Current Status Of Rice Yellow Mottle Disease In Western Kenya

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ABSTRACT

Rice yields in Kenya remain very low due to several constraints with Rice Yellow Mottle Disease (RYMD) caused by Rice Yellow Mottle Virus (RYMV) being the greatest challenge. However, information on pattern of distribution and diversity still remain scanty and hence a hindrance in designing most suitable control measures. A research study was carried out determine the occurrence and distribution of RYMV in rice growing areas of Western Kenya. A survey was conducted in rice growing schemes and farms in five Counties in July 2015 and June 2016 and symptomatic and some asymptomatic leaf samples were collected. Visual RYMD incidence ranged between 2-100%. Serological detection of RYMV in the leaf samples was done by DAS-ELISA. Results showed that 54% and 96% of the 2015 and 2016 samples, respectively were RYMV positive. Selected ELISA positive samples were subjected to molecular analysis. Extraction of total RNA was done using GeneJET Plant RNA Purification Mini Kit. PCR was done using RYMV specific primers and a product of approximately 1000 bp obtained. The coat protein gene was sequenced and sequences (720 bp) were established which identified strain S4 of RYMV. This is useful in monitoring diversity of RYMV in Western Kenya and to assist plant breeders develop resistant rice varieties.

Keywords: Rice, serological and molecular detection, Western Kenya, strain

1. INTRODUCTION

1.1 BACKGROUND

In Kenya, rice is the third staple food after maize and wheat however, rice yields have remained low. Rice production in Kenya is approximated to be between 50,000 -70,000 metric tonnes (USDA, 2016) while national consumption is estimated at between 180,000 -250,000 metric tonnes. Low production is majorly due to pest and diseases coupled with poor agronomic practices. Rice yellow mottle disease (RYMD), caused by the *Rice yellow mottle virus, RYMV*, is the greatest challenge to rice production because it can cause crop loss of up to 100% depending on a number of factors such as rice cultivar, RYMV infection time, RYMV strain, isolates in question and cropping system practiced in rice cultivation (Abo *et al.*, 1998; Luzi-Kihupi *et al.*, 2000). Rice yellow mottle virus (RYMV) belongs to genus *Sobemovirus* and is the important plant virus of rice in Africa. The virus is native to the Africa continent. From the first time observation in 1966 of the virus in Kenya on a local rice variety (Sindano) in a farmer's field near Kisumu on the shores of Lake Victoria, rapid spread of RYMV has been reported throughout Africa in all rice ecological systems (Bakker, 1974, Abo *et al.*, 1998). It is transmitted insect vectors by beetles; order *Coleoptera*, family *Chrysomelidae* like *Sesselia pussilla*, *Chaetocnema pulla*, *Trichispa sericea* and *Dicladispa viridicyanea* (Allarangaye *et al*., 2007; Nwilene *et al*., 2009) and mechanically through agronomic operations like transplanting and in cooperation of re-growths into soil during land preparation (Abo, *et al*., 2000; Fargette *et al*., 2006) and abiotically by wind-mediated contact and irrigation water (Sarra, *et al*., 2004; Sarra, 2005; Abo, *et al*., 1998).

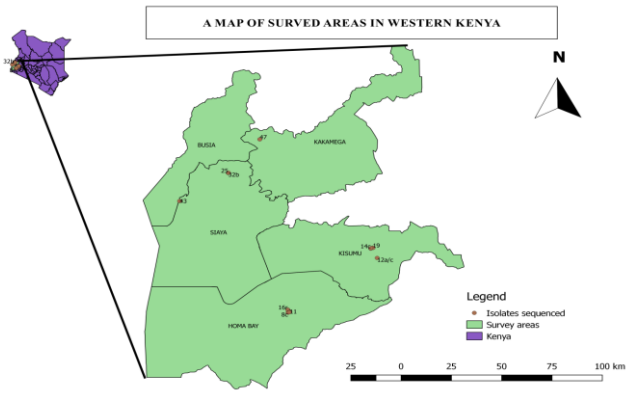
The RYMV is a major threat to food insecurity and this problem is complicated by lack of suitable control strategies that could curb transmission and spread of the virus. Substantive research work has been done on this virus in rice growing countries in West Africa and some East African countries (Uganda, Tanzania and even Rwanda), yet very minimal work has been done on it in Kenya; despite RYMV having a Kenyan origin as it was first identified and reported in Kenya in 1966 (Bakker, 1974). There are six distinct strains of the RYMV that cause the disease (Fargette *et al.*, 2002a), namely; S1, S2 and S3 found in West and Central Africa while S4, S5 and S6 are majorly in East Africa (Traore *et al.*, 2005). The diversity and distribution of RYMV in major rice growing regions of Kenya is currently not well documented. This presents a challenge in the development of the control strategies. Therefore, there was need to determine the current status of RYMD and characterize its causal agent, RYMV in Western Kenya, which is one of the major rice growing regions in Kenya.

2. MATERIALS AND METHODS

2.1 FIELD SURVEY

A survey was done in June 2015 and July 2016 in rice major growing areas in five Counties in Western Kenya (Fig.1). Samples were collected from fields in the following areas Mumias, Matungu and Koyonzo in Kakamega County; Teso-South, Alupe and Bunyalla Irrigation scheme in Busia county; Nyando, Muhoroni and Ahero irrigation Scheme in Kisumu County; Karian, Kagan and Rachuongo in Homabay County and Ujuang'a irrigation Scheme in Siaya County. Symptomatic and asymptomatic leaf samples were collected randomly. Disease score sheet was used to capture disease incidence and severity, rice variety and source of seeds. Sampling points (quadrants of $1M^2$) were set randomly within the farm in a two-way diagonal pattern (Ochola and Tusiime 2011), at each point incidence and severity was determined. A maximum of four sampling points per farm were sampled.

Figure 1: Map of Western Kenya showing areas where samples were collected: Busia, Kakamega, Kisumu and Homa Bay Counties.



SOURCE: Environmental Systems Research Institute (ESRI). (2012). ArcGIS Release 10.1. Redland, CA.

RYMD incidence was calculated as a percentage proportion of infected plants at the sampled point. The incidences were assessed using a modified earlier scale by Raymundo *et al.* (1979). The modified rating scale was as follows; 1-20% low incidence, 21-49% moderate incidence and 50-100% as high incidence. The scale used for scoring disease severity was 0-3 where 0 = no disease symptoms, 1 = mild symptoms, 2 = moderate symptoms and 3 = severe symptoms as previously done by other authors (John and Thottappilly, 1987; Ochola and Tusiime, 2011). The average incidence and severity of the sampled points per farm was used as the actual plot disease incidence and severity. A total of 35 rice leaf samples collected were collected in July 2015 and 85 samples were collected in June 2016. The latitude, longitude and altitude values were recorded using a hand held Global Positioning System (GPS-Entrex venture GARMIN™). The collected data was used to generate a map showing the survey area covered during the study.

2.2 SEROLOGICAL DETECTION

Viral detection serologically was done using the Double -Antibody-Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) protocol according to Pinel *et al*., (2000). Concentration of the virus in the infected leaf was approximated with reference to standardized optical density of a positive isolate from the IRD green house. The antibody used was non discriminatory and worked for all the isolates. An ELISA microplate reader was used to detect the values optical density at 405 nm after 1hour and 2 hours. The isolates that were considered positive were those with optical density values more than twice the value of a negative control (Ochola *et al*., 2011).

2.3 MOLECULAR DETECTION

A total of 19 isolates were selected from the ELISA positive samples based on the location where they were collected to try and find out if there is any diversity in the RYMV. All regions where samples were collected were represented.

Total RNA was isolated from RYMV infected rice leaves that had been frozen using the GeneJET Plant Purification Mini Kit, according to manufacturer's protocol. Speed and time of centrifugation was modified to a small degree on the protocol to improve total RNA quantity and quality. Two primers; the sense primer 3' RYMV II at 10 µM and antisense primer 5' RYMV III at 10 µM were used in transcription and amplification of the targeted gene for the protein coat(Pinel *et al.*, 2000). The sense primer 3' RYMV II was also utilized in the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) to transcribe and amplify genome fragments with the coat protein gene (nt 3447 to 4166) (Pinel *et al.*, 2000). Amplification of the RT-PCR products was done using the 2 primers (primer 3 II' M 5' III) under the following conditions; Denaturation at 94 °C for 5 minutes followed by 30 cycles at 94 °C for 1 minute, Hybridization at 55 °C for 30 seconds, Elongation at 72 °C for 1 minute and finally Final extension at 72 °C for 10 minutes. The resultant reaction mixture was stored at 4 °C. The products of PCR were loaded and visualized in the Ethidium Bromide stained 1% Agarose gel electrophoresis in a buffer

X0.5 Tris Acetate EDTA at 100V for 30 minutes to determine the DNA size.

2.4 SEQUENCING OF THE COAT PROTEIN GENE AND ANALYSIS OF SEQUENCES

PCR amplicons sequencing of the coat protein gene was done with the Taq terminator sequencing Kit (Applied Biosystems) and followed by analysis of CP sequences on an Applied Biosystem 373A sequencer (Pinel *et al.*, 2000; Fargette *et al.*, 2004). Two readings per base (3'to 5' and 5'to 3' directions) led to sequence accuracy of 99.9%. Assembling of sequences was by Seqman (DNASTAR) and sequence analysis was done using by DNASTAR for Apple Macintosh computers (Madison). The sequences of coat protein gene (720 bp) from Western Kenya isolates were compared with reference strains in the EMBL and they were useful in determination of strain of isolates and to monitor intra-strain diversity (Kanyeka *et al.*, 2007).

2.5 DATA ANALYSIS

Data from survey was used to obtain means of each of the described parameters that were recorded (severity and incidence). Analysis of variance (ANOVA) for the differences in the incidences and severity in the various Counties was done. ANOVA was used to obtain least significant difference (L.S.D.) values, which was used to separate the means at $P = 0.05$. Analyses were conducted using statistical analysis software, SAS to obtain correlation between the incidence and severity of RYMD ($r=0.883$; $p<0.0001$).

3. RESULTS

3.1 RYMD SYMPTOMS OBSERVED IN THE FIELD

Various viral symptoms were observed on the rice in the field. They included: yellowing of leaves, stunting, yellow-orange coloration of the field, mottling, and leaf narrowing (Fig.2).

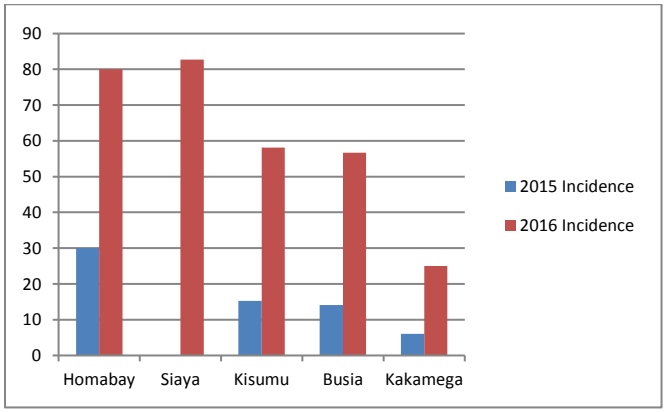
Figure 2: Major RYMV symptoms observed in the rice field; A: Yellow leaves, yellow stripes & brown/orange discoloration of older leaves, B: Leaf narrowing & mottling, C: Stunting, D: leaf necrosis and plant death.

SOURCE: Survey data June, 2015 and July, 2016 in five Western Kenya

3.2 MEAN RYMD INCIDENCE AND SEVERITY

Mean RYMD incidence varied across the counties during the two surveys in June 2015 and in July 2016. Highest mean visual incidence was observed in Siaya County (82.7%) in 2016 and lowest in Kakamega County (6%) in 2015. Other counties where the disease incidence was relatively high were Homa Bay (80%), Kisumu (58.1%) and Busia (56.7%) in 2016 survey. In individual farms, the highest incidence (100%) was at Ujuang'a irrigation scheme in Siaya County on a Pishori rice variety. The lowest disease mean incidence (2%) was recorded on various farms in Busia and Kisumu Counties. Furthermore, there was a significant difference in RYMD incidence between counties ($p<0.0001$). Most farms had a severity of 2 with a few having 1 and 3. There was a positive correlation between viral incidences and severity ($r=0.8843$, $p< 0.0001$) and therefore severity increased with increase in disease incidence.

Figure 3: Mean incidence RYMD in surveyed counties in 2015 and 2016



SOURCE: Survey data June, 2015 and July, 2016 in five Western Kenya

3.3 RICE VARIETIES PLANTED BY FARMERS IN RELATION TO RYMD INCIDENCES

The most popular rice varieties were IR planted by 21.4% mainly in Homa Bay County followed by IR 2793 and IR 2793-8 planted by 16.7% of the farmers mainly in Homa Bay and Kisumu County respectively. The above seeds are mainly supplied by the National Irrigation Board located in Ahero, Kisumu County. Pishori variety is planted by 12% of the farmers mainly in Busia and some parts of Siaya. YS 104 and YS 105 are varieties developed and planted at the Dominion farms in Yala swamp in Siaya County. IR is the most preferred and most popular rice variety among farmers (21.45%) and recorded the highest (65%) disease incidence. This was followed by IR 2793 and IR 2793-8 with disease incidences of 63% and 58% respectively. BR and VR are among the least popular rice varieties with a disease incidence of 5% and severity of 1 (Table 1).

Table1: Ranking of rice varieties planted by farmers in Western Kenya

Variety	Percentage (%) of the Total	Mean RYMD Incidence	RYMD Severity
IR	21.4	65	3
IR 2793	16.7	63	3
IR 2793-8	16.7	58	3

Pishori	12	40	2
Pakistan	4.7	53	3
Achung (local)	4.7	5	1
NERICA 1	2.4	5	1
NERICA 1V	2.4	5	1
Super America 1	2.4	33	2
Sindano (local)	2.4	20	2
SARO 5	2.4	10	1
ITA	2.4	5	1
BR	2.4	5	1
VR	2.4	5	1
YS 104	2.4	0	0
YS 105	2.4	0	0

SOURCE: Survey data June, 2015 and July, 2016 in five Western Kenya

3.4 DETECTION OF RYMV BY ELISA

All rice leaf samples from the field were subjected to ELISA. The results showed that 95 samples out of 120 samples were RYMV positive with ELISA (Table 2). Kagan area in Homa bay County had the highest number (33) of ELISA positive leaf samples followed by Bunyala in Busia County and Ahero in Kisumu County at 18 and 13 respectively while all isolates from Kakamega County were negative with ELISA. All leaf samples collected from Kakamega County tested negative for RYMV with ELISA. ELISA result revealed that 79.2% of the total samples tested were positive with ELISA while only 20.8% were negative.

Table 2: ELISA results of samples from various locations

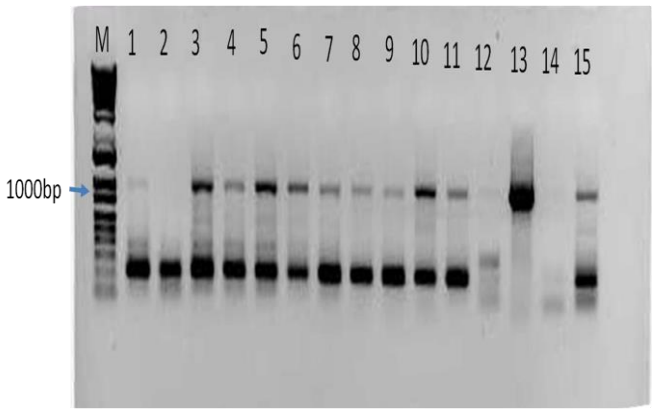
AREA	N	RYMV presence or absent with %			
		RYMV Positive	RYMV + by %	RYMV Negative	RYMV - by %
Kagan	37	33	27.5	4	3.3
Bunyala	22	18	15.8	4	2.5
Ujuanga	14	14	11.7	0	0
Nyando	9	5	4.2	4	3.3
Rachuonyo	5	4	3.3	1	0
Muhoroni	5	4	3.3	1	0.8
Nyangweso	4	4	3.3	0	0
Teso south	4	0	0	4	3.3
Koyonzo	3	0	0	3	2.5
Mumias	3	0	0	3	2.5
Alupe	1	0	0	1	0.8
Ahero	13	13	10.8	0	0
Total	120	95	79.2	25	20.8

SOURCE: DAS-ELISA results from Institut de Recherche pour le development (IRD), 2016

3.5 RT-PCR DETECTION OF RYMV

Amplified fragments of the expected size (1000 bp) were obtained in both 2015 and 2016 isolates after amplification using RYMV specific primers. Nine (9) isolates from 2016 and seven (7) isolates from 2015 group were confirmed to be positive by RT-PCR and were subjected to sequencing (Figure 3).

Figure 3: Gel electrophoresis of RT- products of 12 isolates collected in June 2016.



Lane M- Marker, Isolates in lanes; 1-Kagan, 2-Kagan, 3-Nyangweso, 4- Rachuonyo, 5- Rachuonyo, 6- Ahero, 7- Ahero, 8-Ujuanga,9-Ujuanga,10-Bunyalla,11-Bunyalla,12-Koyonzo,Lane 14-Negative control and Lane 15-Positive Control.

SOURCE: RT-PCR results from Institut de Recherche pour le development (IRD), June, 2016.

3.6 RYMV STRAINS IDENTIFICATION AND DISTRIBUTION

The sequences of coat protein gene of 16 isolates (7 from 2015survey and 9 from 2016 survey) sequenced had the same length of 720 bp. These sequences were found useful in determining isolates strain and RYMV diversity in W. Kenya. The isolates were found to be of RYMV strain S4. No any other strain apart from S4 was recorded in this study. The study of the distribution pattern of the RYMV revealed that strain S4 occurs in the four counties of Western Kenya namely; Busia, Siaya, Homa Bay and Kisumu. However, the virus was found to be absent in Kakamega County.

4. DISCUSSION

One of the major constraints to rice production in Africa is RYMD which is most devastating, causing crop loss of up to 100% (Kouassi *et al.,* 2005). This is hindering rice production in Sub-Saharan Africa where about 100 million people depend on rice for their livelihoods (Nwanze *et al.*, 2006). Control of RYMD is difficult because the virus is highly infectious; however some methods such as application of insecticide to control vectors, cultural practices such as early planting to avoid the peak of vector population and host plant resistance which is the most effective option have been employed to mitigate the spread of the disease. Results of diagnostic survey conducted in western Kenya have shown the wide spread of the disease with incidence levels of up to 100%. Major RYMV symptoms observed in the rice field included yellow leaves, yellow stripes & brown/orange discoloration of older leaves, leaf narrowing & mottling, stunting, leaf necrosis and plant death. The symptoms observed vary depending on the rice genotype cultivated and the age at which the rice crop gets infected (Bakker, 1974; Afolabi *et al*., 2009). The disease also causes reduced tillering, crinkling, malformation and incomplete emergence of the panicles, resulting in sterility and a significant reduction in yields (Albar *et al*., 2003). In susceptible cultivars, there may be general necrosis of tissues, which often leads to death (Fauquet

and Thouvenel, 1977). Severity of infection and the resultant yield loss depends on the rice variety, the age at which the rice gets infected, the RYMV strain involved and the prevailing environmental conditions.

This study has shown that RYMD does occur in all the lowland rice growing areas in Western Kenya, as it was found to occur in the following four counties Kisumu, Homabay, Siaya and Busia. Ocholla and Tusiime (2011), in a similar study in Eastern Uganda found that RYMV was widespread on all the surveyed rice farms; this may be due to cultivation of susceptible varieties seasonally and unavailability resistant varieties. Majority of symptomatic isolates (79.2%) collected from rice fields that were subjected to ELISA; confirmed to be RYMV positive indicating that RYMV is a serious epidemic reported in ecological systems of rice around Africa continent (Abo *et al.,* 1998). Finding of this study however revealed that all isolates that were collected from upland rice mainly from Kakamega County tested RYMV negative with ELISA. This raises a question of the role of irrigation water found in paddy areas in transmission and spread of RYMV. It is documented that irrigation water has a big role in transmission and spread of RYMV. RYMV mechanically is transmitted through agronomic operations like transplanting and in cooperation of re-growths into soil during land preparation (Abo, *et al*., 2000; Fargette *et al*., 2006) and abiotically by wind-mediated contact and irrigation water (Sarra, *et al*., 2004; Sarra, 2005; Abo, *et al*., 1998). Most infections may be as a result of exposing healthy seedlings and plants to virus contaminated and infected material like irrigation water, soil, cattle feces and plants (Abo, *et al*., 1998; Allarangaye, *et al*., 2006; Konate, *et al*., 2001). This may be an explanation why the RYMD was absent in upland rice in this study.

During this study, an individual farm in Siaya County at Ujuanga recorded the highest visual incidence (100%) whereby every rice plant crop on the farm had been attacked while lowest incidence (1%) was observed at a farm in Muhoroni in Kisumu County. A visual mean incidence of 82.7% was observed in Siaya County in 2016 and lowest visual mean incidence (6 %) in Kakamega County in 2015. Other counties where the disease incidence was relatively high were Homabay (80%), Kisumu (58.1%) and Busia (56.7%) in the 2016 survey. These counties practice irrigation low-land cultivation while in Kakamega cultivation is upland and rain-fed. Most farms in the five surveyed counties in Western Kenya recorded relatively high incidences (40% and above). From this study, RYMV is potentially devastating and distinctly African virus specific to lowland rice and indigenous to Africa. This is because RYMD was first reported in rice at Otongolo near Kisumu around Lake Victoria in Western Kenya in 1966 (Bakker, 1974). This explains the existence and spread of the disease in Counties around Lake Victoria in Western Kenya. Since RYMV was first reported in Kenya, it has also been reported in many East and West African countries (Abo *et al.,* 1998).

Symptomatic leaves were collected during survey for serological analysis through ELISA to detect the causative virus RYMV. Some a few asymptomatic leaves were also picked for analysis since the virus could be present and fail to express symptoms. Double sandwich Enzyme Linked Immunosorbent Assay (DAS-ELSA) method

for detection was used since it is cheap and relatively easy to carry out. ELISA results confirmed that 87.4% of the total samples tested were positive with ELISA while only 12.6% were negative (Table 2). The ELISA result revealed that the isolates were serologically similar as found by Ochola and Tusiime (2011) in a similar study in Eastern Uganda. However, since the result showed similarity of the isolates serologically, our result seemed go against the finding of Sere *et al.*, (2007) who stated that isolates originating from a given same host, particular area or field are found to be different serologically. All non-symptomatic leaves that were collected tested RYMV negative. One alternative host plant (*Pennisetum purpureum*) that was symptomatic for RYMV and collected in the rice field tested RYMV positive with ELISA. This is in line with the earlier findings that RYMV has a narrow host range limited to grasses; order *Poaceae*, families *Oryzeae* and *Eragrostidae (*Konate *et al*., 1997; N'Guessan *et al*., 2001, Abo *et al*., 2002). The RYMV virus has ability to survive on alternative host plant such as wild grass until such a time when shift in rice cultivation practices creates suitable condition that favour its transmission (Pinel-Galzi *et al.,* 2006; Sere *et al.,* 2008).

In molecular detection, reverse transcriptase polymerase chain reaction (RT-PCR) confirmed the presence of RYMV in 84.2% of all of the rice leaf samples that were subjected to PCR giving amplified fragments of approximately 1000 bp which was the expected size. The sequences of coat protein gene of 16 isolates sequenced had the same length of 720 bp. These sequences were found useful in determining stain to which isolates belong to and RYMV strain diversity in Western Kenya. All the isolates were found to be RYMV strain S4. No any other strain apart from S4 was reported in this study. This was confirmation of earlier findings that isolates of RYMV strain S4 have been reported to occur solely in East Africa (Pinel *et al.,* 2000). No any other RYMV strain was reported in this study. After laboratory tests and analysis assessment of the distribution of the RYMV revealed that strain S4 occurs predominantly in four counties of Western Kenya namely Busia, Siaya, Homabay and Kisumu but absent in Kakamega County. The breakthrough of this study is that new areas such as Busia, Siaya and Homabay reported RYMV for the first time as it has never been reported before in the same areas. It seems more than 50 years after the discovery of the virus in Kisumu in 1966 (Bakker, 1974) the virus has been spreading and now it exists in four counties of Western Kenya. These findings reveal that long distance spread of RYMV is possible as the virus has spread from the centre of origin along the shores of Lake Victoria at the Kavirondo Gulf (Banwo *et al.,* 2004; Fargette *et al.,* 2006).

RYMD potential effect on the rice cultivation industry in Kenya is shown by its wide distribution and occurrence in the 4 out of the 5 counties rice growing counties of Western Kenya that were surveyed. This finding represents the possibility of this rising plant virus to cause serious epidemics in irrigated rice production systems in the region (Traore *et al.,* 2001; Fargette et al., 2006). This study reveals that four counties (Busia, Siaya, Homa bay and Kisumu) located along the edge and outskirts of Lake Victoria basin recorded high RYMD severities and incidences. This was in line with earlier reports suggesting that the first RYMV observation and

discovery in Western Kenya was along the shores of Lake Victoria (Banwo et al., 2004; Fargette *et al.,* 2006) and the virus has now spread to almost all counties of Western Kenya.

5. CONCLUSION

This study gave important underlying truth of RYMV distribution, occurrence and diversity in Western Kenya. This study has disclosed status of RYMV in Western Kenya and shown that strain S4 is dominant and with a lot of variants (intra-strain diversity). We propose and recommend detailed study of the cause of RYMV strain S4 diversity pattern. Furthermore we recommend that RYMD resistant and tolerant rice varieties are comparatively suitable for cultivation Western part of Kenya.

ACKNOWLEDGEMENT

We acknowledge the Kenya-France PHC Pamoja Project 2016, the National Commission of Science and Technology (NACOSTI) and Association of African Universities (AAU) for their financial support towards this research work.

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