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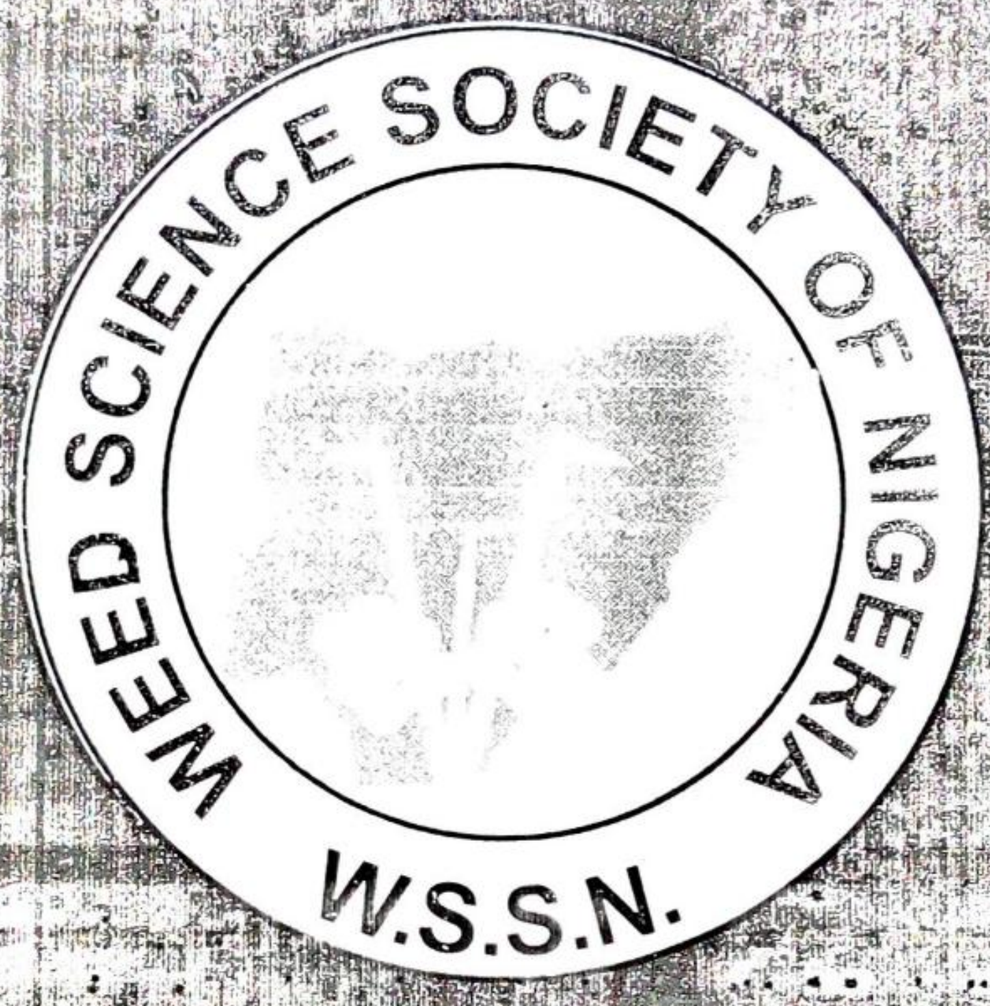

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Research Note

Possible wild hosts of *rice yellow mottle Sobemovirus* in Northern Nigeria

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Abstract

Nine grass weeds with and without the typical mottling and yellowing symptoms of *Rice yellow mottle* (genus *Sobemovirus)* were collected from the infected rice field at Bomo, northern Nigeria. The weed samples were analyzed by direct antibody sandwich enzyme - linked immunosorbent assay (DAS-ELISA). 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 the virus. This is the first report of these weed species as natural reservoirs of the pathogen.

Key words: wild hosts, *rice yellow mottle Sobemovirus,* weeds

Introduction

Rice yellow mottle virus (RYMV) 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).

Infection of rice by RYMV is becoming increasingly important in Nigeria too (Abo *et al.*, 2002). Following its first appearance in 1975 in Niger and Oyo States

(Raymundo and Buddenhagen, 1976) and spread to other parts of Nigeria (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 in rice averaging 25 to 100 % have been recorded (Rossel *et al*., 1982; Alegbejo *et al*., 2006).

RYMV survives on weed species belonging to the family Poaceae, particularly those in the tribe Eragrostidae (Bakker, 1974). Studies have shown that *Cynodon dactylon* (L.) Pers, *Digitaria sanguinalis* (L.) Scop., *Dinebra retroflexa* (Vahl) Ponzer, *Echinochloa colona* (L) Link, *Eleusine indica* Gaertner are experimental hosts of the virus (Bakker, 1974; Raymundo and Buddenhagen, 1976; Okioma *et al*.,1983; Awoderu, 1991; Abo *et al*., 2003). On the other hand, *Ischaemum rugosum* Salisb, *Oryza longistaminata* A.Chev & Roehr and *Panicum repens* L. are natural reservoirs of the pathogen (Bakker, 1974; Awoderu ,1991; Konate *et al*.,1997; Abo *et al*..2002). Considering that the virus is spreading at alarming rate within and between States in Nigeria and the need for effective management of the pathogen, knowledge of its wild hosts is essential. Thus, this study was carried out to identify the weeds that serve as natural hosts of the pathogen.

Materials and Methods

Field surveys and sampling : Monthly field sampling for naturally RYMV - infected grass weeds was carried out at Bomo (11 °11' N, 7° 38 ' E, 695m above sea level) in the northern Guinea Savanna agro- ecological zone of Nigeria, from April, 2005 to June, 2006. Leaves of grass weeds with and without the symptoms of RYMV were collected from the bunds, edges of the field, within the rice field and in the vicinity of RYMV infected plants and stored in the freezer at the Virology Laboratory, Department of Crop Protection, Ahmadu Bello University, Zaria, until used. These samples were then tested in direct antibody sandwich enzyme-linked immunosorbent assay (DAS - ELISA) as described by Clark and Adams (1977).

Direct antibody sandwich enzyme-linked immunosorbent assay: DAS - ELISA was carried out at the Virology Laboratory, Department of Crop Protection Ahmadu Bello University, Zaria, Nigeria. Ten millilitres of the coating buffer (1.59 g sodium carbonate, 2.93 g sodium bicarbonate, 0.20 g sodium azide dissolved in 1 litre H_2O and adjusted to pH 9.6 with hydrochloric acid) was mixed with 10 l of the coating antibody (IgG 0074) and 100 l of the solution was added to each well of the polystyrene microtitre plate. The plate was incubated at 37 °C for 4 hours and then washed three times with phosphate buffered saline - Tween 20 (PBS - T) containing 8.0 g sodium chloride, 0.2 g monobasic potassium phosphate, 1.15 g dibasic sodium phosphate, 0.2 g potassium chloride, 0.2 g sodium azide dissolved in 1 litre H_2O and adjusted to pH 7.4 with sodium hydroxide + 0.5 ml litre -1 Tween 20. Plant extracts were prepared by pooling the leaves of each weed species together and then homogenizing them with 0.1 M phosphate buffer, pH 7.4, at 10 % w/v (1 g of leaves in 10 ml of 0.1M phosphate buffer). The leaf extract of a healthy non

-cereal plant (*Tridax procumbens* L.) was used as healthy control. Each sample was tested in duplicate wells of the microtitre plate. One hundred microlitres of each test sample was added to its respective wells and the plate was incubated overnight at 4 °C. The plate was then washed thrice. Six millilitres of the conjugate buffer [8.0 g sodium chloride 0.2 g potassium chloride, 0.2g sodium azide dissolved in 1 litre H_20 and adjusted to pH 7.4 with sodium hydroxide + 0.5 ml^{-1} Tween 20 + 2 % PVP, 0.2 % egg albumin (Sigma 5253) was mixed with 6 of the antivirus conjugate (AS - 0074, IgG - AP) and 100 l of the solution was added to each well of the test samples. The microtitre plate was then incubated at 37 °C for 4 hours and washed three times. Two tablets of the p - nitrophenyl phosphate (Sigma 104 - 105) was dissolved in 10 ml of the substrate buffer (97 ml diethanolamine, 1 litre H_20, 0.2 g sodium azide and adjusted to pH 9.8 with hydrochloric acid) after which 100 l of the solution was added to each well of the plate. Finally, the plate was incubated at room temperature for 30 minutes. Assessment of the results was done visually. Reaction was accepted to be positive where the colourless p - nitrophenyl phosphate hydrolyzed to a yellow p - nitrophenol.

Results and Discussion

All the weed species tested positive for RYMV while the healthy control was negative (Table 1). This implies that all the test weed species can harbour the virus during the growing season and serve as sources of inocula for secondary spread (Rosenkranz, 1980). It also indicates that the pathogen can survive on them during the off season and then serve as sources of primary inocula at the beginning of the new season (Bakker, 1974). The more important natural reservoirs of the virus are the perennial weeds which can host the pathogen all the year round and for extended number of years. However, the virus can also survive in infected dry leaves of the susceptible annual weeds, resulting in accumulation of inocula. This was most likely responsible for the high incidence (100 %) of the virus observed at the study field during the surveys (data not shown). Therefore, management strategies require in part, timely and effective weed control during and after the growing season. Effective destruction of volunteer plants and debris is also vital in this regard.

The differences observed in the reactions of the various weed species could be attributed to the difference in the pathogenicity and virulence (N' Guessan *et al.*, 200 1) of the strains of the virus invading the various weed hosts. Therefore, these weeds could serve as sources of inocula in immunological and molecular characterization studies aimed at identification of 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. The detection of RYMV in mechanically inoculated *Cynodon dactylon* and *Eleusine indica* has been reported by Awoderu (1991) while this study shows that natural infection is also possible. This is the first report of natural infection of these weeds by RYMV. However, additional research is needed, particularly on those weeds which exhibited weak positive reaction.

Table 1. Results of double antibody sandwich enzyme - linked immunosorbent assay showing reaction profiles of the wild hosts of Rice yellow mottle Sobemovirus at Bomo, Nigeria

Family	Weed species	Growth Habit	Number of samples collected	Sero - reaction
Cyperaceae	*Cyperus esculentus* (L)	P	36	++
	Cyperus rotundus (L.)	P	36	-
	Eleocharis complanata (Boeck)	P	36	+
	Fuirena umbellata (Rottb)	P	36	+
	Kyllinga pumila (Michaux)	P	36	+
Poaceae	*Cynodon dactylon* (L.)	P	36	++
	Eleusine indica (L.)	A	24	-
	Imperata cylindrical (L.)	P	36	++
	Paspalum vaginatum (Sw.)	P	36	+
	Healthy control (*Tridax procumbens* L.)			-

A = annual; P = perennial; + = weak positive reaction; + + = strong positive reaction;
- = negative reaction

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