpr	Fisher probability
M.S	Mean squares
ORF(1-4)	RYMV RNA open reading frame
PBS	Phosphate buffer solution
RYMV	Rice yellow mottle virus
Rymv 1-2	Gene responsible resistance in rice against RYMV-disease
S.S	Sum of squares
S4	RYMV strain four
S5	RYMV strain five
S6	RYMV strain 6
SED	Standard error deviation
$V_{(1---n)}S_0R_{(1-3)}$	Rice genotype (V_{1-n}) uninoculated (S_0) in n^{th} replicate (R1-3)
$V_{(1---n)}S_1R_{(1-3)}$	Rice genotype (V_{1-n}) inoculated (S_0) in n^{th} replicate (R1-3)
V/W	Volume by weight
V0	Control (uninoculated)

Vl	Inoculated
Var.	Varieties
Vpg	Viral genetical protein
VR	Variation factor

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Rice is the staple food for more than half of the world's population, influencing the livelihoods and economies of several billion people (UNDP, 2010). Rice has great potential and plays a critical role in contributing to food and nutritional security, income generation, poverty alleviation and socio-economic growth in Africa (FAO, 2004). It is an important food crop in many African countries and is increasingly preferred over many traditional foods (FAO, 2004). Rice is the second most important cereal crop widely used and consumed in Tanzania after maize (Kadigi, 2003; MAFSC, 2009). The majority of rice farmers depend on rice both for food and income generation, accounting for 60 % of dietary composition of most families in Tanzania (Kanyeka *et al.*, 2007). It provides 21 % of global human per capita energy and 15 % of per capita protein (CABI, 2007).

Africa consumes 11.5 million tonnes of rice per year, 33.6 percent of which is imported (Oteng *et al.*, 2003). The regional contribution by East Africa to rice production has declined by 23.8 % due to disease and other factors like soil fertility, genetic factors and climatic factor, however there is remarkable increase in rice total area cultivated every year (Oteng *et al.*, 2003).

The self sufficiency ratio in rice in Sub-Sahara Africa has decline steadily from 112 % in 1961 to 61 % in 2006, implying the widening of the gap between rice

production and consumption (WARDA, 2007). Population in Tanzania is currently growing at a rapid rate of 2.8 %, the self sufficiency ratio for food is 81 % where as the recommended ratio for Tanzania according to FAO is 120 % (MAFSC, 2009). This results in a continuous increase in demand for food specifically rice and therefore, the need to increase production (Kanyeka *et al.*, 2007). Currently the average yield in Tanzania is estimated at 1.79 tons per hectare which is equivalent to 1.2 tons per ha milled rice, however this is low compared to yields from other countries like Korea, Egypt and Japan, where yields of up to 10 tonnes/ha have been reported (MAFSC, 2009). Rice yield in Tanzania is also below the average rice grain yield in Africa which is 2.1 tonnes/ha, and similarly below the world average rice grain yield of 3.4 tons/ha (MAFSC, 2009).

The major rice production systems in Tanzania are lowland rain-fed, irrigated, and upland systems (MAFSC, 2009). The most predominant rice ecosystem in Tanzania is the rain-fed lowland ecosystem. Most of the small holder farmers grow rice in the Valley of Rufiji River and its three major tributaries Kilombero, Great Ruaha and Luvugu River (MAFSC, 2009). Other valleys where rice is grown include Pangani, Wami, Ruvu and Ruvuma River. Rice is also grown on the flat plains on the shores of Lake Malawi, Lake Victoria, Lake Tanganyika, swamp land in Singida, Dodoma (Bahi swamp), Shinyanga and Mwanza. Upland ecosystem rice is grown on naturally free drained soils, this type of culture is found in such places as eastern slopes of Uruguru and Usambara mountains and southern highlands and northern areas bordering the Rufiji basin. Irrigated lowland rice ecosystem where rice is also grown includes Mbarali, Ruvu and Dakawa. Rice prefers fertile land with good water

retention capacity, characterised by clay loamy soil and heavy soils of valleys are most desirable for rice growing (MAFSC, 2009).

Bucheyeki *et al.* (2011) identified rice production constraints as due to lack of improved varieties, diseases susceptibility, seeds unavailability, drought and high input prices. Luzi-Kihupi *et al.* (2009) reported that one of the reasons for low yield of rice is the occurrence of Rice Yellow Mottle Virus. Kalinga *et al.* (2003) reported that rice yields obtained by many farmers are often only 25 – 30 % of the potential yield of 6 tons and above (MAFSC, 2009). This is largely due to diseases and other factors like soil, rice varieties and climate. Rice Yellow Mottle Virus is a major threat to rice production areas in Tanzania (Kanyeka *et al.*, 2007). Rice yield reduction ranging from 58 to 68 % caused by RYMV was reported in 1986 in the Republic of Niger (Kouassi *et al.*, 2005). Yield losses of 84-97 % were reported in Ibadan, Nigeria (Paul, *et al.*, 2003). In Tanzania, a study conducted by Kanyeka *et al.* (2007) reported yield losses of up 100 % as a result of RYMV.

Viral diseases like RYMV are difficult to prevent, and once established management options are not readily available. The rapid genetic evolution of virus, combined with large-scale dispersal, largely explains our limited success in controlling and eradicating RYMV disease. An important early finding was that RYMV is 'inoculum dependent' That is, the more virus there is in the environment, the severe the disease affects the crop (WARDA, 2000).

The use of resistant rice varieties is the only sustainable approach in managing the disease in Tanzania and in other many African countries (Luzi-Kihupi *et al.*, 2009). Therefore, development and deployment of resistant varieties remains the most effective option for managing RYMV disease. Lack of many resistant and tolerant rice varieties is a set back in improving food security in the country. Thus, identification of resistant and tolerant genotypes will aid in management approaches for RYMV disease and improve rice production in different rice ecosystem in Tanzania. This prompted the need to urgently undertake research on the evaluation of different rice genotypes against rice yellow mottle virus disease.

1.3 Objectives of the Study

The major objective of the study was to identify rice genotypes resistant to Rice Yellow Mottle Virus disease and suitable for farmers use.

The specific objectives of the study were;

1.3.1 To compare pathogenicity of different rice yellow mottle virus strains

1.3.2 To assess biological and economical yield loss due to infection by RYMV.

1.3.3 To determine the reactions of different rice genotypes, when artificially inoculated with RYMV Strains.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rice Taxonomy

Rice belongs to the genus *Oryza* and tribe Oryzeae in the family Poaceae (CABI, 2007). The Oryzeae tribe is believed to have 12 genera with 22 rice species, 20 species are wild while two the *Oryza sativa* (Plate 1a) and *Oryza glaberrima* (Plate 1b) are cultivated rice (Olembo, 2010). Native to sub-Saharan Africa is *O. glaberrima*, thought to have been domesticated from the wild ancestor of *Oryza barthii*. On the other hand, the two strains of *O. sativa* (*Oryza japonica* and *Oryza indica*) are thought to have been domesticated in Asia (Olembo, 2010). Rice is an annual grass with round, hollow, jointed culms (CABI, 2007). The stem is upright, leaf grows from each node, and the small wind pollinated flowers are produced in a branched arching to pendulous inflorescence (Nwilene *et al.*, 2008). The flowers form the grain which is the most important part of rice (Nwilene *et al.*, 2008).

Plate 1a: *Oryza sativa* source (Jaw, 2010)

Plate 1b: *Oryza glaberima* (Jaw, 2010)

2.2 Rice Ecology

Cultivated rice is generally considered semi-aquatic, survives as a perennial, producing many tillers from the nodes (CABI, 2007). The growth of rice from germination to maturity may be divided into three agronomic stages of development; vegetative, reproductive that is, panicle initiation to heading and, grain filling and ripening or maturation stage (Olembo, 2010).

The rice plant is a tropical plant which loves water and needs a warm humid climate, it can not grow at high latitude of 53^0 north and 35^0 south, it can survive at temperatures lower than 13 ^{0}C (55 ^{0}F) (Biology and ecology of rice, (2005). It is adapted to fit a wide range of growing conditions from equatorial, tropics to high altitudes (Salim, *et al.*, 2003). Japonica cultivars are grown predominantly in temperate regions and can germinate and grow under lower temperature (15^0 to 20 ^{0}C) than the tropical and Sub-tropical Indica cultivars (Biology and ecology of rice, (2005).).

2.3 History of Rice Yellow Mottle Virus disease in Africa

Rice yellow mottle virus is the only economic importance rice virus in Africa (Abo *et al.*, 2004). The virus is indigenous to the continent, since its first observation on a local rice variety Sindano in a farmer's field near Kisumu on the shores of Lake Victoria in Kenya in 1966 (Kanyeka *et al.*, 2007). RYMV disease has been reported from several African counties in the East, Central, and West African countries, including the islands of Zanzibar and Madagascar (Fig. 1) (Sarra *et al.*, 2005 Kouassi *et al.*, 2005).

The disease is now widespread in many western and eastern African countries (Opalka *et al.*, 2000). It was described in 1976 in Liberia, Nigeria, Sierra Leone and Tanzania, In the following year, RYMV was reported at several locations in Côte d'Ivoire (Ivory Coast) and in 1980 in Ghana and at Koba in Guinea (Kouassi *et al.*, 2005; Banwo *et al.*, 2004). RYMV was first found to infect only lowland rice in West Africa, however, in 1987 it was also reported for the first time on upland cultivated rice (Kouassi *et al.*, 2005). By the late 1980s, RYMV had been identified in Niger, Burkina Faso, and Mali as well as in Malawi and Rwanda, and it was described in Madagascar in 1989 (Kouassi *et al.*, 2005).

Rice Yellow Mottle Virus also has been recorded in Gambia, Guinea Bissau, Senegal, Mauritania, Zanzibar, Cameroon and Chad (Central Africa) (Kouassi *et al.*, 2005). The origin of RYMV in Africa has been studied by several scientists. The phylogenetic tree inferred from the sequences of RYMV discovered that, RYMV had a characteristic nested structure with an East-to-West orientation of the clades (Hebrard *et al.*, 2005). The study by Kanyeka *el al.* (2007) confirmed speculation that Eastern Arc Mountains is the origin of RYMV. The Eastern Arc Mountains biodiversity hotspot harbours most of the strains found in East African countries (Kanyeka, *et al.*, 2007). From the Eastern Arc Mountains, the virus might have dispersed and differentiated gradually to Central and West Africa along the East to West transects (Kanyeka *et al.*, 2007).

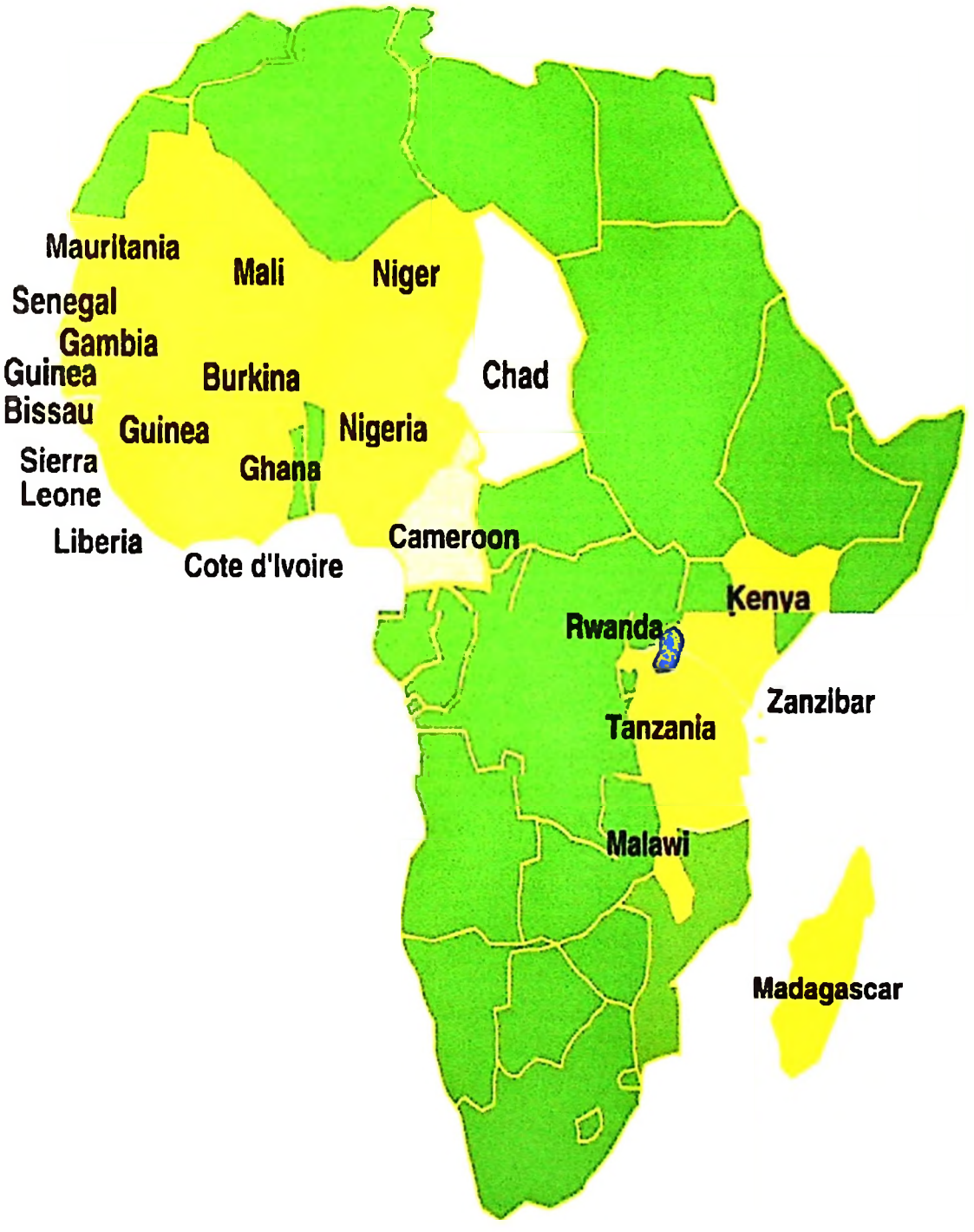


Figure 1: Distribution of Rice Yellow Mottle Virus (RYMV) in Africa; Countries with Rice Yellow Mottle Virus Disease appear in yellow Source: (Kouassi *et al.*, 2005)

2.4 Characteristics of Rice Yellow Mottle Virus of the Genus Sobemovirus

Rice yellow mottle virus belongs to the group iv of positive polarity single strand (+ss) RNA viruses, in the genus *Sobemovirus* (Yassi *et al.*, 1994), and thus it belongs to Picorna-like family of plant viruses (Yassi *et al.*, 1994). The virus belongs to the same group with icosahedral capsids and single stranded, positive-sense RNA genomes, such as Southern bean mosaic virus (SBMV) being the type member (Qu *et al.*, 2000). Rice yellow mottle virus has a distinctive spherical shaped virus particle measuring about 28 ± 3 nm in diameter (Banwo *et al.*, 2004). With at least 15 % single stranded RNA and a molecular weight of 1.41×10^6 daltons (about 4 500 nucleotides) (Banwo *et al.*, 2004).

The type member of the group *Sobemovirus*, specificity is the presence of two extended loops between β-strands βG/βH and βF/βG, CP subunits adopt slightly different conformations depending on their position relative to the symmetry axes (Hebrard *et al.*, 2005). They are characterised by being mechanically transmitted by bettles, the *Chrysomelid* beetles (Nwilene *et al.*, 2008; Banwo *et al.*, 2004), in a semi-persistence manner, that is it does not multiply in the vector (Sarra, 2005). This type of transmission does not require a helper virus for vector transmission (Sarra, 2005), and have a restricted host range mainly the *Gramineae* family (Nwilene *et al.*, 2008). Currently, there are about 12 known insect species which has been confirmed to be involved in transmitting RYMV to different hosts in Africa (Nwilene *et al.*, 2008). The transmission is also by rats and other vertebrates, and abiotically through the soil and during cultural practices by gaining entry into rice plants through injuries of roots during transplanting (Banwo *et al.*, 2004).

2.5 Symptoms and Effects of Rice Yellow Mottle Virus Disease on Rice Growth

Infection by RYMV is mostly systemic, affecting the entire plant and inducing the characteristic symptoms of leaf yellowing of varying intensity, mottling, necrosis and stunted growth (Pinto *et al.*, 1999) (Plate 2). Although the entire plant harbours the virus particles, only a few organs exhibit the symptoms normally on leaves (Biswas *et al.*, 1984). These macroscopic symptoms induced by viruses frequently reflect histological changes within the plant which are necrosis, hypoplasia and hyperplasia (Mathews, 1991).

Further effects due to RYMV disease are, reduced tillering with plant weakening, partial emergence of panicles, delayed flowering with poorly exerted panicle and spikelet sterility (Pinto *et al.*, 1999; Nwilene *et al.*, 2008). The infection also cause decreased biomass production (Froissart *et al.*, 2010). Rice plants of highly susceptible cultivars may die following severe infection (Joseph *et al.*, 2011). Symptom expression may be strongly influenced by light intensity, day length, humidity, temperature and growth stage of the plant (Nwilene *et al.*, 2008).

Generally, older plants exhibit less conspicuous foliar symptoms and less stunting than younger seedlings. Mathews (1991) reported that, the symptoms due to virus infection being localized hypoplasia which is reduced growth and abnormal division of cambial cells. According to Fomba *et al.* (1989), the secondary effects of infection by RYMV include increased severity of infection by fungi such as brown spot (*Cochliobolus miyabeanus*), leaf scald (*Monographella albescens*), sheath rot (*Sarocladium oryzae*) and sheath blotch (*Pyrenochaeta oryzae*).

Plate 2: Symptoms of Rice Yellow Mottle Virus disease on rice leaves Nwilene *et al.*, 2008)

2.6 Infection, Multiplication and Movement of Rice Yellow Mottle Virus in Plant

Movement and multiplication of the virus particles in the host plant are important factors for the disease to develop or colonization to occur (Dawson *et al.*, 1992). The viral RNA possesses various elements that interact directly or indirectly with the host translational machinery and allow them to outcompete cellular messenger RNA's for the scarce translational resource of the host (Sanfacon, 2005).

2.6.1 Infection cycle of Rice Yellow Mottle Virus

Rice yellow mottle virus (RYMV) particles gains entry into rice plants through injuries/wounds and the possible roots of entry are: root damage during transplanting and roots intertwining in the soil, weeding operations, harvesting with sickle , Insects feeding (Nwilene *et, al.*, 2008). It was observed in susceptible rice varieties that, inoculation of RYMV resulted in systemic spread of virus particle in mesophyll and xylem cells (Brigidou *et al.*, 2002). For successful infection, the entry into the cell,

the multiplication of the virus, the movement within the inoculated leaf, the spread within the whole plant, and finally the transmission from plant to plant are important steps (Biswas *et al.*, 1984). The P1 protein of RYMV is required for the infection of plants and is important for the spread of the virus (Sere *et al.*, 2004). The infectivity of FL5-RNA transcripts demonstrated that a VPg molety is not absolutely required for infectivity, as shown for other viruses that possess a VPg contrast, FL5-RNA transcripts required capping to be infectious in rice.

Electron microscopy of infected tissue by RYMV revealed virus particles in vacuoles of xylem parenchyma and mesophyll cells early in the time course of infection. This suggested that vacuoles and other vesicles were the major storage compartments for RYMV particles (Brugidou *et al.*, 2002). The study by Michel *et al.* (2008) revealed that, the period from seedling to booting represented the most vulnerable phase to RYMV infections in rice growth and that yield losses are strongly influenced by host cultivar and time by which infection occurred.

2.6.2 Movement of Rice Yellow Mottle Virus in the whole plant

Invasion of mature vessel elements during xylem maturation provide cell to cell movement (Brigidou *et al.*, 2002). The movement of RYMV therefore, occurs through the xylem parenchyma and sieve elements and the virus migrates through the pit membrane which facilitates systemic virus transport (Opalka *et al.*, 1997). Intracellular transport of cellular macromolecules is facilitated by association with endoplasmatic reticulum (ER) and the cytoskeletal element such as microtubules and microfilaments (Germundsson, 2005). It is through this, the RYMV utilize the

normal intracellular trafficking system of the host to move through the plasmodesmata (Germundsson, 2005).

The movement protein is involved in interacting with the elements of cytoskeleton and other cellullar structures which are involved in intracellular and intercellular movements (Opalka *et al.*, 1997). The movement of RYMV involved chelation of Ca^{2+} from pit membrane of infected cells, by stabilizing the capsid shells and allowing a pathway for spread of RYMV through a destabilized membrane (Opalka *et al.*, 2000). In the RYMV and SBMV a protein encoded by the first open reading frame could be the protein required for local movement and encapsidation is also likely to be essential for phloem-mediated long-distance movement (Brugidou *et al.*, 1995). It is therefore, concluded that RYMV CP are required along with a movement protein(s) for efficient cell-to-cell movement and encapsidation is also likely to be essential for phloem-mediated long-distance movement.

Opalka *et al.* (1997) reported that, RYMV can spread systemically in the leaf cells including epidermal, mesophyll, bundle sheath, and vascular parenchyma cells. In most infected leaf parenchyma cells, the virus moved approximately 1 mm or 8 to 10 cells per day (Opalka *et al.*, 1997). Once the RYMV has entered the phloem, it moves rapidly in it toward growing regions (apical meristems) or other food-utilizing parts of the plant, such as tubers and rhizomes (Palukaitis *et al.*, 2008). As reported earlier that, the P1 of RYMV is required for the infection of plants and is important for the spread of the virus (Sere *et al.*, 2004).

2.7 Transmission of Rice Yellow Mottle Virus

Transmission of RYMV is complex, several means of propagation of the virus have been found and could interact (Sarra, 2005). The virus has been reported to be mechanically transmitted by several species of beetles (Chrysomelidea) (Appendix 1), by rats and other vertebrates, and abiotically through the soil and during cultural practices by gaining entry into rice plants through injuries of roots during transplanting (Abo, 2004; Sarra, 2005). Seed transmission has not been reported (Konaté *et al.*, 2001). Another possible route of entry is through roots intertwining in the soil (Sarra, 2005).

2.8 Current Status of Rice Yellow Mottle Virus Disease

Optimum rice production in Africa cannot be realised mainly due to prevalence of diseases (Jaw *et al.*, 2012). Rice yellow mottle virus (RYMV) is among the most devastating and yield reducing diseases and has remained one of the most problematic diseases in Africa (Jaw *et al.*, 2012). Rice Yellow Mottle Virus, like other plant viruses, is known to be very destructive and a rapidly spreading disease, therefore, threatening the rice production on the African continent (Banwo *et al.*, 2004). The virus is the major threat to more than 3 million hectares of rice in sub-Saharan Africa every year (Mohapatra, 2008 unpublished paper). The disease occurs on both lowland and upland rice rainfed ecosystem as well as irrigated areas (Kanyeka *et al.*, 2007).

It has the potential to devastate all the rice growing ecology, contributing to food scarcity in areas where rice is an important staple food (Mohapatra, 2008

unpublished paper). Yield losses due to RYMV disease fluctuates between 10 and 100 %, depending on plant age prior to infection, susceptibility of the rice variety and environmental factors (Kouassi *et al.*, 2005).

The high variability of the RYMV virus strains, indicates variability of strains from location to location, this proves a major challenge for scientists, because a rice variety that is resistant in one location may be susceptible elsewhere. Currently there are three strains of RYMV in East Africa (S4, S5 and S6) (Kanyeka *et al.*, 2007). In Tanzania, strain S4 occurs in Morogoro, Mwanza, Shinyanga, Mara and Mbeya region, strain S5 is restricted to a few sites only in Kilombero district, strain S6 is widely spread in East Africa (Kanyeka *et al.*, 2007). Therefore, Rice Yellow Mottle Virus disease remains a very serious rice production limitation in most parts of Tanzania and Africa as whole.

2.9 Evolution of Resistance Breaking Rice Yellow Mottle Virus Isolates

Understanding virus evolution is crucial to the development of efficient and stable control strategies, to avoid breakdown of resistance (Gracial-Arenal *et al.*, 2003). Fagette *et al.* (2008) conducted experiments on evolution of coat protein gene of RYMV collected from rice in different parts of Africa, the results showed that an RNA plant virus such as RYMV evolves as rapidly as most RNA animal viruses. Therefore RYMV adapts rapidly to alternative hosts through accumulation of point mutations (Fargette *et al.*, 2008). This is due to the lack of proofreading mechanisms as compared to the dsDNA viruses (Sanjuan *et al.*, 2009). Studies on pathogenicity and molecular diversity of RYMV in Africa, using serological (monoclonal

antibodies), molecular sequencing and phylogenic analysis revealed that, there are several serotypes and phylogenic groups with a well-defined, geographic basis (Kouassi *et al.*, 2005). The study conducted in Tanzania offered a contrasting picture with highly divergent strains of RYMV in each of the different agro-ecologies, all of which differed from those in West/Central Africa (Kanyeka *et al.*, 2007). Tanzanian strains had a specific and restricted geographical range, whereas West African strains had large and partially overlapping geographical distributions, this could reflect strain adaptation to different regional ecosystems (Abubakari *et al.*, 2002).

There are pronounced differences of pathogenicity between different isolates, even within a given strain of a specific region (Fargette *et al.*, 2008). Modifications of the pathogenic characteristics can occur after successive infections in hosts which could lead to resistant-breaking isolates leading to possible emergence of virulent isolates after the deployment of resistant varieties (Fargette *et al.*, 2008). Virulence is the key property of the pathogen, determine emergence and re-emergence of the pathogens, host switch, host range expansion and overcoming resistance (Gracial-Arenal *et al.*, 2003). The wide range of diversity of RYMV strains points to the need for introduction of new genotypes in Tanzania which must be accompanied by pragmatic screening against strains S4, S5 and S6 (Kanyeka *et al.*, 2007).

2.10 Efforts to Reduce Rice Yellow Mottle Virus Disease Effects

Host-resistance genes have been extensively exploited by breeders for the development of virus-resistant plants (Wani *et al.*, 2010). The best hope for reducing rice production losses due to RYMV is the use of resistant varieties (Michel *et al.*,

2008). A gene, Rymv1-2 responsible for high resistance of rice to RYMV, was identified by Laurence Albar, IRD genetist (Albar *et al.*, 2006). Viruses are made up of a small genome coding for a limited number of proteins 5 in the RYMVs (Ventelo-Debout *et al.*, 2008). They therefore, need their host's proteins in order to accomplish each stage of infection cycle (Ventelo-Debout *et al.*, 2008).

One of the proteins that RYMV requires appears to be the *eIF(iso)4G* translation initiation factor coded by the *Rymv1* gene which is involved in viral protein translation and other processes such as the virus's movement within the cell (Alber *et al.*, 2006). Mutation of such gene can confer resistance to RYMV (Alber *et al.*. 2006).

Most cultivated rice varieties are susceptible to the RYMV disease, but a monogenic and recessive high resistance has been found in two varieties (Tog 7291 and Tog 5674) of *O. sativa* and a few varieties of *O. glaberrima* (Traore *et al.*, 2010). The high resistance genes has been identified in rice with *RYMV1* gene which encodes an eukaryotic translation initiation factor eIF(iso) 4G and at least five alleles in west Africa (Traore *et al.*, 2010) while in Tanzania mutagenic resistance was derived from irradiated local variety Supa and introduce rice varieties from Africa rice center and International Rice Research Center (Luzi-Kihupi *et al.*, 2009).

Resistance in rice is controlled by the recessive gene Rymv1-2, which maps on chromosome 4 and encodes the translation initiation factor eIF(iso)4G as reported earlier (Pinel-Galzi *et al.*, 2007). Different degrees of resistance to RYMV have been

detected in *Oryza spp* (Paul, 2003). Resistance to RYMV in *Oryza sativa* is controlled by a few major recessive genes whereas tolerance to RYMV was primarily expression of two dominant genes (Paul, 2003).

The resistance to RYMV is expressed on the one hand, as partial resistance marked by a delay in the appearance of symptoms and in virus accumulation (Ioanidou *et al.*, 2000; Fargette *et al.*, 2002). It has polygenic determinism and has been identified in varieties of *O. sativa* subspecies e.g. Azucena. High resistance to RYMV is characterized by an absence of symptoms, very low virus accumulation and a blockage of virus movement (Ndjiondjop *et al.*, 1999).

Despite all this progress, there are only two known released resistant varieties in Tanzania which are Kalalu and Mwangaza (Luzi-Kihupi *et al.*, 2009; MAFSC, 2009). This calls for further screening and identification of materials, which are resistant for Rice Yellow Mottle Virus disease and suitable for farmers use.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Location of the Experiment

Three pot experiments were conducted in the Horticulture and Crop Science screen houses at Sokoine University of Agriculture. The area is located between latitude $6^0$5'S and 7^0S and longitudes $37^0$39' E, at altitude of 524m asl. The experiments were sorely dependent on irrigation using the tap water. The area average annual temperature ranges from 15 to 30^0C, with coolest months being June and July and warmest being October to December. The experiment was conducted during October 2010 to May 2012.

3.2 Rice Genotypes

Fifty two (52) rice genotypes, including two susceptible checks and two resistant checks were used in the experiment. Three RYMV strains 4, 5 and 6 were used in the study. The 52 rice genotypes together with the RYMV strains 4 and 6 were obtained from the Africa Rice Center regional office in Dar-es-salaam, Tanzania. While RYMV Strain 5 was obtained from Mikocheni Agricultural Research Institute, Dar es-salaam, Tanzania.

3.3 Effect of Rice Yellow Mottle Virus Strain 4, 5 and 6 on Rice Growth

Pathogenicity comparison test for RYMV strains was studied using three (3) rice varieties in first experiment (Table 1), which were tested against RYMV strains 4, 5 and 6.

Table 1: Rice genotypes tested against Rice Yellow Mottle Virus in first experiment

1.	KALALU	- Introduced
2.	SUPA	- Local
3.	SARO-5	- Locally improved

3.3.1 Experimental design

Treatments were allocated at random in a split-plot design arranged in completely randomized design with three replicates, variety being the main factor and RYMV strains being the sub-plot factor. Assignment of treatments was done by using random numbers generated by a scientific calculator.

3.3.2 Statistical model for ANOVA

The model for experiments being

$\chi_{ijk}=\mu+A_i+B_j+(AB)ij+ e_{ijk.}$

where χ_{ijk} is observation of i = i levels of factor A,

j=is j levels of factor B and k is = k experimental unit,

μ is the overall mean,

A_i is levels of factor A,

B_j is levels of factor B,

(AB)ij is interaction effects of j^{th} level of factor A and j^{th} level of factor B,

e_{ijk} is random error term.

3.4 Effect of Rice Yellow Mottle Virus Strain 5 on Growth of Different Rice Genotypes

Twenty five (25) rice genotypes were used in the horticulture screen house, including two susceptible and two resistant rice genotypes as controls. These genotypes were tested against one RYMV Strain-5, which was found to be very virulent strain upon multiplication in the screen house under experiment 3.3 above. Multiplication of RYMV was done in order to make sure that there was virus inoculum which is infective during the experiment period. The list of rice genotypes used in the study are shown below in (Table 2). The objectives were to determine the reactions and the effects of Rice Yellow Mottle Virus (RYMV) disease on biological and economic yields.

Table 2: Rice genotypes screened against Rice Yellow Mottle Virus strain 5 in the second experiment

1. KALALU-Resistant control	10. IR 71026-3-2-3-2-1-1-1	19. IR 77470-3-3-4-2-2
2. MWANGAZA-Resistant	11. IR 73886-9-2-4-2-1-	20. IR 71029-3-1-5-5
3. SUPA-Susceptible control	12. IR 77470-1-7-2-5-3	21. IR 71028-3-1-2-2-3
4. SARO 5-Susceptible control	13. IR 00A 118	22. MATATA G1
5. IR 71030-4-5-5-5	14. IR 69705-1-1-1-4-2	23. IR 77966-1-5-1-B-1
6. IR 73887-1-8-2-1	15. IR 71026-3-2-4-3-5-4	24. IR 71605-2-1-5-3-4
7. NERICA-L 14	16. IR 77470-3-3-3-2-2	25. IR 73885-1-4-3-2-1-4
8. TKM 6	17. IR 71028-3-1-2-5-1	
9. NERICA-L 32	18. NERICA-L42	

3.4.1 Experimental design

Experimental materials for this experiment were laid down by using completely randomized design with three replicates. Assignment of treatment was done at random using random numbers generated by a scientific calculator fx-82TL model.

3.4.2 Statistical model for ANOVA

The model for experiments was $\chi_{ij}=\mu+T_i+e_{ij}$,

where χ_{ij} is the j^{th} observation on treatments,

μ is overall mean,

T_i is treatment effects,

e_{ij} is random error.

3.5 Effect of Rice Yellow Mottle Virus Strain 4 on Performance of Different Rice Genotypes

Thirty one (31) rice genotypes (Table 3) were used in pot experiments, conducted in the Crop Science screen house. The genotypes were tested against RYMV Strain-4. The objectives were to determine reactions and the effects of RYMV disease on biological and economic yields of rice.

Table 3: Rice genotypes screened against Rice Yellow Mottle Virus strain 4 in the third experiment

1. KALALU – Resistant control	13. ADDAY SEL	25. IR 71030-2-2-1-1
2. MWANGAZA - Resistant	14. IR 69734-81-1-1	26. IR 73887-1-8-3-5
3. SARO-5– Susceptible control	15. IR 71605-2-1-1-3-10	27. IR 72928-1-2-4-1
4. SUPA – Susceptible control	16. ITRI MERAH	28. IRR 134
5. IR 73885-1-4-3-2-1-10	17. IR 71605-3-1-1-2-6	29. IR 69705-1-1-3-3-5
6. MATATA G2	18.TN 1	30. IR 72928-1-2-4-1-1-3
7. NERICA-L26	19. IR 81244	31. IR 71027-43-3-2-B-3
8. IR 69705-1-1-3-2-3	20. UTRI JAPAN	
9. IR 69704-4-4-4-8-1-1-1-1	21. IR 69705-1-1-3-2-1	
10. IR 69704-4-8-1-5-1-3	22. IR 72955-6-1-3-4-1	
11. IR 69704-4-8-2-1-1-2	23. IR 69734-16-1-1	
12. IR 1561-228-3-3	24. UTRI MERAH	

3.5.1 Experimental design

The experimental design for this experiment was completely randomized design (CRD) with three replicates. Assignment of treatment was done at random using random numbers generated by a scientific calculator fx-82TL model.

3.5.2 Statistical model for ANOVA

The model for experiments was $\chi_{ij}=\mu+T_i+e_{ij}$,

where χ_{ij} is denotes the observation on j^{th} treatments,

μ is overall mean,

T_i is treatment effects,

e_{ij} is random error.

3.6 Soil Preparation and Sowing

A composite of forest soil was taken from the Crop Museum at SUA. The soil was sterilized by heating in a drum at Hortculture unit to reduce possibility of soil borne pathogens. Four holes were dug in 10 Lt Plastic bucket containing 10 kg of sterile soil and three seeds in each hole were directly sown. Upon germination, seedlings were later thinned to one plant per hole, making four plants per pot.

Each treatment had two pots in each replicate as one set in first experiment (effect of RYMV strain 4, 5 and 6 on growth of rice genotypes), the total number of pots used were 144 for experiment one while in second experiment (effect of RYMV strain 5 on performance of rice genotypes) each treatment had one pot in each replicate making a total of 150 pots. In the third experiment (effect of RYMV strain 4 on performance of rice genotypes), one pot in each replicate was used making a total of 186 pots.

Application of fertilizers containing Nitrogen, Phosphorus and Potassium (NPK) was done in order to improve rice growth. Phosphorus and Potassium compound was applied at a rate of 75kg/ha before plant and Nitrogen in form of Urea was applied at a rate of 100kg/ha in two splits doses at planting as starter dose and as top-dress four weeks after germination.

3.7 Rice Yellow Mottle Virus Inoculum Preparation

Diseased rice leaves from previously inoculated rice plants with RYMV, were used as source of inocula. Phosphate buffer solution (PBS) was added in a 1 000 cm^3

beaker containing sliced rice leaves in a ratio of 1:10 (v/w) was done. The mixture was ground by using an electric blender and then filtered through a double layer cheese cloth to obtain plant leaf sap. The sap was mixed with Carborundum powder in a ratio of 1g Carborundum powder (30 mesh) to 15 ml of plant sap, to create wounds on leaves (Sere *et al.*, 2005).

3.8 Inoculation and Incubation of Experimental Rice Plants

The homogenized sap in plastic bottle was rubbed onto rice leaves of 14 to 18-day-old rice plants using a hand with a piece of cheese cloth as described by (Nwilene *et al.*, 2008; Fomba, 1989). The procedures for inoculation were the same for three experiments. The only difference was the type of RYMV strain used, for first experiment RYMV strains 4, 5 and 6 were used to inoculate three rice varieties. In the second experiment RYMV strain 5 was used to inoculate 25 rice genotypes, while in third experiment RYMV Strain 4 was used to inoculate 31 rice genotypes including two susceptible control varieties (Supa and SARO-5) and resistant control varieties (Kalalu and Mwangaza).

Inoculated and uninoculated rice plants were incubated in the screenhouse for disease development at 25^0-35^0C with a relative humidity of approximate range of 60% to 80%. Observations and assessments of RYMV symptom development were done daily in the first two weeks after inoculation, there after disease assessment was done on weekly basis. Pots in the screen house were arranged far enough not to allow contact among rice leaves to avoid contamination of different treatments.

The use of different RYMV strains in the second and third experiments was based on the fact that, RYMV strain 5 which was used in second experiment lost its infectivity, possibly due to changes in environmental conditions. Further propagation of RYMV strains S4 and S6 collected from Africa Rice office at SUA was done. RYMV strain S4 based on symptom severity was selected to be used in the third experiment.

3.9 Propagation of Inoculum

Each time multiplication of inocula was done by inoculating the 14-day-old susceptible rice plants of variety SARO-5 with required strains and the inocula collected was used to inoculate the test rice genotypes.

3.10 Identification of Other Rice Disease Causing Pathogens (Fungi)

Identification of pathogens caused multiple disease in second experiment were done by taking samples of leaves and stems of diseased rice plants. The Blotter method was used by plating leaf and stems samples in 100 mm sterilized Petri-dishes with three layer of moistened filter papers. The Petri-dishes with samples were incubated in a refrigerator for 7-days at 22 ^{0}C under alternating cycles of 12 hours light and darkness to allow sporulation (Raj *et al.*, 2007). Examinations of fungi spores were done using a compound microscope at different magnification. The identification was based on growth habit and morphological characteristic of the fruiting bodies/spore/cornidia observed under a compound microscope (Balau, 2008).

3.11 Data Collection

3.11.1 Disease severity

Inoculated plants were observed for disease development and data collection for disease score was done from 7 days after inoculation (DAI) and continued at the interval at 7 days for 8 to 9 weeks, by assessing disease severity on rice plant leaves. The scoring was done according to Standard Evaluation System for Rice on the scale of 1-9 for RYMV (IRRI, 1996) (Appendix 2).

3.11.2 Dry matter weight measurement

Dry weight (gm) of one plant from each treatment was taken at the end of the experiment one at random and oven dried at the temperature of 50-60^{0}C. The rice plant material were consecutively weighed for four days by using weighing balance in the Mycology laboratory at the Horticulture unit SUA, the last day readings were taken and used for data analysis.

3.11.3 Plant height measurement

Data on plant height were taken at seven days interval from inoculation of rice and continued weekly for 8 and 9 weeks for experiment one, two and three, respectively. Calibrated ruler in (cm) was used to measure the plant from the soil level to the last leaf (flag leaf). The period of 8 to 9 weeks was considered to be a maximum growth stage on rice disease symptom assessment (Joseph *et al.*, 2011).

3.11.4 Number of tillers per plant

Data collection on tillers per plant was done by counting tiller number from a randomly preselected rice plants, at the interval of 7 days after inoculation and continued for 8 to 9 weeks.

3.11.5 Percentage reduction of growth parameter

Plant height and number of tillers were measured from RYMV inoculated and uninoculated rice plants.

3.12 Panicle exertion, panicle sterility and grain weight

The data on yield (grain weight) and physiological trait (panicle exertion and panicle sterility) involved 31 rice genotypes including standard checks from experiment three. Data for economic yield were collected at the end of the experiment during harvesting.

3.12.1 Percent panicle exertion

Data on fully exerted rice panicles against unexerted/partially exerted panicle per hill were collected before harvesting and calculations on the percentage panicle exertion was done using Micro-soft Exel package.

3.12.2 Percent panicle sterility

Percentage panicle sterility was obtained by counting the total number of seeds per panicle using the seed counter at the Africa Rice office at SUA, followed by removing the unfilled grain/seeds. Counting of filled grains per panicle was done using Elmor seed counter model C1, Serial No. 56002. Percentage panicle sterility

per rice panicle was calculated by taking unfilled grains divided by total grains multiplied by 100 % using the Micro-soft Exel computer programme.

3.12.3 Grain weight

A thousand (1 000) grains were counted using the Elmor seed counter model C1, Serial No. 56002 and weighed on a weight balance; data were recorded as 1 000 grain weight at 12-13% moisture content.

3.12.4 Percent reduction in seed weight and percentage difference in panicle exertion

Percentage loss in seed weight and percentage difference in panicle exertion, was obtained by calculating the mean difference between the uninoculated and the inoculated plants divided by uninoculated.

3.13 Data Analysis

In order to reduce variability within the data, all the raw data from all the experiment were subjected to transformatiom before analysis of variance using the Square root transformation denoted by the formular $(X+0.5)^2$ (Hatibu *et al.*, 2001). Microsoft excel programme was used for data entry, all the data collected from the experiments were subjected to analysis of variance using the procedure described by Hatibu *et al.*,(2001).

Data for experiment one pathogenicity comparison test, were subjected to analysis of variance for Split-plot design using the Genstat statistical analysis package (Glaser

and Biggs, 2010), while data for experiment two and three (Assess biological, economic yield loss due RYMV and determine the reactions due to RYMV infection) were subjected to one-way analysis of variance. The difference was declared significantly different at ($P \leq 0.05$). Means separation tests were done using Duncan Multiple Range Test while mean comparison of inoculated and uninoculated plants were done by performing t-test (Hatibu *et al.*, 2001).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

In general, the 52 different rice genotypes tested, reacted differently upon mechanical inoculation with RYMV inoculum. The effects of RYMV infection were clearly observed as yellowing and stunting on susceptible rice genotypes from the vegetative growth phase to rice maturity (Plate 3a and Plate 3b). The results showed that there were significant ($P \leq 0.05$) reduction in plant height by 12.0 to 63.7 %, number of tillers per plant was 22.38 to 47.93 % and increased disease severity in susceptible rice genotypes (Table 4). There was also a reduction in total dry matter by 19.4 to 22.6%, decreased panicle exertion, increased panicle sterility and decreased grain weight ranged from 65-99 %.

Table 4: The reaction of susceptible rice genotypes to Rice Yellow Mottle Virus disease

	Rice genotypes	Disease score	Reaction class	Rice genotypes	Disease score	Reaction class
1.	TKM 6	9	HS	20. IR 69705-1-1-3-3-5	9	HS
2.	NERICA-L 14	9	HS	21. IR 72928-1-2-4-1-1-3	9	HS
3.	NERICA-L 32	9	HS	22. IR 73885-1-4-3-2-1-10	9	HS
4.	IR 73886-9-2-4-2-1	7	S	23. NERICA-L26	9	HS
5.	IR 00A 118	9	HS	24. IR 69705-1-1-3-2-3	9	HS
6.	IR 71030-4-5-5-5	9	HS	25. IR 69704-4-8-1-5-1-3	9	HS
7.	IR 73887-1-8-2-1	9	HS	26. IR 69704-4-8-2-1-1-2	7	S
8.	IR 71026-3-2-3-2-1-1-1	7	S	27. IR 1561-2-2-8-3-3	9	HS
9.	IR 77470-1-7-2-5-3	9	HS	28. IR 69734-81-1-1	7	S
10.	IR 73885-1-4-3-2-1-4	7	S	29. IR 71605-2-1-1-3-10	9	HS
11.	IR 77966-1-5-1-B-1	7	S	30. ITRI MERAH	7	S
12.	IR 71026-3-2-4-3-5-4	9	HS	31. IR 69734-16-1-1	7	S
13.	IR 77470-3-3-3-2-2	9	HS	32. IR 71605-3-1-1-2-6	7	S
14.	IR 77470-3-3-4-2-2	9	HS	33. TN 1	9	HS
15.	IR 71028-3-1-2-2-3	9	HS	34. IR 81244	9	HS
16.	UTRI MERAH	9	HS	35. UTRI JAPAN	9	HS
17.	IR 71030-2-2-1-1	9	HS	36. IR 69705-1-1-3-2-1	7	S
18.	IR 73887-1-8-3-5	9	HS	37. IR 72955-6-1-3-4-1	7	S
19.	IR 72928-1-2-4-1	7	S			

HS- Highly susceptible rice genotypes and S- Indicates susceptible rice genotypes

Rice genotypes which were resistant/tolerant did not show RYMV disease symptoms or had scant symptoms. The effect due RYMV infection on resistant rice genotypes for plant height, number of tillers, panicle exertion, panicle fertility and grain weight was low compared to the susceptible genotypes (Plates 3a and 3b). These included IR 73886-9-2-4-1-1, IR 71605-2-1-5-3-4, IR 69705-1-1-1-4-2, IR 71028-3-1-2-5-1, IR 71029-3-1-5-5, Matata G1, IRR 134, Matata G2, IR 69704-4-4-4-8-1-1-1, Adday Sel and IR 71027-43-3-2-B-3 (Plates 4a and 4b compare 5a and 5b).

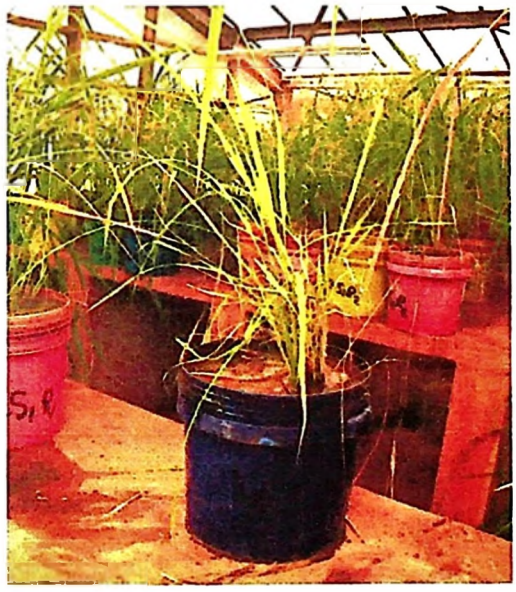


Plate 3a: IR 72928-1-2-4-1-1-3

Plate 3b: Nerica-L 26

Plate 3a and 3b: Symptoms of RYMV disease on IR 72928-1-2-4-1-1-3 and Nerica L. 26 susceptible genotypes

Plate 4a.IRR 134

Plate 4b. Adday Sel

Plate 4a and 4b: Symptoms of RYMV disease on IRR 134 and Adday Sel resistance genotypes

Plate 5a.IRR 134

Plate 5b. Adday Sel

Plate 5a and 5b: Uninoculated IRR 134 and Adday Sel rice genotypes

4.1 Effect of Rice Yellow Mottle Virus Strain 4, 5 and 6 on Rice Growth

4.1.1 Disease severity

Table 5 shows the effect of RYMV strains on three rice genotypes which were used as resistant and susceptible controls. The results indicated that, the genotype Kalalu (resistant control) had a disease severity score of 5 indicating moderately resistance, Supa and SARO 5 both had disease severity score of 9 indicating that they were highly susceptible.

Rice Yellow Mottle Virus disease symptom severity indicated that, there was highly significant ($P < 0.001$) difference between inoculated plants and the uninoculated plants, while no significant variation was observed among uninoculated plants. Inoculated plants indicated no significant difference between SARO-5 and Supa where as Kalalu showed significant difference from the three tested genotypes. RYMV disease symptom severity developed earlier and higher within 7 DAI on the susceptible rice varieties (Supa and SARO-5) inoculated with RYMV strain S5. While for SARO-5 and Supa which were inoculated with RYMV strains S4 and S6, the symptoms were observed during the second week after inoculation. Kalalu showed symptoms within two weeks after inoculation with strain S5 while no symptoms were observed when strains S4 and S6 were inoculated to this rice variety (Table 5).

Rice Yellow Mottle Virus disease symptoms were observed on the fifth day after inoculation and increased rapidly on Supa and SARO-5 inoculated with strain S5, this implies that RYMV strain S5 had higher ability than S6 and S4 to replicate and

spread in the host plant tissues. This is similar to the result obtained by Opalka, (1997) who observed that, 6 days after inoculation RYMV particles had spread systemically to leaves and other tissues of the plant including the epidemal, mesophyll, bundle sheath and vascular parenchyma cells. Symptom expression and spread, has been reported to be the result of displacement of pit membrane by the virus which allows it to be transported to different parts of the plant (Opalka, 1997).

Late appearance of symptoms on SARO-5 and Supa inoculated with RYMV strains S4 and S6, was possibly due to reduced RYMV particle multiplication as a result of unfavourable environmental conditions (Methews, 1991). Strains S4 and S6 inoculation on Kalalu showed no symptom development, this was possibly due to Kalalu variety being resistant to these strains (Luzi-Kihupi *et al.*, 2009). Kalalu when was inoculated with strain S5 showed gradual symptom development, this genotype is probably susceptible to this strain. Joseph *et al.* (2011) and Jaw, (2010) reported that, variations on symptom expression as a result of RYMV infection among rice genotypes is due to genotypic characteristics of the rice plants.

Table 5: The effects of Rice Yellow Mottle Virus strains 4, 5 and 6 on rice varieties tested

Parameter	Rice genotypes	Inocul.	Uninocul.	(P ≤ 0.05)	% Reduction	CV(%)	S.E ±
Plant height	SARO-5	6.598b	8.135d	9.354***	18.89	23	1.5588
	KALALU	6.234a	7.208c	5.917*	13.51		
	SUPA	6.571b	7.870d	7.906**	16.5		
Tillers/plant	SARO-5	0.7583a	0.9700b	21.82***	21.82	35.9	0.32336
	KALALU	1.0354c	1.2573d	17.65**	17.65		
	SUPA	0.7726a	0.9021b	14.35**	14.35		
Dry matter	SARO-5	3.026a	3.909d	8.532***	22.58	10	0.3378
	KALALU	3.516b	3.813c	2.941*	7.78		
	SUPA	3.144a	3.885c	7.359***	19.07		
Disease severity	SARO-5	9 - Highly Susceptible					
	KALALU	5 -Moderately Resistant					
	SUPA	9 - Highly Susceptible					

Means within column followed by the same letter(s) are not significantly different from each other according to DMRT at 5% level. Note: * = significance at P= 0.05,** = Highly significance at P = 0.01 and ***= very highly significance at P = 0.001, NS = non significance difference and CV (%) is coefficient of variation. Inocul = Inoculated plants, Uninocul = Uninoculated plants.

4.1.2 Effect of Rice Yellow Mottle Virus disease on Plant height

Plant height, was highly significant ($P \leq 0.05$) different for the three genotypes tested. Significant ($P \leq 0.05$) differences in pathogenicity were also observed among the RYMV strains when inoculated to the three varieties (Table 6). The differences between inoculated and uninoculated plants for SARO-5, Supa and Kalalu rice genotypes were significant ($P \leq 0.05$). The percent height reduction for inoculated plants when compared with uninoculated among genotypes were 13.5 %, 16.5 % and 18.9 %, for Supa, Kalalu and SARO-5 respectively, implying that plant height of all the rice genotypes were negatively affected by RYMV strains.

The RYMV strains S4, S5 and S6 caused reduction in plant height among the different tested rice varieties (Table 6), but the extent of their effects depended on the type of strain or isolate inflicted by the disease. Virulence comparison among different RYMV strains indicated that, all the RYMV strains differed significantly ($P \leq 0.05$). However the results suggest that RYMV strain S5 was the most virulent strains followed by strain S6 and S4. Since S5 has caused significant reduction on dry matter, number of tillers per plant, plant height and showed the highest disease severity score in comparison to other strains.

This observation indicated that, RYMV was able to cause decreased plant height, but the disease severity depended very much on the rice variety. SARO 5 variety was highly affected in terms of height reduction due to RYMV infection than the resistant control Kalalu, which showed less plant height reduction. The symptom severity on Supa was severe without plant stunting. Similar observations were reported by Paul,

(2003) and Methews, (1991) who reported that reduction in plant height was a general symptom observed for all inoculated plants and the degree of stunting correlated with symptom severity. Onwughalu *et al.* (2010) also observed stunting on the RYMV affected plants and that plant height was significantly affected by RYMV infection at seedling stage which led to 94.4 % yield loss.

4.1.3 Effect of Rice Yellow Mottle Virus disease on dry matter

The dry matter for Supa and SARO-5 varieties were observed to show highly significant ($P \leq 0.05$) difference between inoculated and uninoculated plants (Table 6) while significant ($P \leq 0.05$) differences were observed between inoculated and uninoculated Kalalu rice genotype. Dry matter reduction for Kalalu was 7.86 %, Supa 19.4 % and SARO-5 had 22.6 % being the highly susceptible than the others. The result on dry matter reduction (Table 6) was less on Kalalu variety compared to Supa and SARO-5 varieties; this implies that Kalalu was able to tolerate the effects of the virus load. This study is consistent with the trade-off hypothesis which explains that virulence is an estimated reduction in plant biomass due to infection, or symptom severity (Soledad *et al.*, 2005). The decrease in dry matter observed in this experiment was probably due to the fact that, virus infection leads to reduction of chlorophyll content of the leaf which ultimately cause decreased biomass production in rice plants (Mathews, 1991; Froissart *et al.*, 2010).

4.1.4 Effect of Rice Yellow Mottle virus disease on number of tillers

The reduction in number of tillers per plant (Table 6) due to infection when compared with uninoculated plants was not similar to all tested rice varieties. There

were highly significant ($P \leq 0.001$) differences between inoculated and uninoculated SARO-5, whereas significant ($P \leq 0.01$) differences between inoculated and uninoculated plants were observed on Kalalu and Supa rice varieties. The reduction in the number of tillers per plant for Kalalu and Supa were 17.65 % and 14.35 % respectively, where as Saro had 21.82 %.

The effect of RYMV disease on reduction tillers per plant, showed that there was no significant ($P \leq 0.05$) difference between strains S5 (22.9 %) and S6 (20%) though they had higher percentage reduction than strain 4 (6.31%), this implied that strains S5 and S6 might have had similar effect, however there was a significant ($P \leq 0.05$) difference when the two strains are compared with stain S4. Generally all the strains caused a decrease in number of tillers. The reduced effect of the disease on the number tillers observed on Supa compared to Kalalu variety, might be due contrasting effects caused by the virus, since in some other cases as have been reported by Methews, (1991) viruses cause hyperplasia (increased production of new tissues) that might have led to increased tillers in the inoculated Supa. Another possible reason may be that the variety Kalalu is mere tolerant to some isolates of RYMV, since in this study it has been observed that it was affected by RYMV strain S5 almost equally to the highly susceptible varieties (SARO-5 and Supa). Result have been reported by Kanyeka *et al.* (2007) that RYMV strains regardless of their serological differences caused yield loss through affecting production of tillers. This is similar to what was observed by (Kouassi *et al.*, 2005 and Onwughalu *et al.*, 2010) that, RYMV disease caused reduced tillering of rice at active tillering stage and

ultimately reduced yield. Similarly Alber *et al.* (1998) observed significant correlation between resistance and tillering potential of rice genotypes.

4.1.5 Comparison of virulence among Rice Yellow Mottle Virus strains

Virulence comparison among RYMV strains showed that there were significant ($P \leq 0.05$) differences among strains, with strain S5 showing more virulence than strains S6 and S4 (Table 5 and 6) and (Plate 6a and 6b). The effect of Strain S5 on plant height, number of tillers per plant, disease severity and dry matter per plant was higher than the other RYMV strains (Plate 4a and 4b). The effect of RYMV strains S4 and S6 on dry matter showed non-significant difference. However, RYMV strain S6 showed highly significant ($P \leq 0.001$) difference between inoculated and uninoculated plants on plant height. Strain S4 effect was significantly different between inoculated and uninoculated plants for plant height, number tillers per plant, dry matter and disease severity but the extent of the effect of strain S4 was less compared to S5 and S6. The results suggested that the virulence of RYMV Strain 5 was higher than the other strains, strain S5 might be encoding suppressors of RNA silencing defense, to specifically counteract the RNA silencing-based defence mechanism possessed by the rice varieties (Maule *et al.*, 2007). This is to ensure successful systemic invasion of the host plant (Scholthof, 2005). Strain 5 affected seriously all the varieties, including the resistant control Kalalu. This might be due to the reason that, RYMV strain S5 is both stable and highly virulent (Brugidou *et al.*, 2002). Observation by Waterhouse *et al.* (1999), indicated that, plants have resistance only to closely related virus strains while Alber *et al.* (2006), reported that, virulent isolates belonging to different RYMV strains were able to break the Rymv-1

resistance and that single substitution in Vpgs of RYMV was sufficient to break the resistance (Alber *et al.*, 2006).

Plate 6a: Plant height reduction on SARO-5 rice variety caused by Strain S5

Plate 6b: Plant height reduction on Kalalu rice variety caused by Strain S5

Table 6: Comparison of virulence of Rice Yellow Mottle Virus strains 4, 5 and 6 on three rice genotypes

RYMV STRAIN	Plant height	(P ≤ 0.05)	Number of tillers/plant	(P ≤ 0.05)	Dry matter per hill	(P ≤ 0.05)	D'se severity score
Strain 4	7.307c	3.696*	0.9591b	3.473*	3.702b	2.331*	5
Strain 5	5.970a	15.211***	0.7883a	10.574***	2.804a	14.960***	9
Strain 6	6.141b	13.743**	0.8179a	9.344***	3.702b	9.614**	7
S.E	1.5588		0.32336		0.3378		
CV (%)	23		35.9		10.00		

Means within column followed by the same letter(s) are not significantly different from each other according to DMRT at 5% level. Note: * = significance at P = 0.05,** = Highly significance at P = 0.01 and very highly significance at P = 0.001, NS = non significance difference, MS= Mean squares and CV (%) is coeffient of variation.

4.2 Effect of Rice Yellow Mottle disease on Rice Growth Parameters for Strain 5

4.2.1 Effect of Rice Yellow Mottle Virus strain 5 infection on plant height

Results from second experiment on plant height showed that there were highly significant (P ≤ 0.001) differences among the genotypes tested (Table 7). Mean comparison by t-test between inoculated and uninoculated plants revealed that, three (Nerica L-32, IR 00A118 and Kalalu) out of twenty five (25) rice genotypes showed very highly significant (P ≤ 0.001) difference between inoculated and uninoculated plants. Eight genotypes Mwangaza, IR 71030-4-5-5-5, IR 73887-1-8-2-1, IR 73885-1-4-3-2-1-4, IR 77470-3-3-3-2-2, Nerica-L 42, SARO 5 and IR 71028-3-1-2-2-3 showed high significant (P ≤ 0.01) difference between inoculated and uninoculated plants. The remaining fourteen (14) rice genotypes, including the susceptible control

Supa showed no significant difference between inoculated and uninoculated plants (Table 7). Reduction in plant height was observed to vary within the range of 8.22 to 39.12 %. This implies that different genotypes had different resistance levels to Rice Yellow Mottle Virus disease. But Nerica L-32 and Kalalu rice genotypes showed an increase in plant height upon inoculation by 40.76% and 30.6 %, respectively (Fig. 2). This implies that the effect of RYMV and other fungal pathogens stimulated growth in these varieties due to prolonged vegetative lag phase as well as anatomical and histological changes resulting from virus infection (Onwughalu *et al.*, 2010).

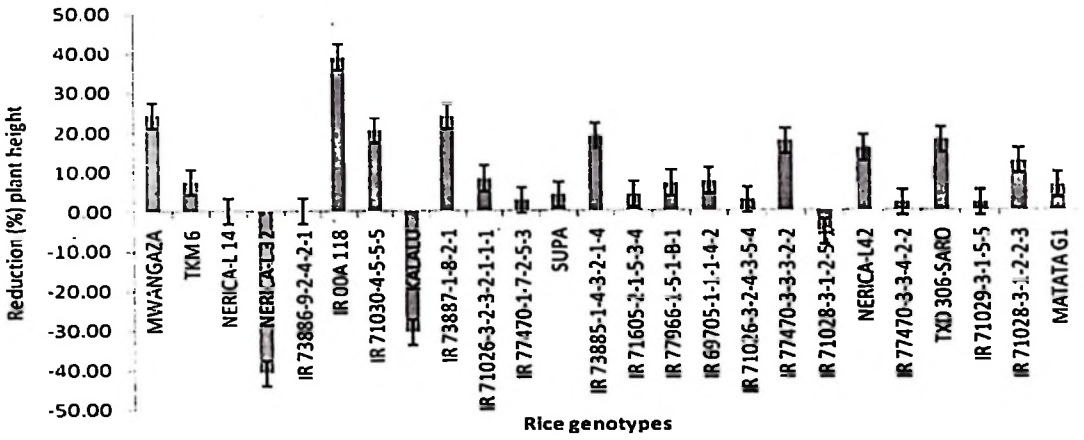


Figure 2: Percentage redution in plant height of rice genotypes caused by Rice Yellow Mottle Virus S5 infection

- Plants with negative plant height reduction, implied increase in plant height upon RYMV inoculum inoculation.

Table 7: Effect of Rice Yellow Mottle Virus disease on plant height of different rice genotypes for Strain 5

Rice genotypes	Plant height (cm)		(P ≤ 0.05)
	uninoculated	inoculated	
1.MWANGAZA	6.002gh	4.546df	7.702**
2.TKM 6	5.530ef	5.122il	2.159NS
3.NERICA-L 14	6.037h	6.031m	0.030NS
4.NERICA-L 32	3.900a	5.490l	8.298***
5.IR 73886-9-2-4-2-1	4.859bd	5.301jl	2.343NS
6.IR 00A 118	4.685bc	2.852a	9.315***
7. IR 71030-4-5-5-5	5.992gh	4.767ei	6.482**
8.KALALU	5.028cd	6.567n	8.142***
9.IR 73887-1-8-2-1	4.726bc	3.589b	6.013**
10.IR 71026-3-2-3-2-1-1-1	5.469ef	5.019gk	2.380*
11.IR 77470-1-7-2-5-3	4.996cd	4.862ej	0.696NS
12.SUPA	6.467i	6.203mn	1.406NS
13.IR 73885-1-4-3-2-1-4	5.662fg	4.586dg	5.712**
14.IR 71605-2-1-5-3-4	5.622fg	5.388kl	1.226NS
15.IR 77966-1-5-1-B-1	4.558b	4.238cd	1.692NS
16.IR 69705-1-1-1-4-2	5.221de	4.829ei	2.071NS
17.IR 71026-3-2-4-3-5-4	5.074cd	4.941fj	0.702NS
18.IR 77470-3-3-3-2-2	4.842bd	3.989c	4.515**
19.IR 71028-3-1-2-5-1	5.587ef	5.887m	1.586NS
20.NERICA-L42	4.998cd	4.204cd	4.203**
21.IR 77470-3-3-4-2-2	4.901bd	4.996gk	0.696NS
22.TXD 306-SARO	5.527ef	4.542df	5.213**
23.IR 71029-3-1-5-5	4.526b	4.613dh	0.460NS
24.IR 71028-3-1-2-2-3	5.758fh	5.034hk	3.835**
25.MATATA G1	4.787bc	4.480de	1.626NS
S.E.	1.4956	1.6109	
CV(%)	28.6	33.0	

Means within column followed by the same letter(s) are not significantly different from each other according to DMRT at 5% level. Note: * = significance at P = 0.05,** = Highly significance at P = 0.01 and *** = very highly significance at P = 0.001, NS = non significance difference and CV (%) is coefficient of variation.

4.2.2 Effect of Rice Yellow Mottle Virus strain 5 on number of tillers

The number of tillers per plant showed significant (P ≤ .001) differences among the tested genotypes (Table 8). However, the differences between inoculated and uninoculated plants for this parameter were not significantly different except for IR 71030-4-5-5-5 and IR 71028-3-1-2-2-3, IR 77470-3-3-4-2-2, IR 71026-3-2-4-3-5-4 and IR 71605-2-1-5-3-4. The effect of RYMV disease on reducing the number tillers per plant ranged from 0 to15 % (Fig. 3), the reduction in number of tillers could be

the result of reduced metabolites due to RYMV and fungal infection. Infected plants opted to complete their life cycles in expense of profuse tillering. IR 71605-2-1-5-3-4, Kalalu, Supa, IR 77966-1-5-B-1, IR 69705-1-1-1-4-2 and IR 71028-3-1-2-5-1 showed an increase in the number of tillers for inoculated plants as the result of RYMV infection. The results are in agreement with Methews (1991) who reported that, viruses cause hyperplasia (increased production of new tissues) that might have led to increased tillers in the inoculated rice plants. The results on rice plant height and number of tillers per plant were not consistent between inoculated and uninoculated rice genotypes. This might be due to multiple disease infection observed, which prompted to the identification work of the pathogens that caused the diseases.

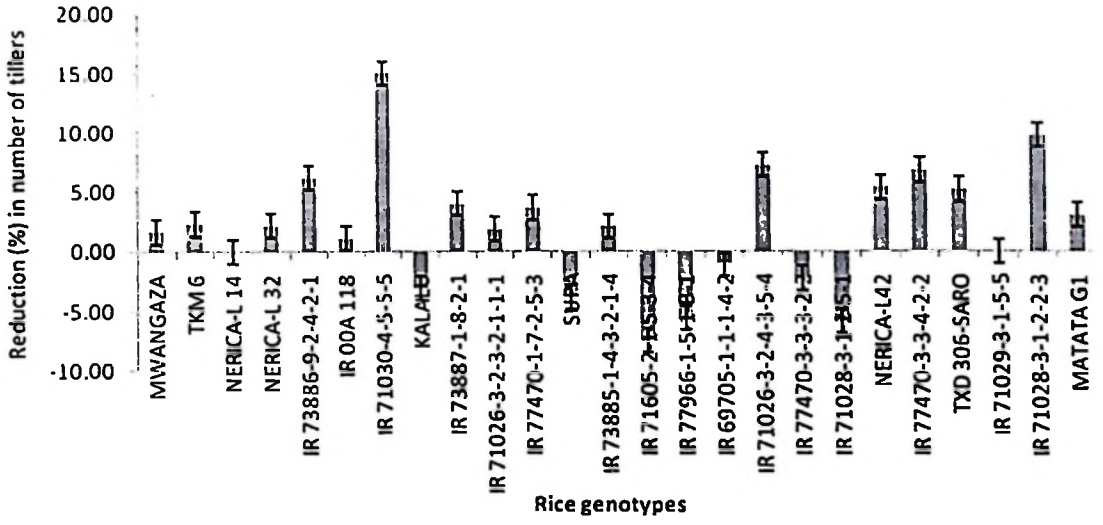


Figure 3: Percentage reduction in number of tillers of rice genotypes caused by Rice Yellow Mottle Virus strain 5

- Plants with negative number of tiller reduction, implied increase in number of tillers upon RYMV inoculum inoculation.

Table 8: Effect of Rice Yellow Mottle virus disease on tillering of different rice genotypes for Strain 5

Rice genotypes	Tillers/Plant		(P ≤ 0.05)
	Uninoculated	inoculated	
1.MWANGAZA	0.719a	0.7071a	0.190NS
2.TKM 6	0.7263ac	0.7432ac	0.839NS
3.NERICA-L 14	0.7109a	0.7109ab	0.00NS
4.NERICA-L 32	0.7109a	0.7263ab	0.761NS
5.IR 73886-9-2-4-2-1	0.7071a	0.7510ac	2.176NS
6.IR 00A 118	0.7148a	0.7071ab	0.380NS
7. IR 71030-4-5-5-5	0.8562g	0.7277ab	6.372***
8.KALALU	0.7071a	0.7289ab	1.082NS
9.IR 73887-1-8-2-1	0.7404ae	0.7109ab	1.462NS
10.IR 71026-3-2-3-2-1-1-1	0.7649bf	0.7505ac	0.713NS
11.IR 77470-1-7-2-5-3	0.7488ae	0.7212ab	1.366NS
12.SUPA	0.7071a	0.7342ac	1.344NS
13.IR 73885-1-4-3-2-1-4	0.7222ab	0.7071a	0.749NS
14.IR 71605-2-1-5-3-4	0.7666bf	0.8239e	2.813**
15.IR 77966-1-5-1-B-1	0.7342ad	0.7752cd	2.033NS
16.IR 69705-1-1-1-4-2	0.8671g	0.8762f	0.452NS
17.IR 71026-3-2-4-3-5-4	0.7716cf	0.7157ab	2.771**
18.IR 77470-3-3-3-2-2	0.7148a	0.7311ab	0.808NS
19.IR 71028-3-1-2-5-1	0.7625bf	0.8073de	2.223NS
20.NERICA-L42	0.7510ae	0.7109ab	1.986NS
21.IR 77470-3-3-4-2-2	0.7834ef	0.7301ab	2.640**
22.TXD 306-SARO	0.7637bf	0.7239ab	1.973NS
23.IR 71029-3-1-5-5	0.7109a	0.7109ab	0.00NS
24.IR 71028-3-1-2-2-3	0.8087f	0.7301ab	3.900***
25.MATATA G1	0.7781df	0.7546bc	1.165NS
S.E.	0.17022	0.16950	
CV(%).	22.7	21.7	

Means within column followed by the same letter(s) are not significantly different from each other according to DMRT at 5% level. Note: * = significance at P = 0.05,** = Highly significance at P = 0.01 and *** = very highly significance at P = 0.001, NS = non significance difference and CV (%) is coefficient of variation.

4.2.3 Rice Yellow Mottle Virus disease symptoms severity for Strain 5

Rice Yellow Mottle Virus disease severity was observed in the second experiment on the effect of Strain 5 on performance of rice genotypes. The RYMV disease scores for 7 genotypes included resistant controls (Mwangaza and Kalalu) and the tested rice genotypes IR 71029-3-1-5-5, IR 71605-2-1-5-3-4, IR 69705-1-1-1-4-2, IR 71028-3-1-2-5-1 and Matata G1 were 1 to 5 (Fig. 4), showed no significant difference between the inoculated and uninoculated plants. These rice genotypes showed faint or low

RYMV symptoms, this indicated that there were restricted multiplication and spread of the RYMV particles within the plant body in these rice genotypes. Similar results were reported by Ndjiondjop *et al.* (1999) that highly resistant rice varieties were characterized by absence of symptoms, low virus accumulation and blockage of virus movement. Similarly Jaw (2010) reported that virus content in susceptible and resistant lines using ELISA test showed to be closely related to visual scoring of symptoms. This also supports the truth that, the rice genotypes with lower disease severity score might be resistant to RYMV disease infection (IRR, 1996).

Contrary to this conclusion Michel *et al.* (2008) reported that, good selection for resistant or tolerant genotypes can not be limited to cultivar foliar reactions. Jaw *et al.* (2012) also reported that, symptoms in screening tests is not enough to distinguish lines for disease resistance, because symptoms can be masked in some cultivars and with some isolates. Therefore, there is a need to further undertake research on these rice genotypes found to be resistant or tolerant to confirm their reactions. From the second experiment on the effect of RYMV strain 5 on rice genotypes, nineteen (19) out of 25 rice genotypes tested including the susceptible controls (SARO 5 and Supa) showed disease severity score of 7 and 9, indicating susceptible to highly susceptible reactions. This implied that these genotypes possess all the components necessary for viral replication and systemic infection. Therefore, they were unable to restrict the multiplication and spread of virus within the plant (Dawson *et al.*, 1992). Opalka *et al.* (1997) also reported that RYMV in susceptible rice genotypes can spread systemically in leaf cells and rapidily spread in all actively growing regions, on the

expense of the host's photosynthetic products. This is similar reports by Paul, (2003) that much damage on susceptible varieties was a result of RYMV infection.

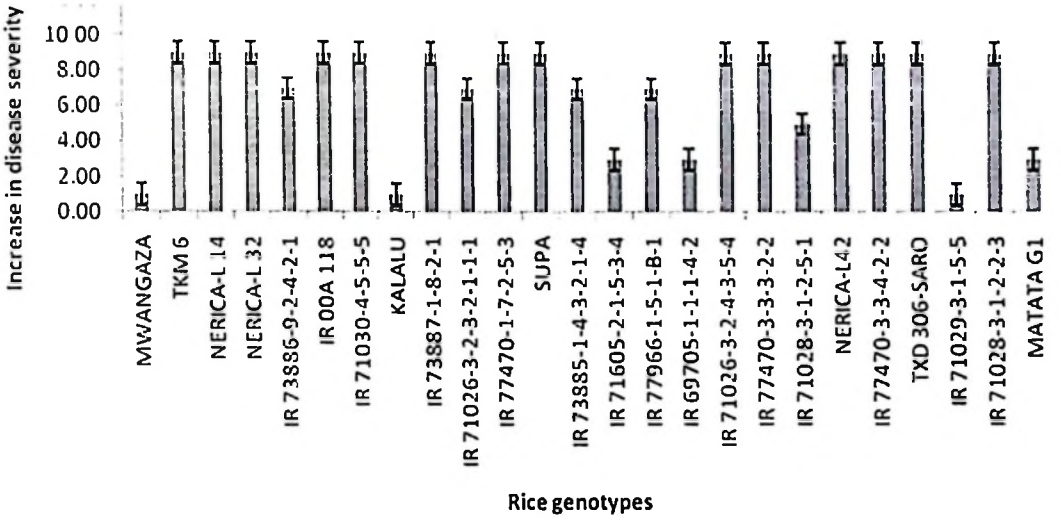


Figure 4: Rice Yellow Mottle Virus disease severity symptoms on rice genotypes caused by Strain 5

4.2.4 Identification of other rice pathogens

Following multiple disease infection observed in the second experiment, identification of disease causing pathogens was done by culturing rice leaf and stem samples (Plate 6a and 6b) in water soaked blotter (Plate 7a, 7b, 7c and 7d). Results indicated the presence of several fungal disease causing pathogens, which included *Fusarium moniliforme* that causes seedling blight/damping off followed by *Verticillium sp.* which causes Verticillium wilt disease in rice, *Bipolaris oryzae* which causes brown spot disease and *Magnoportheca oryzae* (rice blast) (Plate 7a, 7b, 7c and 7d).

The disease causing pathogens, largely interfered with the growth of the test plants in the screen house as a result, the experiment could not reach maturity. The presence of RYMV in the rice plants might have also encouraged the wide spread of secondary infection. This was also reported by Fomba *et al.* (1989) that, secondary effects of RYMV infection included increased severity of fungal infections such as brown spot (*Cochliobolus miyabeanus*), leaf scald (*Monographella albescens*), sheath rot (*Sarocladium oryzae*) and sheath blotch (*Pyrenochaeta oryzae*). Application of Copper sulphate powder to seeds prior to sowing and post emergence application of Dithane at 50mg/20Lts of water, aimed at reducing fungal and bacterial disease causing pathogens did not have any effect on disease development.

Therefore, it can be concluded that, apart from RYMV disease there were other pathogens such as *Fusarium moniforme, Bipolaris oryzae, Magnoportheca oryzae and Verticillium sp* which caused serious yield loss. The observed secondary infection to the inoculated rice genotypes in the screen house was probably due to residue viable spores of fungi from previous experiments, seed borne pathogens that could not be killed by chemical application and wind movement in and out of the screen house that moved spores from other areas.

Plate 7a **Plate 7b**

Plate 7a and 7b: Infected rice plant leaf and stem samples by fungal pathogens

Plate 7c: Plated rice leaf samples by blotter paper method for identification

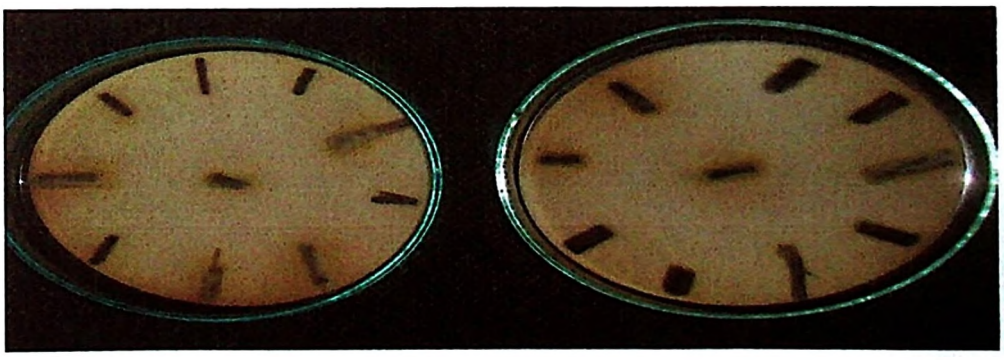


Plate 7d: Germinating culture of fungi on rice leaf and stem samples for identification of secondary infecting pathogens on rice genotypes

The Blotter paper method was preferred to Nutrient agar in that, it is cheap, reliable and allow few specific pathogen to grow, which have infected that particular specific host (Toma *et al.*, 2013). Therefore, the chances for contamination are less with Blotter paper method than Nutrient agar.

4.3 Effect of Rice Yellow Mottle Virus strain 4 on Rice Growth Parameters (Third experiment)

The effect of RYMV disease on plant height and number of tillers per plant among the 31 tested rice genotypes, revealed that there were statistically significant variations in responses among the rice genotypes tested (Table 9 and 10). This implies that different rice genotypes responded differently to RYMV disease as the result of genotypic differences among them.

4.3.1 Effect of Rice Yellow Mottle Virus strain 4 on Plant height

Comparison between inoculated and uninoculated rice plants on plant height (Table 9) indicated that, three rice genotypes were very highly significant ($P \leq 0.001$) different. Significant ($P \leq 0.01$) differences on plant height were also observed on 19 rice genotypes which included Mwangaza (resistant control) and Supa and SARO 5 (susceptible controls). Other 10 rice genotypes showed significant ($P \leq 0.05$) difference to non-significant difference in terms of their plant heights. Rice genotypes showed non significant difference were Matata G2, Adday Sel, Nerica L26, IR 69705-1-1-3-2-3 and Kalalu. Height reduction of inoculated rice plants that showed significant ($P \leq 0.05$) difference ranged from 12.0 to 63.7 %, while height reduction of inoculated plants that showed non significant ($P \leq 0.05$) difference ranged from 0.1

and 3.9 % (Fig. 5). The rice genotypes which showed non-significant difference reacted the same as Kalalu (resistant control) upon RYMV inoculation. These genotypes showed high ability to resist the effects of RYMV invasion, possibly by inhibiting the replication and spread of the virus, since viruses depend intimately on plant regulatory and metabolic processes to multiply and spread within the host (Dawson *et al.*, 1992).

Observations on resistant control (Mwangaza) indicated that there was severe reduction in rice plant height. This was possibly due to attack by bacterial leaf blight observed on the plant leaves two weeks after inoculation. One of the pots planted with Mwangaza was seriously affected by bacterial leaf blight, this might have contributed to reduction in plant height. The other tested rice genotypes on plant height reacted the same as the susceptible controls (Supa and SARO-5), which were seriously affected by RYMV disease. This implies that RYMV particles were able to multiply in the plant and spread throughout all the important food producing sites and actively growing parts of plant (Dawson *et al.*, 1992). This might have led to reduction in dry matter production and partitioning, ultimately reduced plant height. Alber *et al.* (1998), Kouassi *et al.* (2005) and Kanyeka *et al.* (2007) reported that plant height reduction and yield loss was due to RYMV disease. Onwughalu *et al.* (2010) also reported similar observation that plant height was significantly affected by virus infection at seedling stage.

Table 9: Effect of Rice Yellow Mottle Virus disease on rice plant height for Strain 4 from third experiment

Rice genotypes	Plant height (cm)		(P ≤ 0.05)
	uninoculated	inoculated	
1.IRR 134	7.337fj	6.030gl	4.253**
2.TXD 306-SARO	7.405fj	5.399ck	5.995**
3.IR 69705-1-1-3-3-5	7.882im	6.528kl	4.187**
4.IR 72928-1-2-4-1-1-3	8.061im	3.504ab	14.093***
5.IR 73885-1-4-3-2-1-10	6.989eh	5.916gl	3.319**
6.MATATA G2	7.804a	7.812m	0.023NS
7.NERICA-L26	5.945ac	5.980gl	0.107NS
8.IR 69705-1-1-3-2-3	6.585ce	6.322jl	0.812NS
9.IR 69704-4-4-4-8-1-1-1-1	7.677hl	6.343jl	4.126**
10.IR 69704-4-8-1-5-1-3	7.274ei	5.039ci	6.911**
11.IR 69704-4-8-2-1-1-2	7.640gl	5.004ch	8.151**
12.IR 1561-228-3-3	5.680ab	4.848cg	2.629*
13.ADDAY SEL	6.722df	7.137lm	1.284NS
14.IR 69734-81-1-1	7.560gk	5.535dk	6.262**
15.IR 71605-2-1-1-3-10	7.368fj	4.336bd	9.375***
16.ITRI MERAH	5.995ac	4.146bc	5.408**
17.IR 71027-43-3-2-B-3	7.640gl	2.776a	15.041***
18.IR 69734-16-1-1	6.685df	5.882fk	2.484*
19.KALALU	5.452a	5.440ck	0.037NS
20.MWANGAZA	8.395km	6.226hl	5.999**
21.IR 71605-3-1-1-2-6	7.005eh	6.022gl	3.042*
22.TN 1	7.937jm	5.384ck	8.293***
23.IR 81244	7.937im	4.664bf	10.121**
24.UTRI JAPAN	8.418lm	6.588km	5.659**
25.IR 69705-1-1-3-2-1	7.269ei	5.680ek	4.805**
26.IR 72955-6-1-3-4-1	8.460m	6.257il	6.810**
27.SUPA	8.570m	5.197cj	10.430***
28.UTRI MERAH	7.429fj	5.680ek	5.409**
29.IR 71030-2-2-1-1	6.020ac	4.555be	4.528**
30.IR 73887-1-8-3-5	6.191bd	5.206cj	3.254*
31.IR 72928-1-2-4-1	6.888eg	4.629be	6.988***
SED	2.3964	2.4299	
CV(%)	33.6	44.3	

Means within column followed by the same letter(s) are not significantly different from each other according to DMRT at 5% level. Note: * = significance at P = 0.05,** = Highly significance at P = 0.01 and very highly significance at P = 0.001, NS = non significance difference and CV (%) is coefficient of variation.

- From above there is high coefficient of variation CV (%), though data transformation was done, this might have been contributed by rice genetical differences and the effect of RYMV disease among rice genotypes tested.

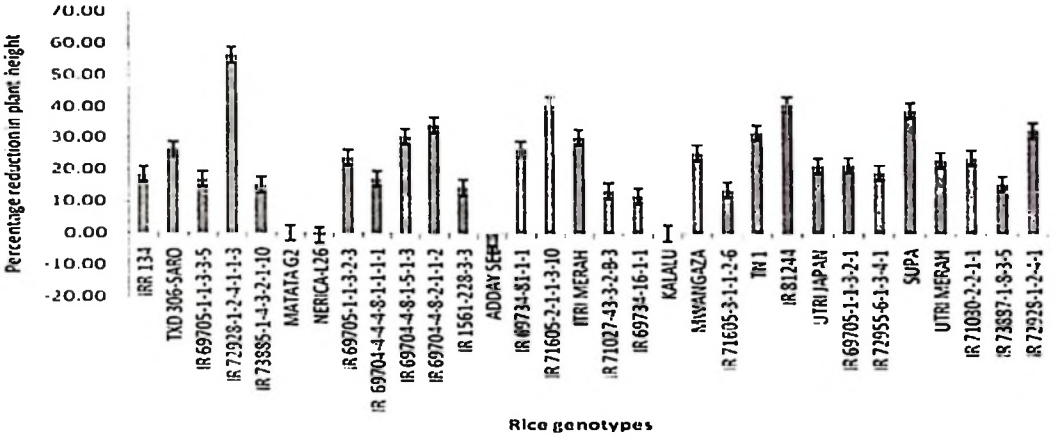


Figure 5: Percentage reduction in plant height of rice genotypes caused by Rice Yellow Mottle Virus strain 4

4.3.2 Effect of Rice Yellow Mottle Virus strain 4 infection on number of tillers

The number of tillers per plant indicated very highly significant ($P \leq 0.001$) difference among the 31tested rice genotypes (Table 10). Mean comparison between inoculated and uninoculated plants on the tested rice genotypes indicated that, six (6) out of 31 rice genotypes showed very highly significant ($P \leq 0.001$) difference on number of tillers per plant including SARO 5 (susceptible control) where as twelve (12) genotypes showed highly significant ($P \leq 0.01$) difference in terms of number of tillers. The other 13 rice genotypes showed significant ($P \leq 0.05$) differences to non-significant differences on number of tillers per plant (Table 10). The genotypes which showed non-significant differences were IRR 134, Matata G2, IR 73885-1-4-3-2-1-10, Nerica L26, IR 69704-4-4-4-8-1-1-1, Adday Sel, IR 73887-1-8-3-5, IR 71605-3-1-1-2-6 as well as Kalalu and Mwangaza (resistant controls).

Reduction of number of tillers for inoculated plants that showed significant ($P \leq 0.05$) differences ranged from 22.38 to 47.93 % , while reduction of number of tillers of

inoculated rice plants that showed non significant ($P \leq 0.05$) differences ranged from 0.69 to 18.06 % (Fig. 6).

Significant variation between inoculated and uninoculated rice plants observed among the tested 31 rice genotypes, on number of tillers might be due to RYMV infection. The variation of the effect was not uniform among the tested rice genotypes possibly due to varietal genetic differences.

Pinto *et al.* (1999) and Mogga *et al.*, (2012) reported that the effect of RYMV on tillering was reductions in the number of tillers of susceptible rice genotypes. Jaw *et al* (2012) also observed similar result that, there were significant reductions in number of tillers among the susceptible lines upon infection by RYMV. The rice genotypes IRR 134, MATAT G2, IR 69704-4-4-4-8-1-1-1-1, Adday Sel and IR 73887-1-8-3-5, showed non-significant variation in tillering, implying that the virus was unable to affect the ability of these genotypes to produce tillers. These genotypes underwent less percentage reduction in tillering compared to susceptible controls.

Rice genotype IR 73887-1-8-3-5 showed non-significant ($P \leq 0.05$) difference on reduction of number of tillers, but it showed a disease severity score of 9. The reason might be that, infection by RYMV to this rice genotype had led to increased production of tillers, in order to increase the survival ability of this plant. The observation agrees with Mathews, (1991) who reported that, the effect of virus on plants being abnormal division of cambial cells which might lead to increased tillers production.

Table 10: Effect of Rice Yellow Mottle Virus disease on the number of tillers for Strain 4

Rice genotypes	Number of tillers/plant		(P ≤ 0.05)
	uninoculated	inoculated	
1.IRR 134	1.683ce	1.384ck	2.750NS
2.TXD 306-SARO	1.940eh	1.174ah	7.016***
3.IR 69705-1-1-3-3-5	2.225ij	1.724kl	4.588**
4.IR 72928-1-2-4-1-1-3	1.720ce	0.982ac	6.763***
5.IR 73885-1-4-3-2-1-10	1.625cd	1.364dk	2.394NS
6.MATATA G2	2.095cd	1.929l	1.524NS
7.NERICA-L26	1.601cd	1.514gk	0.799NS
8.IR 69705-1-1-3-2-3	2.095eh	1.626jl	2.620**
9.IR 69704-4-4-4-8-1-1-1-1	1.586cd	1.575il	0.096NS
10.IR 69704-4-8-1-5-1-3	2.127hj	1.191ah	8.577***
11.IR 69704-4-8-2-1-1-2	2.044fj	1.348cj	6.377**
12.IR 1561-228-3-3	1.717ce	1.056ae	6.054**
13.ADDAY SEL	1.496bc	1.525hk	0.263NS
14.IR 69734-81-1-1	2.348j	1.488gk	7.889**
15.IR 71605-2-1-1-3-10	1.843dg	1.169ah	6.178**
16.ITRI MERAH	1.278b	0.892a	5.059**
17.IR 71027-43-3-2-B-3	1.934eh	1.007ad	8.502***
18.IR 69734-16-1-1	2.007fi	1.449gk	5.117*
19.KALALU	0.979a	0.962ab	0.157NS
20.MWANGAZA	1.017a	0.929ab	0.721NS
21.IR 71605-3-1-1-2-6	1.626cd	1.566il	0.551NS
22.TN 1	2.113g j	1.435lk	6.215**
23.IR 81244	1.833df	1.282bj	5.059**
24.UTRI JAPAN	2.051fi	1.226ai	7.567***
25.IR 69705-1-1-3-2-1	2.006fi	1.544il	4.145*
26.IR 72955-6-1-3-4-1	1.676ce	1.164ah	4.698**
27.SUPA	1.4480bc	1.151ag	3.022*
28.UTRI MERAH	1.843dh	1.089af	7.065***
29.IR 71030-2-2-1-1	1.532bc	1.063ae	4.294**
30.IR 73887-1-8-3-5	1.777df	1.485ab	2.866NS
31.IR 72928-1-2-4-1	1.683ce	1.226ai	4.309**
CV(%)	33.1	35.8	
SED	0.8895	0.7053	

Means within column followed by the same letter(s) are not significantly different from each other according to DMRT at 5% level. Note: * = significance at P = 0.05, ** = Highly significance at P = 0.01 and *** = very highly significance at P = 0.001, NS = non significance difference and CV (%) is coefficient of variation.

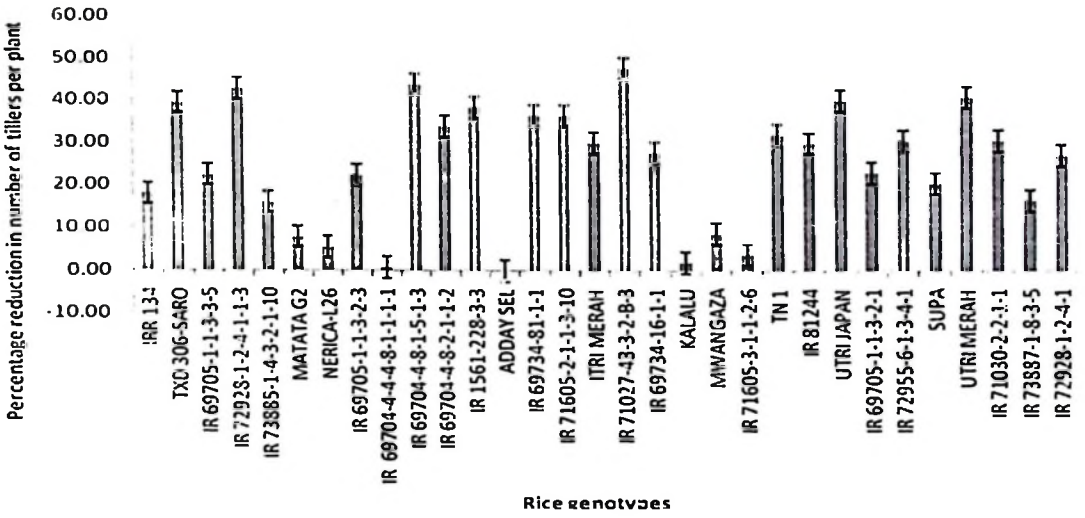


Figure 6: Percentage reduction in number of tillers of rice genotypes caused by Rice Yellow Mottle Virus strain 4

4.3.3 Rice Yellow Mottle Virus disease symptoms for Strain 4

Rice Yellow Mottle Virus disease severity scores showed that, there were significant variations in plant reactions among 31 tested rice genotypes (Fig. 7). Five rice genotypes included IRR 134 (Plate4b), Matata G2, Adday SeL (Plate 4c), IR 71027-43-3-2-B-3 showed no significant difference between inoculated and uninoculated plants. The maximum disease severity score was 3, indicating highly resistant rice genotypes as the resistant control (Plate 4a). Kalalu and IR 69704-4-4-4-8-1-1-1 rice genotypes showed significant ($P \leq 0.01$) difference between inoculated and uninoculated plants and had a maximum score of 5 indicating moderate resistance. Michel *et al.* 2008 reported that a RYMV disease resistant variety had no any visible symptom of the disease and no reduction of grain yield. This is due to inhibition of virus multiplication and a movement within the host plant (Ghesqueiere *et al.*, 1997: Ndjiondjop *et al.*, 1999). Kalalu and IR 69704-4-4-4-8-1-1-1 showed symptoms, though low compared to susceptible controls implying that multiplication and spread

of viruses were restricted to some extent in these genotypes, this might have led to delayed symptoms appearance and virus accumulation (Ioanidou *et al.*, 2000: Fargette *et al.*, 2002).

Twenty four rice genotypes (Fig. 7) showed very highly significant ($P \leq 0.001$) difference between inoculated and uninoculated plants with scores of 7 and 9 indicating that they were moderately to highly susceptible, this included IR 72928-1-2-4-1-1-3 and Nerica-L 26 (Plate 3a and 3b) as susceptible controls SARO-5 and Supa. It is reported that virus invades the plant, induce symptoms and reduce yield (Dawson *et al.*, 1992). Similarly, Mogga *et al.* (2012) observed strong correlation between the mean disease score and reduction in grain weight where as N'Guessan, (2001) observed significant relationship between symptom intensity and yield loss. This was due to reduced photosynthetic activities as a result of change in chloroplast structure caused by reduced content of photosynthetic pigments (Matthews, 1991).

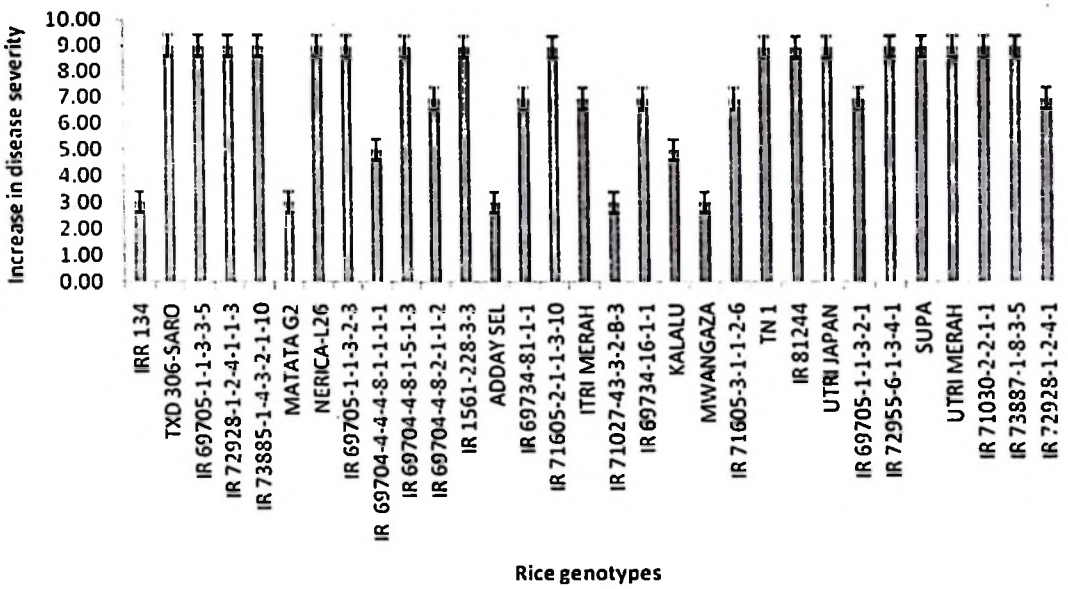


Figure 7: Increase in Rice Yellow Mottle Virus disease severity on rice genotypes caused by Strain 4

4.3.4 Effect of Rice Yellow Mottle Virus disease on rice yield components

The effect of RYMV disease on panicle exertion, panicle sterility and grain weight showed significant variation among the 31 rice genotypes tested. This implies that partial panicle exertion, increased panicle sterility and reduced grain weight will lead to yield loss.

4.3.4.1 Effect of Rice Yellow Mottle Virus strain4 infection on rice panicle exertion

Panicle exertion varied significantly ($P \leq 0.05$) different among 31 rice genotypes inoculated with RYMV while non-significant difference were observed among uninoculated plants (Table 11). Comparison between inoculated and uninoculated plants showed that, twenty four (24) rice genotypes showed significant ($P \leq 0.05$) difference on panicle exertion.

The panicle exertion percentage differences ranged from 41.51 to 99.29 % with IR 72928-1-2-4-1-1-3, Supa and SARO-5 being among the genotypes that showed poorly exerted particles (Fig. 8). The reason for that significant variation in panicle exertion observed was probably due to RYMV infection on the different rice genotypes. This indicated that, RYMV disease was able to affect yield through reducing or delaying the panicle to open fully and reach maturity.

In such situation the rice grain filling stage is either delayed or inhibited to an extent that, the panicle produced did not reach maturity normally as uninfected rice genotypes (Pinto *et al.*, 1999). These conditions lead to immature seeds and incomplete panicle exertion (Pinto *et al.*, 1999). Onwughalu *et al.* (2011) reported that virus infection delayed flowering which might be the reason for low percentage panicle exertion in rice genotypes. Delayed flowering and incomplete panicle exertion might be a result of host resource competition between host itself and the invading virus. This agrees with what has been reported by Onwughalu *et al.* (2011) that poor exerted panicles bearing sterile and discoloured spikelets are associated with the RYMV disease. The study reported by Nwilene *et al.* (2008) also showed that infected rice plants exhibited delayed flowering with poorly exerted panicles.

The remaining seven rice genotypes tested showed non-significant variation between inoculated and uninoculated plants on panicle exertion. The genotypes included Mwangaza, Kalalu, Matata G2, IRR 134, IR 69704-4-4-4-8-1-1-1, Adday Sel, IR 71027-43-3-2-B-3 (Table 11). These genotypes had 0 to 31.35 % difference in panicle exertion between inoculated and uninoculated plants (Fig. 8). Since these genotypes

had similar reaction to the resistant controls, it is therefore possible that, the genotypes might be possessing resistant genes that have the ability of inhibiting the effect of rice yellow mottle virus infection (Nwilene *et al.*, 2008).

Table 11: Effect of Rice Yellow Mottle Virus disease on panicle exertion of different rice genotypes for Strain 4

Rice genotypes	Percent (%) panicle exertion		t-test
	Inoculated	Uninoculated	
1.IRR 134	8.622fh	9.923b	0.882NS
2.TXD 306-SARO	2.840ad	10.025b	4.875***
3.IR 69705-1-1-3-3-5	3.987af	10.025b	4.096***
4.IR 72928-1-2-4-1-1-3	0.707a	10.025b	6.322***
5.IR 73885-1-4-3-2-1-10	4.463af	10.025b	3.774**
6.MATATA G2	9.806h	9.890b	0.057NS
7.NERICA-L26	4.517af	9.792b	3.579**
8.IR 69705-1-1-3-2-3	2.864ad	9.967b	4.820***
9.IR 69704-4-4-4-8-1-1-1-1	9.353gh	10.025b	0.456NS
10.IR 69704-4-8-1-5-1-3	2.335ac	9.681b	4.984***
11.IR 69704-4-8-2-1-1-2	5.571bh	9.803b	2.872**
12.IR 1561-228-3-3	0.707a	10.025b	6.322***
13.ADDAY SEL	10.025h	10.025b	0.00NS
14.IR 69734-81-1-1	1.607ab	10.025b	5.711***
15.IR 71605-2-1-1-3-10	4.919ch	9.141ab	3.507***
16.ITRI MERAH	2.312ac	9.883b	5.711***
17.IR 71027-43-3-2-B-3	5.853bh	8.526a	1.814*
18.IR 69734-16-1-1	6.842ch	9.952b	2.110*
19.KALALU	9.697h	9.580b	0.080NS
20.MWANGAZA	10.025h	9.895b	0.088NS
21.IR 71605-3-1-1-2-6	8.053eh	9.885b	1.243NS
22.TN 1	2.623ad	10.025b	5.022***
23.IR 81244	7.330dh	9.212ab	1.277**
24.UTRI JAPAN	5.250ah	10.025b	3.240**
25.IR 69705-1-1-3-2-1	5.864bh	10.025b	2.823**
26.IR 72955-6-1-3-4-1	3.800ae	8.561a	3.230**
27.SUPA	0.707a	9.904b	6.240***
28.UTRI MERAH	7.896eh	10.025b	1.444*
29.IR 71030-2-2-1-1	0.707a	10.025b	6.322***
30.IR 73887-1-8-3-5	5.325ah	9.912b	3.112**
31.IR 72928-1-2-4-1	4.717ag	10.025b	3.601**
CV (%)	47.9	35.6	
SED	2.4992	0.5524	

Means within column followed by the same letter(s) are not significantly different from each other according to DMRT at 5% level. Note: * = significance at P = 0.05,** = Highly significance at P = 0.01 and *** = very highly significance at P = 0.001, NS = non significance difference and CV (%) is coefficient of variation.

- From above there is high coefficient of variation CV (%), though data transformation was done, this might have been contributed by rice genetical differences and the effect of RYMV disease among rice genotypes tested.

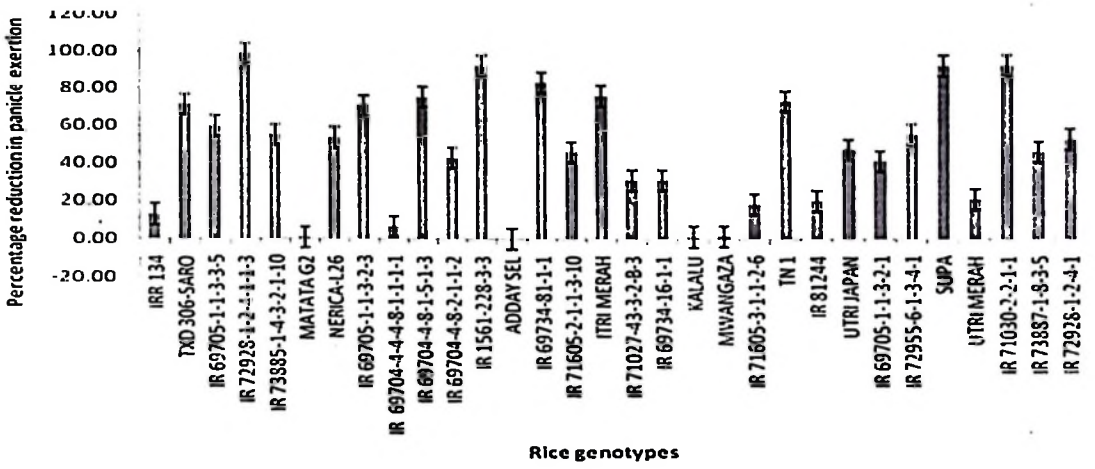


Figure 8: Percentage reduction in panicle exertion of rice genotypes caused by Rice Yellow Mottle Virus strain 4 infection

4.3.4.2 Effect of Rice Yellow Mottle Virus strain 4 infection on rice panicle sterility

Panicle sterility among inoculated rice genotypes showed significant ($P \leq 0.001$) differences (Table 12). Comparison test on panicle sterility between inoculated and uninoculated plants indicated that, eleven (11) rice genotypes showed very highly significant ($P \leq 0.001$) difference where as fifteen (15) rice genotypes indicated highly significant ($P \leq 0.01$) difference on panicle sterility between inoculated and uninoculated. The percentage mean differences between inoculated and uninoculated plants ranged from 35 to 62 % (Fig. 9), indicating that, RYMV increased percentage panicle sterility among inoculated plants. The variation on panicle sterility may be due to varietal genetic differences, since different rice genotype responded differently upon inoculation. Nwilene *et al.* (2008) reported that RYMV disease causes delayed flowering with poorly exerted panicles and spikelet sterility in rice. These results are also in accordance to what Pinto *et al.* (1999) reported, that RYMV disease caused panicle sterility.

Rice genotypes IRR 134, MATATA G2, Kalalu, Adday Sel and IR 71027-43-3-2-B-3 showed non-significant ($P \leq 0.05$) difference on panicle sterility. The percentage mean difference between inoculated and uninoculated plants on panicle sterility ranged from 4.05 to 22.14 % (Fig. 10). This implies that the genotypes showed high ability to resist the virus load upon infection. Alber *et al.* (2006) and Paul, (2003). reported that resistance to RYMV infection in *Oryza spp.* is controlled by few major recessive genes. Resistance in rice genotypes is characterised by mild RYMV disease symptom expression and limited yield loss (Ghesquiere *et al.*, 1997). Therefore, these above named genotypes might be possessing genes for resistance against RYMV.

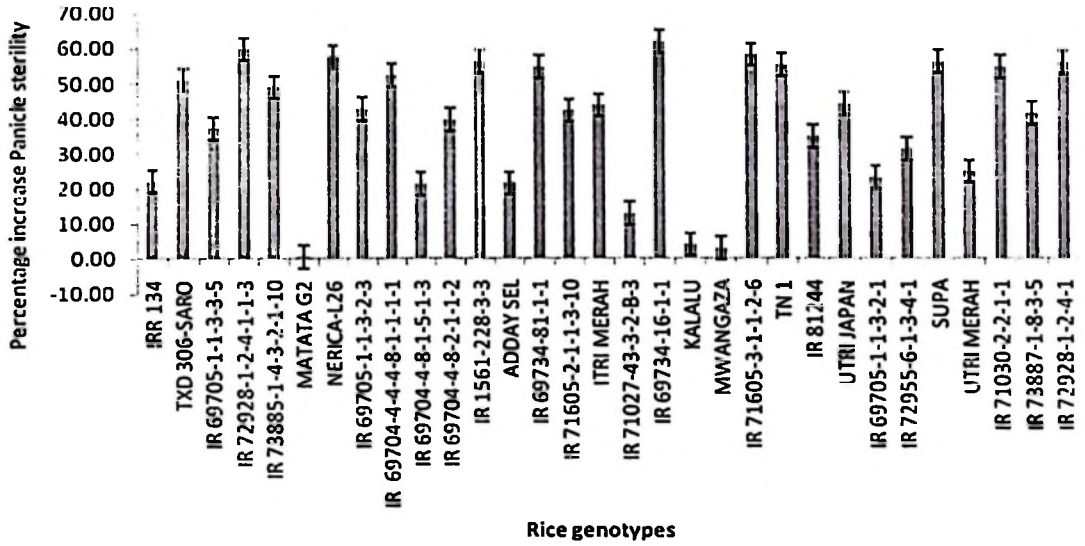


Figure 9: Percentage increase in panicle sterility of rice genotypes caused by Rice Yellow Mottle Virus strain 4

Table 12: Effect of Rice Yellow Mottle Virus disease on panicle sterility of different rice genotypes for Strain 4

Rice genotypes	Percent (%) panicle sterility		(P ≤ 0.05)
	Inoculated	uninoculated	
1.IRR 134	5.135ac	3.574ac	1.137NS
2.TXD 306-SARO	8.220bf	4.017ad	2.816***
3.IR 69705-1-1-3-3-5	8.938df	5.606ad	2.427**
4.IR 72928-1-2-4-1-1-3	10.025f	4.017ac	4.376***
5.IR 73885-1-4-3-2-1-10	7.285bf	3.713ac	2.602**
6.MATATA G2	6.051ad	6.399cd	0.253NS
7.NERICA-L26	8.736cf	3.700ac	3.668***
8.IR 69705-1-1-3-2-3	9.288df	5.341ad	2.875**
9.IR 69704-4-4-4-8-1-1-1-1	7.827bf	3.719ac	2.993**
10.IR 69704-4-8-1-5-1-3	8.009bf	6.297bd	1.296NS
11.IR 69704-4-8-2-1-1-2	8.795df	5.285ad	2.556*
12.IR 1561-2-2-8-3-3	10.025f	4.357ad	4.128***
13.ADDAY SEL	5.094ab	3.993ac	0.802NS
14.IR 69734-81-1-1	10.001f	4.509ad	4.00***
15.IR 71605-2-1-1-3-10	8.494bf	4.893ad	2.623**
16.ITRI MERAH	9.838ef	5.515ad	3.149**
17.IR 71027-43-3-2-B-3	8.024bf	6.977d	0.695NS
18.IR 69734-16-1-1	8.674bf	3.278a	3.950***
19.KALALU	6.297ae	6.042ad	0.186NS
20.MWANGAZA	3.363a	3.949ad	0.155NS
21.IR 71605-3-1-1-2-6	8.035bf	3.340ab	3.419***
22.TN 1	8.132bf	3.615ac	3.290***
23.IR 81244	8.840df	5.751ad	2.250*
24.UTRI JAPAN	8.768df	4.886ad	2.827**
25.IR 69705-1-1-3-2-1	7.207bf	5.539ad	1.215*
26.IR 72955-6-1-3-4-1	7.984bf	5.485ad	1.820*
27.SUPA	9.500df	4.142ac	3.970***
28.UTRI MERAH	7.214bf	5.418ad	1.308*
29.IR 71030-2-2-1-1	10.025f	4.509ad	3.924***
30.IR 73887-1-8-3-5	6.633af	3.868ac	2.013**
31.IR 72928-1-2-4-1	8.322bf	3.655ac	3.460***
CV(%)	30.0	23.1	
SED	1.855	1.422	

Means within column followed by the same letter(s) are not significantly different from each other according to DMRT at 5% level. Note: * = significance at P = 0.05,** = Highly significance at P = 0.01 and *** = very highly significance at P = 0.001, NS = non significance difference and CV (%) is coefficient of variation.

4.3.4.3 Effect of Rice Yellow Mottle Virus strain 4 on rice grain weight

Results on grain weight reduction among the 31 tested rice genotypes showed very highly significant (P ≤ 0.001) differences, implying that there were variations in response among genotypes (Table 13). Comparison between inoculated and

uninoculated plants showed that, twenty (20) rice genotypes had highly significant ($P \leq 0.05$) differences on grain weight reduction, this included the susceptible controi SARO-5 and Supa rice genotypes. Percentage grain weight reduction ranged from 48.00 to 86.02 % (Fig. 10). This result indicates that the virus was able to inflict disease to the plant, which resulted in failure of the plant in partitioning of biomass produced to the developing seeds causing a reduction in grain weight. The same results have been reported by Michel *et al.* (2008) that, rice varieties infected with RYMV disease resulted in reduction of seed weight. Similarly Onwughalu *et al.* (2011) observed a 1 000 grain weight loss ranging from 17 to 100 %. The reason for grain weight differences might be caused by virus infection which affects starch deposited in the endosperm (Michel *et al.*, 2008).

Eleven rice genotypes showed non-significant ($P \leq 0.05$) differences between inoculated and uninoculated plants (Table 13). These genotypes showed weight reduction ranging from 1.01 to 43.69 % (Fig. 10). This indicated that these rice genotypes were resistant and less affected by RYMV disease, possibly the RYMV particle were unable to interfere with the portioning of biomass toward developing seeds. This agrees with observations reported by Onwughalu *et al.* (2011) that, resistant rice varieties showed no decrease in grain weight. Michel *et al.* (2008) observed that, symptom severities were related to grain weight reduction which was varietal related in the test rice varieties. He proposed evaluation on the effects of RYMV disease using couple of symptoms and grain weight criteria on different rice genotypes. Ndjiondjop *et al.* (1999) reported that, the severity RYMV disease symptoms was associated with increased in ELISA absorbance in infected leaves.

By using the above results on grain weight, symptoms, panicle exertion and sterility from third experiment, the following rice genotypes can be concluded to be possessing genes that confer resistance to RYMV disease, Matata G1, IRR 134, Matata G2, IR 69704-4-4-4-8-1-1-1, Adday Sel and IR 71027-43-3-2-B-3.

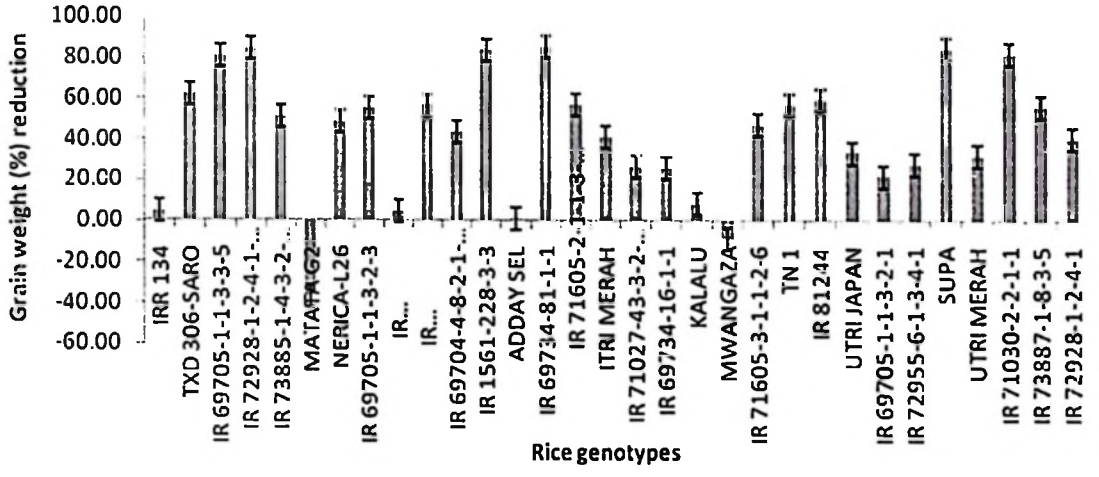


Figure 10: Percentage reduction in grain weight of rice genotypes caused by Rice Yellow Mottle Virus strain 4

Table 13: Effect of Rice Yellow Mottle Virus disease on grain weight of different rice genotypes for Strain 4

Rice genotypes	1000 grain weight (gm)		(P ≤ 0.05)
	inoculated	uninoculated	
1.IRR 134	4.411bc	4.628c	0.225NS
2.TXD 306-SARO	1.961ab	5.163c	2.757***
3.IR 69705-1-1-3-3-5	0.859a	4.527bc	3.796***
4.IR 72928-1-2-4-1-1-3	0.707a	4.673c	4.00***
5.IR 73885-1-4-3-2-1-10	2.240ab	4.582bc	2.421**
6.MATATA G2	4.673bc	3.42ab	1.287NS
7.NERICA-L26	2.425ab	4.747c	2.401**
8.IR 69705-1-1-3-2-3	1.981ab	4.450bc	2.553**
9.IR 69704-4-4-4-8-1-1-1-1	4.621bc	4.855c	0.242NS
10.IR 69704-4-8-1-5-1-3	2.063ab	4.799c	2.828**
11.IR 69704-4-8-2-1-1-2	2.484ab	4.412bc	1.994**
12.IR 1561-2-2-8-3-3	0.707a	4.553bc	3.976***
13.ADDAY SEL	4.793bc	4.842c	0.050NS
14.IR 69734-81-1-1	0.707a	5.057c	4.497***
15.IR 71605-2-1-1-3-10	1.994ab	4.678c	2.775**
16.ITRI MERAH	1.862ab	3.172a	1.862**
17.IR 71027-43-3-2-B-3	3.114ac	4.254bc	1.179NS
18.IR 69734-16-1-1	4.788bc	5.146c	0.370NS
19.KALALU	3.359ac	4.700c	0.386NS
20.MWANGAZA	5.752c	5.299c	0.468NS
21.IR 71605-3-1-1-2-6	4.359bc	4.471bc	0.116NS
22.TN 1	2.033ab	4.759c	2.819**
23.IR 81244	1.898ab	4.718c	2.915**
24.UTRI JAPAN	3.063ac	4.627c	1.617*
25.IR 69705-1-1-3-2-1	4.439bc	4.540bc	0.105NS
26.IR 72955-6-1-3-4-1	3.427ac	4.763c	1.381NS
27.SUPA	0.764a	5.275c	4.664***
28.UTRI MERAH	3.076ac	4.531bc	1.504*
29.IR 71030-2-2-1-1	0.707a	4.094ac	3.502***
30.IR 73887-1-8-3-5	2.033ab	4.670c	2.727**
31.IR 72928-1-2-4-1	2.687ac	4.530bc	1.906*
% CV	52.6	13.2	
SED	1.561	0.6073	

Mean within column followed by the same letter(s) are not significantly different from each other according to DMRT at 5% level. Note: * = significance at P = 0.05,** = Highly significance at P = 0.01 and *** = very highly significance at P = 0.001, NS = non significance difference and CV (%) is coefficient of variation.

- From above there is high coefficient of variation CV (%), though data transformation was done, this might have been contributed by rice genetical differences and the effect of RYMV disease among rice genotypes tested.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

Of the 52 rice genotypes tested in this study, 11 rice genotypes were found to be promising in terms of resistance to RYMV disease. These included IR 73886-9-2-4-1-1, IR 71605-2-1-5-3-4, IR 69705-1-1-1-4-2, IR 71028-3-1-2-5-1, IR 71029-3-1-5-5 Matata G1, IRR 134, Matata G2, IR 69704-4-4-4-8-1-1-1, Adday Sel and IR 71027-43-3-2-B-3. Growth and performance of these genotypes were similar to that of Mwangaza (Resistant control). Since the experiments were conducted in the screen house, field experiments are needed to further confirm the resistant of these genotypes under field conditions.

Among the strains of RYMV, strain 5 shown to be more virulence than the rest of the strains. The effects of Strain 5 on rice plant height, number of tillers per plant and dry matter per plant was higher than the other RYMV strains. This study also revealed that, Kalalu which was included in all experiments might be possessing tolerance characteristics rather than resistant to some virulent isolates of strain 5. Mwangaza was observed to be resistant to all three (RYMV strain 4, 5 and 6) Rice Yellow Mottle virus strains.

5.2 Recommendations

i. The study revealed that periodical screening of rice genotypes against RYMV using virulent strains is important in identifying resistant rice material for production and breeding purposes.

ii. The study revealed that, Strain 5 was very virulent, it is therefore, important to prevent the spread of this strain to other rice growing areas.

iii. Further investigation is required for the promising rice genotypes which have been reported in this study, in order to confirm their resistance, especially in RYMV hot spot areas and under field conditions. This will enable researchers to identify resistant genotypes which can ultimately be used by farmers.

iv. Genetic studies to identify genes conferring resistance in tolerant genotypes are recommended.

REFERENCES

Abo, E. M., Alegbejo, M. D., Sy, A. A. (2004). Evidence of non-transmission of rice yellow mottle virus (RYMV) through rice seed. *Tropicultura* 22(3): 116-121.

Abubakar, Z., Ali, F., Pinel, A., Traore, O., Nguessan, P., Notteghem, J., Kimmins, F., Konate, G., Fargette, D. (2002). Phylogeography of rice yellow mottle virus in Africa. *Journal of general virology* 84(3): 733-743.

Albar, L., Bangretz-reyser, M., Hebrand, E., Ndjiondjop, M. N., Ghesquiere, A. (2006). Mutations in Eif (ISO) 4G translation initiation factor confer high resistance to rice yellow mottle virus. *The plant journal 47: 417-426.*

Alber, L., Lorieux, M., Ahmed, N., Rimbault, I., Pinel, A., Sy, A. A., Fargette, D., Ghesquiere, A. (1998). Genetic basis and mapping of resistance to rice yellow mottle virus. QTLs identification and relationship between resistance and plant morphology. *Journal of theory and Applied Genetics* 97 : 1145-1154.

Balau, A. (2008). Saprophytic and parasitic micomycetes on sugar beet (Beta vulgaris L.). university de stiinte Agricole Si medicina veritinera lasi, Lucrari stiintifice. *Seria of Agronomia* 52: 607-610.

Banwo, O. O., Alegbejo, M. D., Abo, E. M. (2004). Rice yellow mottle virus genus Sobemovirus; A continental problem in Africa. *Plant protect Science*. 40(1): 26-36.

Biology and ecology of rice, (2005). The biology and ecology of rice (*Oryza sativa*) in Australia. Australian government department of health and aging, office of gene technology. [www.ogtr.gov.au/internet/ogtr/publishing.nst .../rice3/.../biologyrice1] site visited on 24/4/ 2011

Biswass, S. B., Biswas, A. (1984). An introduction to viruses. Third ed Vikas publishing house PVT ltd. New Delhi India. 678 pp.

Brugidou, C., Holt, C., Yassi, N. A., Zhang, S., Beachy, R., Fauquet, C. (1995). Synthesis of an infectious full-length cDNA clone of Rice Yellow Mottle Virus and mutagenesis of the coat protein. *Journal of virology* 206: 108-115.

Brugidou, E., Opalka, N., Yeager, M., Beachy, N. R., Fauquet, C. (2002). Stability of rice yellow mottle virus and cellular compartmentalization during infection process in *Oryza sativa* (L). Virology 297: 98-108.

Bucheyeki, L. K., Shennkawa, E., Kadadi, D., Lobulu, J. (2011). Assessment of rice production constraints and farmers preferences in Nzega and Igunga district, Tanzania. Journal of advance in development research 2: 30-37.

CABI (2007). Compedium for agricultuural crops protection. Oxford press, London. 282pp.

Dawson, O. W., Hilf, E. M. (1992). Host range determinations of plant viruses. *Annual review on plant physiology, Molecular Biology* 43: 527-555.

FAO (2004). International Year of Rice, Rice is Life. Food and Agriculture Organization of United Nations, Viale delle termedi caracalla Rome, Italy. Proceedings of the FAO rice conference, 12-13th February, 2004. 36pp.

Fargette, D., Pinel, A., Rakotomalala, M., Sangu, E., Traore, O., Sereme, D., Sorgho, F., Issaka, S., Hebrard, E., Sere, Y., Kanyeka, Z., Konante, G. (2008). Rice yellow mottle virus an RNA plant virus, evolves as rapid as most animal RNA virus. *Journal of virology* 82(7): 3584-3589.

Fargette D, Pinel A, Traoré O, Ghesquière A and Konaté G. (2002). Emergence of resistance-breaking isolates of *Rice yellow mottle virus* during serial inoculations. *European Journal of Plant Pathology* 108: 585–591

Fomba, S. N. (1989). Screening for seedling resistance to rice yellow mottle virus in some rice cultivars in Sierra leone. *The american phytopathological society* 72: 641-642.

Froissart, R., Doumayron, J., Vuillaume, F., Alizon, S., Michalakis, Y. (2010). The virulence transmission tradeoff in vector –borne plant viruses. a review of non-existing studies. *Plant molecular Biology* 43: 527-555.

Glaser, A., Biggs, C. (2010). Introduction to statistical methods in GenSTAT. BiggsVSN International, 5 Waterhouse, Waterhouse, Hemel Hempstead UK. [https://www.vsni.co.uk/email/capetownslides/anova.pdf] site visited on 20/5/2010.

Germundsson, A. (2005). Transgenic resistence to PMTV and PVA provides novel insights to viral long distance movement. A Thesis for award of Doctoral of Philosophy at the Swedish university of agricultural science, Uppsala, 105 pp.

Ghesquiere, A., Albar, L., Lorieux, M., Ahmadi, n., Fargette, D., Huang, N., McCouch, S. R., Nottegham, J. L. (1997). A major quantitative trait locus for rice yellow mottle virus resistance maps to a cluster of blast resistance genes on chromosome 12. *Phytopathology* 87: 1243-1249.

Gracial-Arenal, F., Fraile, A., Malpila, M. J. (2003). Variations and evolution of plant virus populations a review. *Introduction to Microbiology* 6: 225-232.

Hatibu, N., Reuben, S., Sibuga, K.P., Katule, A., Msumali, G. P. (2001). Research skill: project formulation, experimental design, data analysis and reporting. TERP-II-SUA Project. Published by Tanzania Agriculture Research Project Phase two-SUA Morogoro. 261pp.

Hebrard E., Pinel-Galzi, A., Catherinot, V., Labesse, G., Brugidou, C., Fargette, D. (2005). Internal point mutations of the capsid modify the serotype of rice yellow mottle virus. *Journal of virology* 79(7): 4407-4414.

IRR (1996). Standard evaluation system for rice. 4th edition, Inger genetic resource. IRR P. O. Box 933.1099 Manila, Phillipines. 20pp.

Jaw, A. (2010). Screening and molecular characterization of near-isogenic lines for resistance to rice yellow mottle virus. A dissertation for Master of Science degree award at Kwame Nkrumah university, 98 pp.

Jaw, M. N., Ndjiopnndjop, R., Akromah, Sere, Y. (2012). Identification of near isogenic lines resistance to Rice Yellow Mottle Virus. *African crop science journal* 20: 163-168.

Joseph, A., Olufolajji, D. B., Nwilene, F. E., Onasanya, A., Omole, M. M., Onasanya, R. O., Sere, Y. (2011). Genotypes by environment interaction of chlorophyll reduction in rice cultivars screened for resistance to RYMV disease with vectors transmission method. International Journal of Agricultural Research, 6(9): 691-698.

Ioanidou, D., Lett, J., Pinel, A., Assigbetsé, K., Brugidou, C., Ghesquière, A., Nicole, M. and Fargette, D. (2000). Responses of *Oryza sativa japonica* subspecies to infection with *Rice yellow mottle virus*. *Physiological and Molecular Plant Pathology* 57: 177–188.

Kadigi, R. M. J. (2003). Rice economics at the local and national level; the case of Usangu plains in Tanzania. River basin management research in Tanzania (RIPARWIN). RIPARWIN Project, Soil-Water Management Research Group (SWMRG), Sokoine University of Agriculture. P.O Box 3003, Morogoro/Tanzania.19 pp.

Kalinga, G. M. (2003). Tanzania country paper Agriculture in wetlands. [http://www.fao.org/DOCREP/003/x6611E/x6611e02d.htm] site visited on 17th /10/2010.

Kanyeka. Z. L., Sangu. D. E., Penel-Galzi. A., and Herbart. E. (2007). Distribution and Diversity of Local Strains of Rice Yellow Mottle Virus in Tanzania. *African Crop Science Journal* 15(4): 201-209.

Konaté, G., Sarra, S. and Traore, O. (2001). Rice yellow mottle virus is seed borne but not seed transmitted in rice. *European Journal of Plant Pathology* 107: 361-364.

Kouassi, N. K., N'guessan, P., Albar. L., Fauquet, C.M., Brugidou, C. (2005). Distribution and characterization of rice yellow mottle virus, a threat to african farmers. *American journal phytopathological society plant disease* 89(2): 124-131.

Luzi-Kihupi, A., Zakayo, A., Tusekelege, H., Mkuya, M., Kibanda, N. J. M., Khatibu, K.J., Maerere, A. (2009). Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations Conference, Rome, 26 October, 2009. 385-387pp.

MAFSC (2009). National rice development strategy of the United Republic of Tanzania, Ministry of Agriculture, Food Security and Cooperative. [http:// www.kilimo.go.tz] site visited on 17th/10/ 2010.

.Methews, R. E. E. (1991). Plant virology, third edition. Department of Cellular and Molecular Biology. Academic press Incorporation publishers. Haurcourt Brace Javanovich, 834 pp.

Michel, Z., Hilaire, T. K., Mongomake, K., Soury, I. (2008). Screening rice (Oryza sativa L.) varieties for resistance to rice yellow mottle virus. *Scientific research essay*. 3(9): 416-424.

Mogga, M. L., Lano, J., Asea, G., Gibson, P., Edema, R. (2012). Reaction of rice cultivars to a virulent RYMV strain in Uganda. *Journal of African crop science* 20: 51-57.

Mohapatra, S. (2008). The rice AIDS of Africa. Rice today (unpublished paper). [http://www.warda.cgiar.org/publications/AR2000/F3.htm] site visited on 5/11/2011.

Maule, A., Caranta, C., Baulton, M. I. (2007).source of natural resistance to plant viruses: status and prospects. *Molecular plant pathology* 8(2): 123-231.

Ndjiondjop, N., Albar, L., Fargette, D., Fauquet, C., Ghesquière, A. (1999). The genetic basis of high resistance to *Rice Yellow Mottle Virus* (RYMV) in cultivars of two cultivated rice species. *Plant Disease* 83: 931–935.

N'Guessan, P.(2001). Distribution, pathogenicity and interactions of two strains of rice yellow mottle virus in forested and savannah zones of West Africa. *Journal of Plant Disease*. 85: 59-64.

Nwilene, F. E., Sere, Y., Ndjiondjop, M. N., Abo, M. E., Traore, A. A., Asidi, A. N., Onasanya, A., Togola, A., Angunbiade, T.A. (2008). Rice Yellow Mottle Virus (RYMV) and its insect vectors ecology and control. Field Guide and Technical Manual for WARDA, CGIAR, Cotonou, Benin, 36 pp.

Nwilene, F. E., Oikeh, S. O., Agumbiade, T. A., Oladinaji, O., Ajayi, O., Sire, M., Gregorio, G. B., Togola, A., Toure, A. D. (2008). Growing low land rice; Production handbook. Africa Rice Center (WARDA), 01B. P. 2031, Cotonou, Benin. 44 pp.

Olembo, N., Mmboyi, F., Oyugi, K. (2010). Success story in crop improvement in Africa. The case of rice in Sub-Sahara Africa. Biotechnology stakeholders forum, Nairobi, Kenya. [www.absfafrica.org] visited on 8/23/2010.

Onwughalu, J.T., Abo, M.E., Okoro, J.I.K., Onasanya. A., Sere, Y. (2010). Rice yellow mottle virus infection on the performance of rice (Oryza sativa L.) relative to time of of infection under screenhouse condition. Journal of applied science 10(13): 1341-1344.

Opalka, N., Brugidou, C., Bonneau, C., Nicole, M., Beachy, R. (1997). Movement of rice yellow mottle virus between xylem cells through pit membranes. *Natural Academic Science* 95: 3323-3328.

Opalka, N., Tihone, M., Brugidou, C., Kumar, A., Beachy, N. R., Fauquet, M. C., Yeager, M. (2000). Structure of native and expanded Sobemoviruses by electro-Cryo-microscopy and image reconstruction. Journal of molecular biology 54: 197-211.

Oteng, J. W., Sant-Anna, R. (2003). Rice production in Africa. Current situation and issues. University of Ghana. FAO office, Accra-Ghana. [http://www. fao.org/docre /003/x22243t/x2243/05.html] site visited on 8/10/2010.

Palukaitis, P., Carr, J. P. (2008). Plant resistance response to viruses. *Journal of plant pathology* 90(2): 153-171.

Paul, C.P., NG, N. G., Ladeide, T. A. O. (2003). Mode of gene action of inheritance for resistance to rice yellow mottle virus. *African crop science journal* 11(3): 143-150.

Pinel-Galzi, A., Rakotomalala, M., Sangu, E., Sortho, F., Kanyeka, Z. (2007). Theme and variation in the evolutionary pathways to virulence of a RNA plant virus species. *Plos pathological* 3(11): 1761-1770.

Pinto, M. Y., Rosan, A. K., Baulcombe, D. (1999). Resistance to rice yellow mottle virus (RYMV) in cultivated Africa rice varieties containing RYMV transgenic. *Nature Biotechnology* 17: 702-707.

Qu, C., Lijas, L., Opalka, N., Brugidou, C., Yeager, M., Beachy, N. R., Fauquet, M. C., John, E. J., Lin, T. (2000). 3D Domain swapping modulates the stability of member of an icosahedral viruses group. *Journal of Molecular Biology* 8(10): 1095-1103.

Raj, M. H., Nayaka, S. C., Niranjana, S. R., Shetty, H. S. (2007). Health status of farmers saved paddy, sorghum, sunflower and cowpeas seeds in Karnataka, India. *Agricultural sciences* 3(2): 167-177.

Salim, M., M. Akram, M.E. Akhtar and M. Ashraf. 2003. Rice, A production Hand Book. Pakistan Agricultural Research Council, *Islamabad.* 70 pp.

Sanfacon, H. (2005). Replication of positive strand RNA viruses in plant; Contact points between plants and virus components. *A review Canadian journal of Botany* 83: 1529-1549.

Sanjuan, R., Agudero-Romero, P., Elena, S. F. J. (2009). Upper-limit mutation rate estimation for a plant RNA-viruse. Biotechnology letters 5: 394-396.

Sarra, S. (2005). Novel insighs in the transmission of rice yellow mottle virus in irrigated rice. PhD thesis, Wageningen University. Netherlands. *Journal of Plant science* 87: 803-808.

Scholfthof, H. B. (2005). Plant virus transport: Motions of functional equivalent trends in plant science. *Journal of Plant science* 10(8): 336-382.

Sere, Y., Onasanya, A., Afolabi, A. S., Abo, E. M. (2004). Evaluation and potential of double immunodifusion Gel assay for serological characterization of Rice yellow mottle virus isolates in West Africa. *Journal of biotechnology* 4(2): 197-205.

Soledad, S., Fraile, A., Malpica and Garcial-Arenal, F. (2005). Analysis of host adaptation and its relationship with virulence in Cucumber Mosaic Virus. *American phytopathology Society* 95(7):827-833.

Toma, F., Abdullah, N.Q. (2013). Isolation and identification of fungi from spices and medicinal plants. *Environmental and Earth Science*. 5(3): 131-138.

Traoré, O., Pinel-Galzi, A., Issaka, S., Poulicard, N., Aribi, J., Aké, S., Ghesquière, A., Séré, Y., Konaté, N., Hébrard , E., Fargette, D. (2010). The adaptation of Rice yellow mottle virus to the eIF(iso)4G-mediated rice resistance. *Virology*, 408(1):103-108.

UNDP (2010). Sustainable rice production systems, 'Green commondities facility' Human Development Report. *Advances in Global Change Research* 35: 53–54.

Ventelo-Debout, M., Tranchant-Dureuil, C., Nguyen, T. H., Bangratz, M., Sire, C., Delseny, M., Brugidou, C. (2008). Rice yellow mottle virus stress responsive genes from susceptible and tolerant rice genotypes. *Plant biology* 26: 1-12.

Wani, S. H., Sanghera, S.G. (2010). Genetic engineering for viral disease management in plants. *Natulae scientic biologica*e 2(1): 20-28.

WARDA (2000). Rice yellow mottle virus. WARDA annual report. Mali west Africa. [http://www.warda.cgiar.org/publications/AR2000/F3.htm] site visited on 13/10/2010.

WARDA (2007). Overview of recent developments in the Sub Sahara African rice sector; Africa rice center (WARDA), 01 B.P. 2031 Cotonou, Benin. [http://www.warda.cgiar.org/publications/AR2000/F3.htm] site visited on 13/10/2010.

Waterhouse, P.M., Smith, N. A., Wang, M. (1999). Virus resistance and gene silencing: killing the messenger. *Trend in plant science* 4(11): 1360-1385.

Yassi, M. N. A., Ritzenthaler, C., Brigidou, C., Farquet, C., Beachy, R. (1994). Nucleotides sequence and genome characterization of rice yellow mottle virus RNA. *Journal of general virology* 74: 249-257.

APPENDICES

Appendix 1: Insects which transmit RYMV in rice

Plate 7a: *Oxya hyla*

Plate 7b: *Chnootriba similis*

Plate 7c: *Conocephalus longipennis*

Plate 7d: *Locris ubra*

Plate 7a, b, c and d: Vectors for RYMV (source from Nwilene *et al.*, 2006).

Appendix 2: Standard evaluation system for Rice Yellow Mottle Virus disease

Score	Observable characteristics	Reaction types
1	No symptoms	Highly resistant
3	Green leaves but with sparse dots patches and less than 5% height reduction.	Resistant
5	Green leaves or pale with mottling and 6-25% height reduction and delayed flowering	Moderately resistant
7	Leaves pale or yellow 25-75% height reduction and delayed flowering	Susceptible
9	Leaves turn yellow or orange□ 75%, no flowering and some dead plants.	Highly susceptible

(Source: IRRI, 1996)

Appendix 3: Analysis of variance for different parameters for Strain 4, 5 and 6 from first experiment

a) Analysis of variance for rice plant height

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	2	64.699	32.350	13.28	<.001
RYMV strain	3	808.095	269.365	110.54	<.001
Variety.RYMV strain	6	35.426	5.904	2.42	0.025
Error	1423	3467.522	2.437		
Total	1423	4375.743			

b) Variate: Number of tillers per plant

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	2	23.0360	11.5180	113.14	<.001
RYMV strain	3	13.9034	4.6345	45.52	<.001
Variety.RYMV strain	6	0.9983	0.1664	1.63	0.134
Error	1423	144.8679	0.1018		
Total	1423	182.8057			

Variate: Dry matter

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	2	139.176	69.588	14.14	<.001
Strain	2	1328.575	442.858	90.00	<.001
Variety.Strain	6	202.722	33.787	6.87	<.001
Error	167	821.779	4.921		
Total	178	2492.252			

Appendix 4: Analysis of variance for the second experiment

ANOVA for uninoculated plant means second experiment

a. Variate: plant height

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
VARIETY	24	1137.051	47.377	21.18	<.001
Residual	3351	7495.944	2.237		
Total	3375	8632.994			

b. Variate: Tillers_per plant

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
VARIETY	24	6.40537	0.26689	9.21	<.001
Residual	3351	97.09228	0.02897		
Total	3375	103.49765			

ANOVA for inoculated plant with Strain 5 from second experiment

c. Variety: Plant height

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	24	2109.439	87.893	33.87	<.001
Residual	3365	8732.099	2.595		
Total	3389	10841.538			

d. Variate: Tillers per plant

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	24	5.45926	0.22747	8.83	<.001
Residual	3365	86.68212	0.02576		
Total	3389	92.14137			

Appendix 5: Analysis of variance for growth parameters from third experiment

ANOVA for uninoculated plant means

1. Variate: Plant height

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	30	2654.054	88.468	15.41	<.001
Residual	3310	19008.255	5.743		
Total	3340	21662.309			

2. Variate: Number of tillers per plant

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	30	313.7831	10.4594	13.22	<.001
Residual	3310	2618.9483	0.7912		
Total	3340	2932.7314			

ANOVA for inoculated with Strain 4 plant means from third experiment

Variate: Plant height

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	30	3424.783	114.159	19.34	<.001
Residual	3313	19560.610	5.904		
Total	3343	22985.393			

3. Variate: Number of tillers per plant

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	30	214.4759	7.1492	14.37	<.001
Residual	3313	1648.0008	0.4974		
Total	3343	1862.4767			

Appendix 6: ANOVA for economic and physiological traits from third experiment

ANOVA for uninoculated

1. Variate: Percentage parnicle exertion

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	30	14.4920	0.4831	1.58	0.064
Residual	62	18.9178	0.3051		
Total	92	33.4099			

2. Variate: Percentage sterilty

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	30	91.520	3.051	1.51	0.086
Residual	62	125.405	2.023		
Total	92	216.926			

3. Variate: 1000 grain weight

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	30	17.7746	0.5925	1.61	0.058
Residual	62	22.8673	0.3688		
Total	92	40.6419			

ANOVA for inoculated plant with Strain 4 for third experiment

4. Variate: Percentage panicle exertion

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	30	828.364	27.612	4.44	<.001
Residual	62	385.143	6.212		
Total	92	1213.507			

5. Variate: Percentage panicle sterilty

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	30	228.899	7.630	2.22	0.004
Residual	62	213.424	3.442		
Total	92	442.322			

6. Variate: 1000 Grain weight

Source of variation	D.f.	S.S.	M.S.	V.R.	F pr.
Variety	30	189.932	6.331	2.60	<.001
Residual	62	151.134	2.438		
Total	92	341.066			