Identification of Weed hosts of *Rice yellow mottle Sobemovirus* at Sayen Gobirawa, Northern Nigeria

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Abstract:
A study was carried out to identify the weed hosts of Rice yellow mottle Sobemovirus (RYMV) at Sayen Gobirawa, northern Nigeria during the 2005/06 cropping season. Weed species with or without the typical symptoms of the virus were collected in and around the RYMV-infected rice field. The detection of the pathogen was based on double antibody sandwich enzyme - linked immunosorbent assay (DAS-ELISA) and bioassays of cultivated plants. All the weed species (Cynodon dactylon (L.) Pers, Cyperus esculentus L., Cyperus rotundus L., Eleocharis complanata Boeck, Eleusine indica (L.) Gaertner, Fuirena umbellata Rottb., Imperata cylindrica L., Kyllinga pumila Michaux and Paspalum vaginatum Sw.) tested positive for RYMV. The present results suggest that some weed grasses belonging to the families Cyperaceae and Poaceae could serve as natural hosts of RYMV and therefore could play a significant role in the spread of the disease.

Keywords: Weed hosts; *Rice yellow mottle Sobemovirus;* Sayen Gobirawa; Nigeria

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INTRODUCTION

Rice yellow mottle Sobemovirus is positive sense RNA virus (Bakker, 1970; 1974). The virus was first reported in 1966 at Otonglo, Kenya, near Lake Victoria in East Africa (Bakker, 1970). It was later found in West Africa (Raymundo and Buddenhagen, 1976), southern Africa and Madagascar (Reckhaus and Randrianangaly, 1990). In 2001, it was noticed in Central Africa (Traore *et al.*, 2001) and by 2002 it was already in Europe (Koklu and Yilmaz, 2004). The virus is spreading rapidly between and within the countries of the world.

Infection of rice by RYMV is becoming increasingly important in Nigeria too (Abo *et al.*, 2002). Following its first appearance in 1978 at Ibadan in Oyo State (IITA, 1979) and spread to other parts of the country (Awoderu, 1991; Singh *et al.*, 1997; Abo *et al.*, 2002; Alegbejo *et al.*, 2006), the incidence ranges from 5 to 100 % (Rossel *et al.*, 1982; Awoderu, 1991; Alegbejo *et al.*, 2006). Consequently, yield losses averaging 25 to 100 % have been recorded (Rossel *et al.*, 1982; Alegbejo *et al.*, 2006). Various weed species have been implicated in the epidemiology of the virus. Bakker (1974) reported that the pathogen was probably on grass weeds prior to rice cultivation and then spread to it. Additionally, Okioma *et al.* (1983) and Awoderu (1991) reported that some wild grass species which occur abundantly around rice fields are important reservoirs of the pathogen. Earlier studies reported that RYMV has a narrow host range which is restricted to species in the Poaceae, tribes Oryzae and Eragrostidae (Bakker, 1974). Experimental hosts of the virus are the *Cynodon dactylon* (L.) Pers., *Digitaria sanguinalis* (L.) Scop., *Dinebra retroflexa* (Vahl) Ponzer, *Echinochloa colona* (L.) Link and *Eleusine indica* (L.) Gaertner (Bakker, 1974; Okioma *et al.*, 1983; Awoderu, 1991; Konate *et al.*, 1997. Abo *et al.*, 2003). On the other hand, *Ischaemum rugosum* Salisb and *Oryza longistaminata* A. Chev and Roehr have been reported as its natural weed hosts (Bakker, 1974; Awoderu, 1991; Konate *et al.*, 1997; Abo *et al.*, 2002). Knowledge of weed hosts is essential for the characterization of virus, detection of new strains and sustainable management strategies. The major objective of this study was to identify the weeds that serve as natural reservoirs of RYMV.

Materials and Methods

Field surveys and samplings

Weeds with symptoms such as leaf mottling yellowing, blistering, curling, narrowing distortion, vein clearing and stunting were collected randomly from Sayen Gobirawa (11°1'N, 7°40'E, 650 m above sea level). Weeds without symptom of infection were also collected. The samples were packed in plastic bags, labeled and stored in the freezer (-20 °C) at the Virology Laboratory of the Department of Crop Protection, Ahmadu Bello University, Zaria, until used. Samples were collected from April, 2005 to June, 2006. They were taken from the rice field, edges and vicinity of the RYMV-infected plants. For each weed species 36 symptomatic and symptomless samples were collected. In all, nine weed species were studied.

Serology

The serological detection of the virus was based on double antibody sandwich enzyme linked immunosorbent assay (DAS ELISA) as described by Clark and Adams (1977). Each weed species was evaluated in one homogenate, in duplicate wells of the polystyrene microtitre plate. The monoclonal antibody against RYMV (AS 0478/11) supplied by Dr. S. Winter of the Plant Virus Collection Centre, DSMZ - Braunschweig, Germany was used. The Oyo RYMV isolate (DSMZ 0478) was used as a positive control while leaf extract of healthy non cereal plant (*Tridax procumbens* L.) served as negative control. The various buffers used include coating buffer (1.5 g sodium carbonate, 2.93 g sodium bicarbonate, 0.20 g, sodium azide pH 9.6, conjugate buffer (PBS-T, 2 % PVP + 0.2 % egg albumin per litre) and substrate buffer (97 ml diethanolamine, 0.2 g sodium azide per H_2O, pH 9.8). The experiment was carried out twice.

Bioassay tests

Crude extract from symptomatic and symptomless samples was prepared separately by homogenizing leaves with 0.1M phosphate buffer, pH 7.4 in 1:10 ratio (w/v). Carborundum powder (600 mesh) was added to the extract of each sample and the homogenate rubbed onto leaves of 2 week old six seedlings of the rice cultivar Bouake 189, which is highly susceptible to RYMV. Three seedlings of each species were left uninoculated to serve as controls. The test plants were kept in a screen house at 22 30 °C and inspected for symptoms daily. A total number of 6 observations were made at weekly interval.

Back inoculation

The leaves of plant species that remained symptomless were back assayed on Bouake 189 seedlings and observed for 4 weeks. Three uninoculated seedlings served as controls.

Results and Discussion

Reaction of the weed samples in serology and bioassay tests 1

The entire weed species tested positive for RYMV while the healthy controls were negative (Table 1). When the plants were inoculated with extract of symptomatic samples none of the weed species induced completes susceptibility. A susceptibility of 50 % (3/6) resulted from the extract of the weed species *Cyperus esculentus* L., *C. rotundus* L., *Fuirena umbellata* Rottb., and *Kyllinga pumila* Michaux within the family Cyperaceae. In the family Poaceae, the weed species *Cynodon dactylon* (L.) Pers, *Eleusine indica* (L.) Gaertner and *Imperata cylindrica* L. induced 50 % infectivity. Conversely, *Eleocharis complanata* Boeck and *Paspalum vaginatum* Sw. resulted in a susceptibility of 33.3 % (2/6) within the Cyperaceae and Poaceae family, respectively (Table 2). When the plants were treated with extract of symptomless samples *I. cylindrica* induced 100 % (6/6) infection while a susceptibility of 83.3 % (5/6) was induced by *E. indica* (Table 2). Infectivity of 66.7 % (4/6) resulted from *C. dactylon, F. umbellata, K. pumila* and *P. vaginatum* while the weed species *C. esculentus, C. rotundus*, and *E. complanata* produced 50 % infection (Table 2). When the result of the bioassay test was considered in general terms, 51.7 % (31/60) members of the Cyperaceae showed the typical symptoms of RYMV. In contrast, 62.5 % (30/48) species of the Poaceae induced the disease. In all the back inoculation tests the indicator plants exhibited the typical symptoms of the virus.

Table 1: Reaction of the various weed species in double antibody sandwich enzyme linked immunosorbent assay

Weed Species	Sero-reaction
Experiment 1	
Cynodon dactylon	+
Cyperus esculentus	+
Cyperus rotundus	+
Eleocharis complanata	+
Eleusine indica	+
Fuirena umbellate	+
Imperata cylindrical	+
Kyllinga pumila	+
Paspalum vaginatum	-
Healthy Control	
Experiment 2	
Cynodon dactylon	+ +
Cyperus esculentus	+ +
Cyperus rotundus	+
Eleocharis complanata	+ +
Eleusine indica	+
Fuirena umbellata	+
Imperata cylindrica	+ +
Kyllinga pumila	+ +
Paspalum vaginatum	+
Healthy Control	-

+ = Light yellow colouration
+ + = Deep yellow colouration
- = Colourless

Reaction of the weed samples in serology and bioassay tests 2

All the weed species tested positive for the virus (Table I). In the bioassay tests it was observed among the members of Cyperaceae that the level of infection 66.7 % (4/6) produced by *K. pumila* was the highest when the plants were inoculated with extract of symptomatic samples (Table 2). Fifty % susceptibility resulted from inoculation with extract of *C. esculentus, E. complanata*, and *F. umbellata*. On the other hand, susceptibility of 33.3 % was produced by *C. rotundus*. Among the species of Poaceae inoculation of the test plants with symptomatic samples resulted in complete infection by extract of *I. cylindrica*. On the other hand, susceptibility of 66.7, 50.0 and 33.3 % each was recorded upon inoculation with *C. dactylon, E. indica*, and *P. vaginatum*, respectively. When the plants were treated with extract of symptomless samples the members of Cyperaceae *C. esculentus, F. umbellata*, and *K. pumila* induced the highest (83.3 %) susceptibility while *C. rotundus* and *E. complanata* produced 66.7 % infection (Table 2). Within the members of Poaceae, inoculation with extract of *C. dactylon* resulted in the highest (83.3 %) level of susceptibility. However, the use of *E. indica, I. cylindrica* and *P. vaginatum* produced 66.7 % infection each (Table 2). Considering the level of infection in general terms 63.3 % (38/60) members of the Cyperaceae induced RYMV symptoms while 66.7 % (32/48) species of the Poaceae produced the disease in the test plants (Table 2).

The detection of the virus in all the weed species indicates their importance in the ecology and survival of the pathogen. Additionally, the ability of the virus extract from the symptomless weed samples to induce RYMV symptoms suggests their significant role in the epidemiology of the disease. Rosenkranz (1983) reported that symptomless hosts of a virus can be particularly troublesome if they act as overwintering hosts and remain unknown since bioassay has to be carried out in order to confirm their presence. Therefore, timely and effective control of the weeds is essential. The susceptibility of all the perennial weed species is of special interest since they are capable of harbouring the pathogen all the year round and for extended number of years. The role of the susceptible annual weeds is also of great concern since the virus can survive in infected dry leaves (Bakker, 1970). Bakker (1974) stated that perennial weeds provide avenues for the virus to survive during the off season and then serve as sources of inocula at the beginning of the new season. Additionally, the annual weeds could also harbour the virus during the growing season and serve as sources of inocula for secondary spread (Rosenkranz, 1980). These were probably responsible for the high RYMV incidence (100 %) observed during the field surveys (data not shown).

The detection of RYMV in mechanically inoculated *Eleusine indica* (L.) Gaertner bas been reported by Awoderu (1991) while this study shows that natural infection is also possible. However, this report is not in agreement with the earlier work of Abo *et al.* (2003), who reported it as a non-host of RYMV. This might be due to the difference in the pathogenicity of the isolates involved (Fargette *et al.*, 2000; N'Guessan *et al.*, 2001). Also, the detection of the virus in *Imperata cylindrica* contrast to the findings of Abo *et al.* (2003), could also be attributed to the difference in the pathogenicity of the isolates (Fargette *et al.*, 2001; N'Guessan *et al.*, 2001) evaluated. The report of RYMV in the mechanically inoculated *Cynodon dactylon* was earlier reported by Awoderu (1991) while this study reports natural infection by the pathogen. The differences observed in the symptoms expressed upon inoculation with extract of the various weed species could be attributed to the difference in the virulence (N'Guessan *et al.*, 2001) of the strains of the virus invading the weeds.

Table 2: Reaction of Bouake 189 rice plants to extract of weed samples collected from RYMV infected field at Sayen Gobirawa in 2005/2006

Weed species		EXPERIMENT I								EXPERIMENT 2							
		Number of plants infected when inoculated with extract of:				Back inoculation				Number of plants infected when inoculated with extract of:				Back inoculation			
	LC	ST	C	SL	C	ST	C	SL	C	ST	C	SL	C	ST	C	SL	C
Cyperaceae																	
Cyperus esculentus	P	3/6	0/3	3/6	0/3	4/6	0/3	5/6	0/3	3/6	0/3	5/6	0/3	5/6	0/3	6/6	0/3
C. rotundus	P	2/6	0/3	3/6	0/3	3/6	0/3	4/6	0/3	2/6	0/3	4/6	0/3	3/6	0/3	5/6	0/3
Eleocharis complanata	P	2/6	0/3	3/6	0/3	3/6	0/3	3/6	0/3	3/6	0/3	4/6	0/3	3/6	0/3	3/6	0/3
Fuirena umbellata	P	3/6	0/3	4/6	0/3	3/6	0/3	4/6	0/3	3/6	0/3	5/6	0/3	3/6	0/3	6/6	0/3
Kyllinga pumila	P	3/6	0/3	4/6	0/3	4/6	0/3	4/6	0/3	4/6	0/3	5/6	0/3	5/6	0/3	5/6	0/3
Poaceae																	0/3
Cynodon dactylon	P	3/6	0/3	4/6	0/3	3/6	0/3	5/6	0/3	4/6	0/3	5/6	0/3	4/6	0/3	6/6	0/3
Eleusine indica	A	3/6	0/3	5/6	0/3	4/6	0/3	5/6	0/3	3/6	0/3	4/6	0/3	3/6	0/3	5/6	0/3
Imperata cylindrical	P	3/6	0/3	6/6	0/3	3/6	0/3			6/6	0/3	4/6	0/3			4/6	0/3
Paspalum vaginatum	P	2/6	0/3	4/6	0/3	3/6	0/3	3/6	0/3	2/6	0/3	4/6	0/3	3/6	0/3	5/6	0/3

A = Annual species; P = Perennial species; C = Control; LC = Life Cycle; ST = Symptomatic sample; SL = Symptomless sample

Therefore, these weeds could serve as sources of inocula in immunological and molecular characterization studies aimed at identifying the strains of the virus (Rosenkranz, 1987) as well as their distribution. Interestingly, inocula could also be obtained from these weed species by plant breeders to confer resistance on the RYMV susceptible rice cultivars.

Although it has been reported that weed hosts of RYMV are mainly in the Poaceae family, (Bakker, 1974) RYMV is highly variable so that biological and serological differences among its isolates are not uncommon (Konate *et al.*, 1997; N'Guessan *et al.*, 2000, 2001; Pinel *et al.*, 2000). This was probably responsible for infection of the weed species belonging to the family Cyperaceae.

Conclusion

The results of this study indicated that weed species in the family Cyperaceae could also harbour the virus. However, this is the first report of RYMV on *Cyperus esculentus*, *Cyperus rotundus*, *Eleocharis complanata*, *Fuirena umbellata, Imperata cylindrica, Kyllinga pumila*, and *Paspalum vaginatum*. Future natural weed hosts studies should evaluate plant species in other families.

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