

Evaluation and Deployment of Rice Genotypes Resistant to Rice Yellow Mottle Virus Disease in the Southern Highlands of Tanzania

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Abstract: Rice yellow mottle virus (RYMV) is the most important biotic stress undermining productivity of the crop envisaged to spur economic growth and improvement of livelihoods in Tanzania. In efforts to exploit economic and eco-friendly natural host resistance for sustained disease management, reactions of 60 promising rice genotypes selected from various germplasm to a mixture of isolates of the virus under artificial inoculation in the Southern highlands zone represented by Kyela district were studied. Twenty of the most promising entries were further verified on farm at two lowland rainfed and two upland ecologies in the district. Resistance or susceptibility of the genotypes was determined by severity of symptoms as scored on a standard international evaluation scale ranging from 1 to 9 and plant height reduction measured as the difference in height between inoculated and uninoculated plants. Results indicated that RYMV isolates used differed by their virulence and the varieties by their vertical resistance. Observed reactions ranged from highly susceptible to highly resistant. About 50% of the genotypes showed a high level of resistance while 20% were highly susceptible. The intermediate reactions were 0% resistant, 10% susceptible and 20% moderately resistant. Susceptible cultivars recorded up to 75% reduction in height and death of plants whereas those resistant had mild symptoms and negligible plant height reduction. Highly resistant genotypes were immune to infection by the virus across all locations. Five of the highly resistant cultivars namely Salama M55, M57, M19, M35, IITA 235 were acceptable to farmers in terms of cycle length, plant and grain types. Further research to enable official release for commercial use and wide scale accessibility of seed to farmers was deemed necessary to ease damage from RYMV. The varieties can also be deployed as sources of resistance in cross breeding programmes to improve the local susceptible cultivars whose background is already acceptable to farmers. Exhaustive screening of germplasm collections to identify diverse sources of novel genes for resistance was also recommended together with verification of high resistance of genotypes across regions.

Key words: RYMV, resistant varieties, management.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world food crops and the most important staple cereal after maize in eastern, central and west Africa where per capital consumption is increasing rapidly due to high rates of population growth, increased urbanization and associated shifts in dietary patterns (ECCARN, 2006). Rice development is envisaged to spur economic growth, alleviate poverty and food insecurity in the country (MAFC, 2008). It is produced in almost all regions in a total of 720,000 ha by small holder farmers under lowland rainfed (72%), upland (20%) conditions while only 8% is under irrigation by large farmers (MAFC, 2008). The country is only 80% self sufficiency because productivity is low averaging 1.2 to 2.4 t/ha compared to world average of 3.4t /ha and potential of 10 - 11 t/ha that can be realized under optimum conditions (FAOSTAT, 2010; Kashenge *et al.*, 2012). Low productivity is attributed to poor yielding traditional varieties that succumb to a series of biotic and abiotic stresses. Rice yellow mottle virus (RYMV) is currently the most serious biotic constraint of the crop which strikes in the most productive low lands and irrigated rice cultures in Tanzania and Africa at large. Yield losses fluctuate between 10% - 100% depending on age of plant at infection, nature of variety used and environmental factors.

The disease is caused by a Sobemovirus which is endemic to the African continent. It is characterized by yellowing and mottling of leaves, poor panicle exertion, stunting, spikelet sterility and plant death. It was first observed at Otonglo Kenya in 1966 but in the next two decades it was recorded in all major rice producing countries in Sub-Saharan Africa. In Tanzania, it was first noted at Mkindo irrigation scheme, Morogoro in mid 1980s but epidemic outbreaks resulting into 75% to 100% crop losses started in Kyela district during 1995/96 cropping season. The virus survives in perennial grasses of the *Oryzae* and *Eragrostidae* families, crop stubbles and ratoons during offseason while it is naturally transmitted by insect vectors (*Chrysomelidae* beetles, grasshoppers), mechanically through plant injuries and abiotically by wind and irrigation water (Traore *et al.*, 2009).

An integrated disease management (IDM) approach has been imperative in all national and regional endeavors to achieve economical, eco-friendly and sustainable control of the disease. While various cultural and chemical measures against the virus and vectors respectively have been generated in different production environments, development of host genetic resistance,

the backbone of IDM is challenged by high diversity of the virus causing erosion and breakdown of resistance (Soko *et al.*, 2010). Through the use of monoclonal antibodies six main serotypes of the virus have been identified and molecularly characterized into six virulent strains that are adapted to different geographic zones in Africa. Three of them (S1, S2, S3) are distributed in west Africa and the rest (S4, S5, S6) in east Africa and Madagascar (Amacho *et al.*, 2009).

In addition, new resistant breaking isolates or pathovars have been isolated in Tanzania which is considered the centre of origin of the viral population (Kanyeka *et al.*, 2007). Deployment of host resistance is also constrained by lack of strong resistance in many agronomically farmer preferred rice cultivars. High resistance to the virus controlled by recessive genes *rymv 1 - 2* has been found in *Oryza glaberrima* and very few *O. Sativa indica* accessions (Rakotomalala *et al.*, 2008; Thienele *et al.*, 2010; Ownghalu *et al.*, 2011). In order to broaden the gene pool for durable genetic control of the pathogen continued efforts in identification and characterization of diverse sources of genes for resistance to RYMV are deemed necessary alongside the identification and promotion of other management technologies. Novel genes for resistance or tolerance to RYMV that may be inherent in the wealth of rice landraces and national germplasm collections have not been fully explored. The aim of the study was to identify resistance rice genotypes from a selected germplasm on the basis of the reaction profile of the cultivars artificially inoculated with the virus.

MATERIALS AND METHODS

Varietal screening was done in Kyela district which is a hot spot site for RYMV in the Southern Highlands of Tanzania (SHT). Initially about 200 rice genotypes comprising of germplasm collections at Uyole Agricultural Research Institute (ARI) and introductions from Sokoine University of Agriculture (SUA) breeding programme and International Rice Research Institute (IRRI) were evaluated for resistance on station at Kikusya Kyela during 2012 cropping season. Out of the lot, a total of 60 entries were selected and validated in 2013 from which 20 genotypes were selected and verified on farm through farmer fields schools (FFS) in four villages. Two of the latter (Lugombo, Mpunguti) represented lowland while the other (Kisale, Kikusya) had upland rice ecosystems. A randomized complete block experimental design in three replications was used on station and two replications in FFS. Plot size on station measured 40cm x 200 cm and on-farm 1.0m x 3.5m. Direct seeding at 3 seeds/hole at spacing of 20 x 20cm between and within rows was used in all areas. Fertilizers triple super phosphate (TSP), and Calcium ammonium nitrate (CAN) were

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broadcasted at blanket rates of 30kg P₂O₅ and 40kg N/ha respectively in planting furrows and later urea (20kg N/ha) was top dressed three weeks after germination. All screenings were done under artificial inoculation using inoculums from the viral population at the respective site.

Inoculum preparation and inoculation

Inoculums were obtained by crushing leaves of infected plants from surrounding fields in water (200g/l water). Inoculation was done 45 days after germination (dag) by rubbing the whole seedling from the base to the top with a cotton swab soaked before hand in the viral solution. Plants in one replication at each site were not inoculated to ensure seed recovery and measurement of other parameters apart from RYMV disease.

EVALUATION OF RESISTANCE TO RYMV

Disease incidences, severity and plant height reductions were assessed at 14, 28 and 50 days post inoculation. Incidence was measured as percentage of plants showing visible symptoms. Symptom severity was scored on a scale of 1 to 9 according to the standard evaluation system by IRRI (2002) where score of 1 denoted highly resistant (HR), 3 resistant (R), 5 moderately resistant (MR), 7 susceptible and 9 highly susceptible reactions accompanied by plant death. Plant height reduction in percentage was calculated using the formula: $100 \times (HO - Hi)/HO$ where HO = height of uninoculated control and Hi = Height of inoculated. Other data were yield variables, farmers opinions and preferences in the on-farm tested entries. Farmers assessed agronomic qualities of the genotypes at grain maturity stages using farmer's own set criteria on 1 - 3 scale where 1 = Very good or Highly acceptable, 2 = Good/Acceptable; 3 = Poor/Not acceptable. Preferred local cultivars were included as checks in all tests. On station data were analyzed by Genstat 13 statistical software and means were separated by Duncan's New multiple range test.

RESULTS

Reactions of various germplasm sets to RYMV at Kikusya on station during 2013 season are shown on Table 1 and 2. Disease incidences and severity between the entries were significantly different both at 21 and 50 days post inoculation. Most (66%) of the tested upland NERICA varieties showed resistant to highly resistant reactions and only 34% were susceptible to the Kikusya viral population (Table 1). Only Nerica 2, 9 and 11 exhibited very susceptible reactions.

The other set of elite germplasm screened showed lower levels of resistance compared to the upland Nericas as shown in Table 2. All genotypes from IRRI were highly susceptible except two (IR72955-6-1-3-4-1, IR71605-3-1-1-2-6) which were moderately resistant. About 33% of the elite entries showed highly resistant (HR) reactions, 9% were moderately resistant (MR) and the rest were highly susceptible (HS). Susceptible varieties including local checks did not give any seed yield (Table 4).

Performance of 20 promising entries at 4 locations on-farm is shown on Table 3. Some of the genotypes showed differential reactions to RYMV isolates at different locations showing susceptibility in some areas but resistant at other locations (Figure 1). About 11 entries including Mwangaza resistant check (Luzi *et al.*, 2008) gave HR scores (1-3) across four locations while 3 were MR and the rest were susceptible. The viral population at Lugombo and Mpunguti lowland areas appeared to be more virulent compared to the upland strains at Kikusya and Kisale. Even Kalalu, considered resistant in previous years succumbed to infection by the Lugombo strain. Gigante, an *Oryza sativa* variety reported to have high resistance to the virus in West Africa was highly susceptible at all locations. The rating of the genotypes by farmers is also indicated on Table 3. Most of the resistant and MR genotypes Salama M-19, Moroberekan, Kalalu, Lunyuki, Salama M55, Salama M57, Mwangaza, FARO II, Salama M55, IRAT 252, IITA 235, SSD-5, IRAT 256 had agronomic traits that were acceptable to farmers but the Salama entries M55, M19, M57, M35, IITA 235 and Mwangaza were highly acceptable to farmers with consequence of stealing from the experimental plots. The others Moroberekan, Lunyuki, FARO II and IRAT 252 were HR at all locations but had poor seed types despite good yields (Table 4).

Although the upland Nericas 8, 11, 13 appeared resistant to RYMV, they were seriously infected with other diseases principally sheath rot (*Sarocladium oryzae* (Sawala) Gums and Hawksworth) and showed very poor performance with respect to adaptability in the lowland areas. Their short stature and grain types were also not favorable to farmers even in the upland cultures. Inoculated genotypes showed height reductions ranging from 5 - 74% depending on the degree of susceptibility (Figure 2).

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Table 1: Reactions of improved upland Nericas to RYMV inoculation at Kikusya 2013

Name of Entry		Disease incidence (%)		Disease severity (0 – 9)		Reaction category (Mean)
		21DAI	50DAI	21DAI	50DAI	
1.	BORA KUPATA	0	0	0	0	HR
2.	NERICA 13	0	0	0	0	HR
3.	KARAFUU	0	0	0	0	HR
4.	NERICA 4	0	50	0	5	MR
5.	NERICA 14	0	0	0	0	HR
6.	MELI	0	0	0	0	HR
7.	NERICA 5	0	0	0	0	HR
8.	NERICA 2	20	70	3	7	MR
9.	MEROCA 17	0	0	0	0	HR
10.	NERICA 6	4	40	5	6	MR
11.	NERICA 9	100	100	8	9	HR
12.	NERICA 10	1	75	3	6	R
13.	NERICA 1	0	0	0	0	HR
14.	NERIKA 14	0	0	0	0	HR
15.	NERICA 3	0	0	0	0	HR
16.	NERICA 12	0	0	0	0	HR
17.	NERICA 7	0	0	0	0	HR
18.	NERICA 16	0	0	0	0	HR
19.	JUMLI MOSHI	0	80	0	8	HS
20.	CHEREHANI	60	90	7	8	HS
21.	SENA	100	100	9	9	HS
22.	MWANGAZA	0	0	1	1	HR
23.	WAHIWAHI	80	100	9	9	HS
24.	KISHINGO	70	100	7	9	HS
25.	NERICA 8	0	0	0	0	HR
26.	NERICA 18	-	-	-	-	-
27.	INDIA RANGIMKIA	100	100	7	9	HS
Mean		10	29.6	2.3	2.5	HR
Local check(SUPA) Mean		100	100	9	9	HS
CV %		35	32	29	28	
SE		0.169	0.234	0.0782	0.0663	

Table 2: Response of 19 elite germplasm to RYMV inoculation at Kikusya 2013

Name of Entry		Disease incidence (%)		Disease Severity (0 - 9)		Mean reaction Category
		21DAI	50DAI	21DAI	50DAI	
1.	UTRI MERAH1	0	100	0	8	HS
2.	KALALU	0	0	0	0	HR
3.	MWANGAZA	0	0	0	0	HR
4.	ADDA SOL	0	100	0	9	HS
5.	UTRI MERAH ₂	0	100	0	8	HS
6.	UTRI RAJAPAN	0	90	0	8	HS
7.	TXD 306	60	100	7	7	S
8.	IR 69705-1-1-3-2-1	70	80	6	7	HS
9.	IR 69705-1-1-3-3-5	100	100	8	8	S
10.	IR 71605-2-1-1-3-10	80	80	6	6	MR
11.	IR 72955-6-1-3-4-1	80	80	5	5	HS
12.	IR 69704-4-8-1-1-1-1	90	90	8	8	MR
13.	IR 71605-3-1-1-2-6	100	100	5	5	HS
14.	IR 69734-81-1-1	70	80	8	8	S
15.	IR 69705-1-1-3-2-3	50	50	6	6	HS
16.	IR 69734-16-1-1	90	90	8	8	HS
17.	IR 73886-9-2-4-2-1	30	70	7	7	HR
18.	Salama M – 19	0	0	1	1	HR
19.	Moroberekan	0	0	1	1	HS
20.	SSD – 5	50	70	6	7	MR
21.	Gigante	70	100	7	9	HS
22.	Salama M1	5	6	3	4	R
23.	Lunyuki	0	0	1	1	HR
24.	IRAT 256	2	2	3	4	S
25.	Salama M34	3	2	3	3	MR
26.	Bouake	50	60	6	6	HR
27.	Salama M55	20	30	4	6	MR
28.	Salama M57	0	0	1	1	HR
29.	BG 380 – 2	25	30	4	4	HR
30.	FARO II	0	0	1	1	HR
31.	Salama M35	0	0	1	1	HR
32.	IRAT 252	5	5	2	2	HS
33.	IITA 235	0	0	1	1	HR
34.	Local check Rangi 2	80	100	7	9	HS
35.	Local check SARO 5	70	95	7	7	HS
36.	Local check URO 1	90	100	8	9	HS
37.	Check Kilombero	90	100	8	9	HS
Mean entries		40.5	48	4.7	5.4	
Mean local checks		91	99	7.5	9	
CV%		30	34	29	28	
SE		0.015	0.072	0.042	0.058	

HS = Highly susceptible; HR = Highly resistant; R = Resistant; S = Susceptible; MR = Moderately resistant

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Table 3: Performance of 20 promising rice genotypes inoculated with RYMV strains across four locations in Kyela district 20156

Rice genotype		Severity RYMV score (1 - 9)					Mean % height reduction	Disease score category	Mean performance rating by farmers
		Mpu-nguti	Kisale	Kiku-sya	Lugo-mbo	Mean			
1	Salama	1	1	1	1	1	24	HR	HA
2	Moroberekan	1	1	1	1	1	19	HR	A
3	Nerica 1	4	8	1	9	5.2	39	MR	P
4	Kalalu	5	1	7	7	5.0	11	MR	A
5	SSD 5	2	1	7	8	4.5	24	HS	A
6	Gigante	7	8	8	7	7.5	74	S	P
7	Salama M ₁	7	7	5	7	6.5	40	HS	P
8	Lunyuki	3	1	1	1	1.5	19	MR	A
9	IRAT 256	3	9	4	3	4.5	34	MR	P
10	Bouake	6	4	6	7	5.8	42	HR	P
11	Salama M55	4	4	6	1	3.0	42	HR	A
12	Salama M57	1	1	1	1	1.0	13	HS	HA
13	Rangi Mbili	8	8	7	7	7.5	53	HR	P
14	Mwanganza	1	1	1	1	1.0	5	S	HA
15	BG380-2	9	3	7	7	6.5	48	HR	P
16	FARO II	2	2	1	1	1.5	11	HR	HA
17	Salama M35	1	1	1	3	1.5	49	HS	A
18	Nerica L - 25	8	7	6	8	7.3	58	HR	P
19	IRAT 252	2	1	1	4	2.0	17	HR	A
20	IITA 235	3	2	1	4	2.0	21	HR	HA
Kihogo red check		9	8	8	9	8.8	81	HS	
Mean entries		3.6	3.6	3.4					

HA = Highly Acceptable
A = Acceptable (Average)
P = Poor (not accepted)
HS = Highly susceptible
HR = Highly resistant
MR = Moderately resistant
R = Resistant
S = Susceptible

Table 4: Plant height, yield and two components of promising selected genotypes at Kikusya 2015

S/N	Rice genotype	Height (cm)	Tillers/Plant	Yield (kg/ha)	1000 kernel wt(g)
1	Salama M19	89 a	9 a	2688 b	29 a
2	Moroberekan	94 b	8 a	1275 d	26 b
3	Gigante	99 c	13 b	1233 d	23 c
4	Salama M1	105 e	13 b	2525 b	26 b
5	Lunyuki	97 c	10 a	3108 a	30 a
6	IRAT 256	90 a	11 a	2920 b	27 b
7	Salama M55	94 b	10 a	1963 c	27 b
8	Salama M57	98 c	7 c	1275	28 a
9	Irat 252	94 b	6 c	2213 c	30 a
10	IITA 235	88 a	7 c	1818 c	28 a
11	Mwangaza	81 d	7 c	1588d	26 b
12	Zambia	87 a	10 a	963e	
	Mean	89	8	1600	28
	CV%	17	23	26	9

Means followed by same letters in a column are not statistically significantly different.

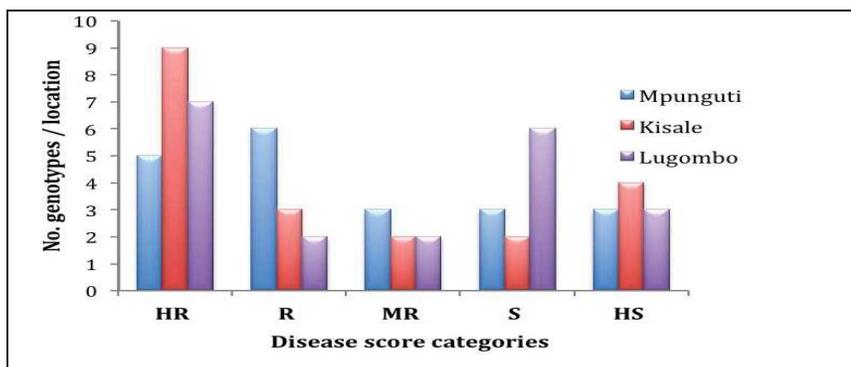


Fig. 1: Reaction of 20 genotypes to RYMV inoculation at three locations

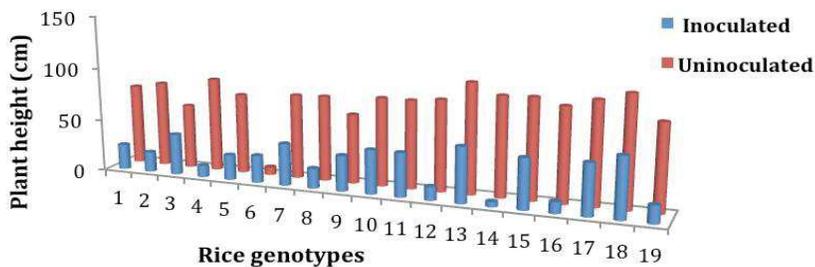


Fig 2: Mean Percentage height reduction among cultivars inoculated with RYMV across 4 locations

DISCUSSION

Quite significant differences in responses to inoculation among rice genotypes evaluated on station and on farm were observed (Table 1 - 4). All the popular local genotypes and lines from IRRI originating in Asia were highly susceptible to RYMV compared to upland genotypes such as Nerica 8, 11 - 13 which showed resistance (Table 1 - 2). These results support other findings that few resistant genotypes have been identified among the popular lowland adapted *indica* rice genotypes (Thiemele *et al.*, 2010; Rakotomalala *et al.*, 2008). Strong resistance to RYMV has been found mostly in the native African rices *Oryza glaberrima* and partial resistance in japonica upland cultivars (Zouzou *et al.*, 2008; Samado *et al.*, 2008). A certain degree of cultivars by strain virulence interaction was observed among genotypes screened between the two ecosystems (Table 3, Fig. 1). Some genotypes were susceptible to viral strains in one location but resistant elsewhere indicative of differences in virulence between isolates in the two locations and race specific resistant mechanisms as reported by

Dago *et al.* (2015), Amacho *et al.* (2009) and Issaka *et al.* (2012). Viral isolates from the lowland ecosystems represented by Mpunguti and Lugombo sites appeared more virulent or aggressive by attacking more of the tested genotypes than those from Kisale and Kikusya upland ecology (Fig. 1). Comparable findings were reported by Soko *et al.* (2010), Issaka *et al.* (2012) and Ochola *et al.* (2011). According to Amacho *et al.* (2011), RYMV is highly variable. Three of the major strains of the virus *S4*, *S5*, *S6* in addition to a “new” resistance breaking strain have been characterized from different rice growing areas in Tanzania whereby *S4-l* serotype and variants were prevalent in Mbeya and Lake Nyasa (Kanyeka, 2007).

As such they may be attributed to sporadic disease epidemics reported in the hot spot lowland rice growing areas in Kyela district although full characterization has not been done. Resistance conferred by the major genes *rymv* 1-2 identified in the *O. sativa* Gigante from west Africa was overcome by pathovars in all locations. Studies by Soko *et al.*, (2015) proved that continuous cropping of same productive rice cultivars contributes to the emergence of more virulent and aggressive isolates. Based on severity of symptoms expressed at 50 days post inoculation (dpi) genotypes were differentiated into 4 groups comprising of HS (20%), S (10%), MR (20%) and HS (50%) (Table 3, Fig 1). Apart from premature death, susceptible cultivars were characterized by major height reduction

up to 74% (Figure 2). Resistance at host plant level is characterized by reduced virus multiplication, reduced symptom expression, insignificant height reduction and high yield (Traore *et al.*, 2010; Onwghalu *et al.*, 2011). Highly resistant genotypes exhibited no symptoms at all locations and were considered immune (Rakotomalala *et al.*, 2009).

The study identified 9 rice genotypes which comprised of a local collection, Lunyuki and other introductions namely Salama M55, M57, M35, Mwangaza, IITA 235, Moroberekan, FARO II and IRAT 252 which were HR or immune. Five among these Salama M57, M19, M55, IITA 235, M35 were acceptable to farmers in terms of yield, cycle length, height, apart from RYMV resistance. Kalalu variety released as resistant in 2008, succumbed to infection at all sites whereas Mwangaza was quite stable (Luzi – Kihupi *et al.*, 2008; 2009). Moroberakan had poor yield while Lunyuki had quite high yields but grain type was not very favorable. Some improvement is desirable for this variety and further research to enhance availability of seed of the genotypes which combined durable resistance to RYMV with other traits acceptable to farmers to minimize damage from RYMV. The highly resistant varieties can also be deployed as resistance sources for improving the farmer preferred local varieties that possess good culinary traits in breeding ongoing cross breeding programmes.

CONCLUSION

From the study nine genotypes including a landrace were considered to have broad resistance to RYMV of which five had agronomic traits preferred by farmers. Further on farm research validation and promotion for two years to enable official release of these genotypes for wider use and availability of seed to farmers is very necessary to mitigate impact of RYMV on productivity and broaden genetic base to slow down emergence of new virulent strains. There is also a need to deploy the genotypes as resistance sources, in breeding programmes for improving the farmer preferred local varieties that possess good culinary traits. Unfortunately, screening work was based on RYMV isolates from Kyela district only as it was considered to harbour the most virulent strains. Because of the biological diversity of the virus, it is quite necessary to validate resistant varieties in relation to a wider range of isolates through tests in other major rice growing zones in the country before final release can be made.

ACKNOWLEDGEMENT

We gratefully acknowledge the National commission for Science and Technology (COSTECH) through National Fund for Advancing Science

and Technology (NFAST) for financial support, Uyolet agricultural research Institute and Kyela district council for logistics support.

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