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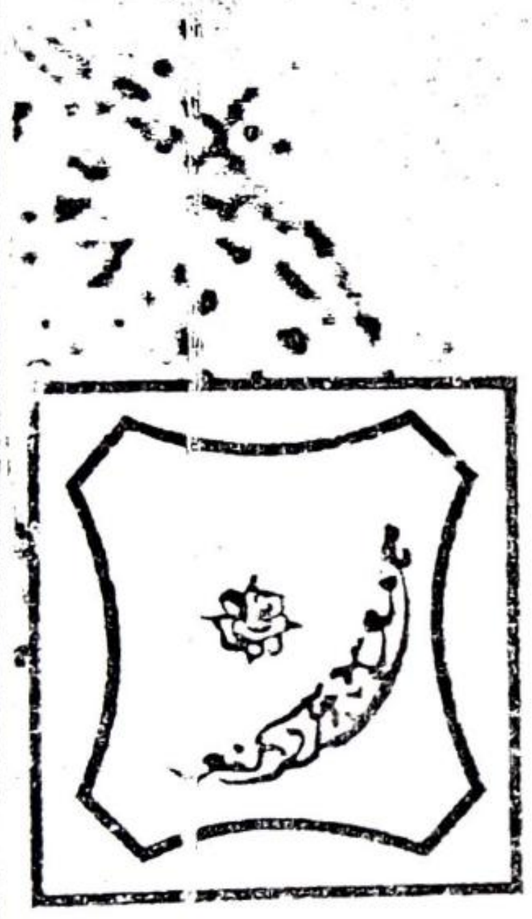

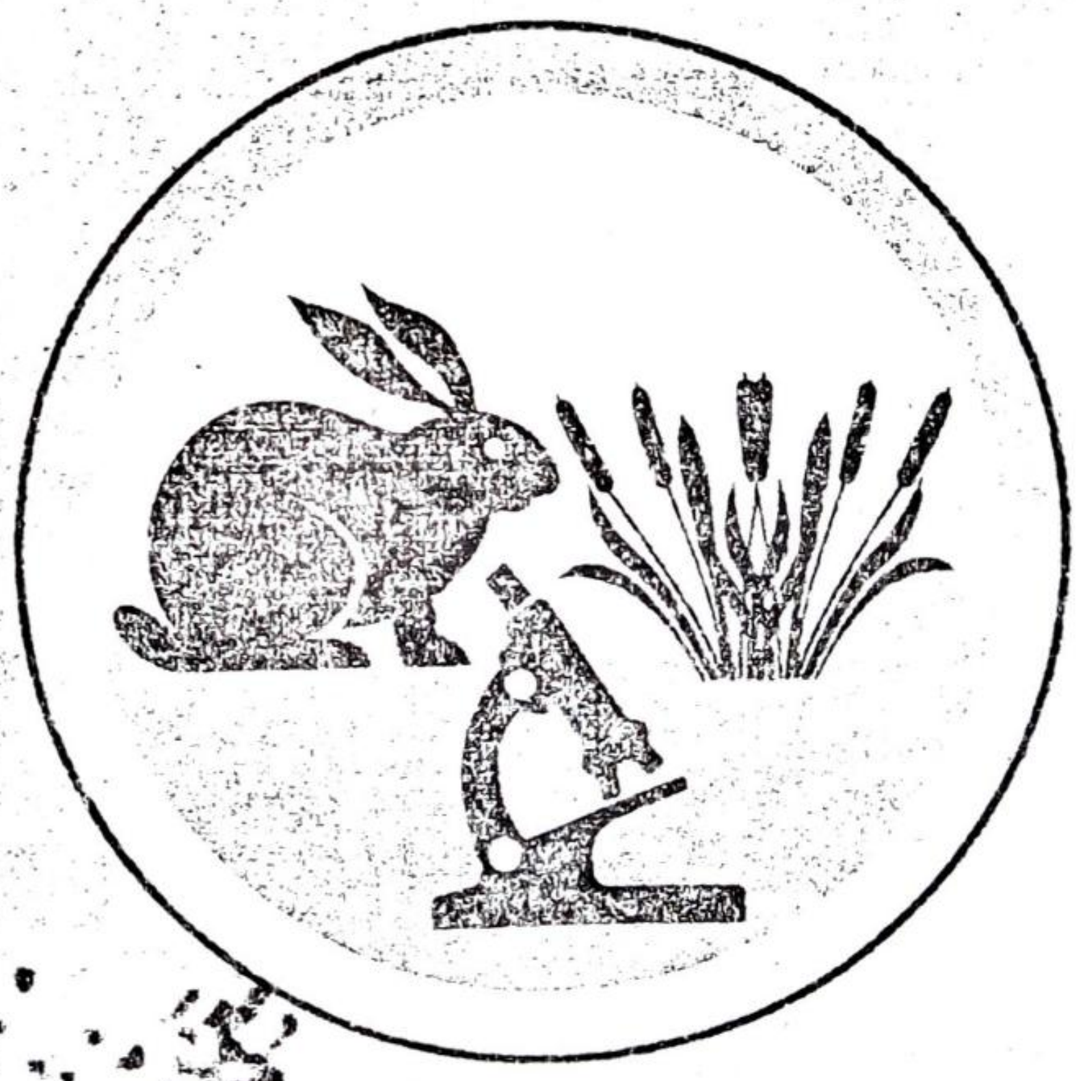

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PRELIMINARY STUDIES ON THE SEROTYPES AND SERO-DISTRIBUTION OF Rice yellow mottle Sobemovirus IN NORTHERN NIGERIA

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ABSTRACT

Twenty-seven field isolates of Rice yellow mottle Sobemovirus were collected from different locations in northern Nigeria, with one from Cameroon isolates and two from Niger Republic, during the 2005 and 2006 cropping years. The isolates were typed serologically in the Triple Antibody Sandwich Enzyme-linked Immunosorbent Assay (TAS-ELISA) test. The test showed that two serotypes (SA and SB) now exist in the northern Nigeria with SB dominating. These serotypes are entirely different from those obtainable in Cameroon and Niger Republic.SA and SB were found in Kaduna State, in northern Guinea Savanna while only one serotype (SA) was found in Katsina and Sokoto States in the Sudan Savanna agro-ecological zone of Nigeria. This is the first report of having more than one serotype of the virus in Nigeria. The information provided on the serotypes and their distribution could be used in rice breeding programme to develop resistant cultivars.

Keywords: Serotypes, Sero-distribution, Rice yellow mottle virus, Northern Nigeria

INTRODUCTION

Rice yellow mottle virus (RYMV) was first noticed in Otonglo, Kenya in 1966 (Bakker, 1970). In Nigeria, it was first identified in 1975 (Raymundo and Buddenhagen, 1976) in Niger and Oyo States. However, it has since spread to Anambra and Imo States (Awoderu, 1991), Akwa-Ibom, Ebonyi and Sokoto States (Singh *et al.*, 1997), Bauchi, Gombe and Kano States (Singh *et al.*, 1997; Abo *et al.*, 2002) and recently, to Zamfara State (Alegbejo *et al.*, 2006).

RYMV attack can be identified by mottling and yellowing of the leaves of infected plants, reduced tillering, stunting, poor panicle development, sterility, non-uniform flowering, spikelet and grain discoloration, and death of plants (Bakker, 1970, 1971, 1974; Rossel *et al.*, 1982; Fargette *et al.*, 2002).

Six serotypes are known to exist: S1, S2 and S3 are present in West Africa while S4, S5 and S6 are in East Africa (Pinel *et al.*, 2002 ; Banwo *et al.*, 2002; Fargette *et al.*, 2002; Pinel-Galzi and Fargette, 2006). However, only one serotype (S1) has been found in Nigeria (Mansour and Baillis, 1994) and this was from three isolates collected over 15 years ago from the south western Nigeria (Mansour and Baillis, 1994; Fargette *et al.*, 2002).

At present, there is scanty information on the serotypes of the virus, which must have evolved over the years in Nigeria. However, such an understanding is a powerful tool required by plant breeders in order to develop resistant cultivars, which to date, remains the most effective and sustainable measure in the management of the disease. Therefore, more research is needed to determine the distribution and differentiate the serotypes in view of their epidemiological importance.

MATERIALS AND METHODS

Collection of isolates and maintenance of virus

Leaves of, and whole rice plants showing the mottling and yellowing symptoms of the RYMV were collected during the vegetative stage of the crop, in April, 2005 and June, 2006 (Table 1). The infected rice leaves were preserved in the freezer while the live plants were maintained in the screenhouse at the Department of Crop Protection, Ahmadu Bello University, Zaria, Nigeria. The virus was recovered from the infected leaves by back inoculation to the highly susceptible rice *Oryza sativa indica* cultivar Bouaké 189. The inoculum was prepared by pooling the infected leaf tissue of each isolate together and then homogenizing at the ratio of 1:10 w/v (1g of leaves in 10 ml of 0.1M phosphate buffer) in 0.1M phosphate buffer, pH 7.4 using sterile mortars and pestles (Luzi-

Kihupi *et al.*, 2000; Sarra *et al* ., 2004). Carborundum powder (600 mesh) was added to the inoculum at the rate of 5mg ml $^{-1}$ (Konate *et al.*, 1997) and seedlings were inoculated three weeks after sowing (WAS) by dipping a piece of cheesecloth in the homogenate and then using it to gently streak the upper surface of the older leaves thrice (Ndjiondjop *et al.*, 1999). The inoculated leaves were then washed with distilled water (Noordam, 1973). NPK fertilizer (15-15-15) was applied at the rate of 2.5g to each pot at 4WAS. The test plants were incubated in the screenhouse at 22 30 °C and observed for symptoms expression. Leaves of the test plants showing mottling and yellowing symptoms of the RYMV were harvested four weeks after inoculation (WAI) and then used as sources of inocula during the serotyping.

Serology

Determination of the serotypes of RYMV was carried out at the Virology Laboratory, Department of Crop Protection, Ahmadu Bello University, Zaria, Nigeria.. Four monoclonal antibodies (AS 0478 /11, AS 0478/12, AS 0478/21 and AS 0478/22) raised against Nigerian isolates of RYMV were used. All the monoclonal antibodies (MAbs) were obtained from Plant Virus Collection Centre, DSMZ Braunschweig, Germany. The isolates were tested in the triple antibody sandwich enzyme linked immunosorbent assay (TAS - ELISA) as described by Clark and Adams (1977).

Triple antibody sandwich enzyme linked immunosorbent assay

Fifty microlitres of the purified coating antibody (IgG, AS-0074) was mixed with 50ml of the coating buffer (1.59g sodium carbonate, 2.93g sodium bicarbonate,0.20g sodium azide dissolved in 1 litre H_20 and adjusted to pH 9.6 with hydrochloric acid) and 100l of the solution was added to each well of the five polystyrene microtitre plates. The coated plates were then incubated for 4 hours at 37°C after which they were washed three times with phosphate buffered saline Tween 20 (PBS-T) containing 8.0g sodium chloride, 0.2g monobasic potassium phosphate, 1.15g dibasic sodium phosphate, 0.2g potassium chloride, 0.2g sodium azide dissolved in 1 litre H_2O and adjusted to pH 7.4 with sodium hydroxide + 0.5 ml^{-1} Tween 20. The microtitre wells were blocked by adding solution of 2% skimmed milk dissolved in PBS T to each well. The plates were emptied of their contents, blotted on the tissue paper and again washed thrice. Antigen was prepared from each test isolate as described earlier and 100l aliquots of each was added to its designated wells in the plates. The Oyo isolate (DSMZ 0478) and leaf extract of a healthy non-cereal plant (*Tridax procumbens* L.) was used as infected and healthy control, respectively. Each test sample was evaluated in duplicate wells. The plates were then incubated at 4°C. After further washing, 160 l of each monoclonal antibody (MAb) was diluted in 8 ml of the conjugate buffer (8.0g sodium chloride, 0.2g monobasic potassium phosphate, 1.15g dibasic sodium phosphate, 0.2g potassium chloride, 0.2g sodium azide dissolved in 1 litre H_2O and adjusted to pH 7.4 with sodium hydroxide + 0.5 ml^{-1}Tween 20 + 2 % PVP + 0.2 % egg albumin (Sigma A- 5253) and 100l of the resulting solution was added to its respective wells. Besides, 100l of the polyclonal antibody (DSMZ 0074) was tested in the duplicate wells of the healthy control. The plates were incubated at 37°C for 4 hours and then washed three times. Forty microlitres of RAM-AP was dissolved in 40ml of the conjugate buffer and 100l of the solution was added to each well of the plates. The plates were incubated at 37°C for 2 hours and then washed thrice. Ten tablets of p-nitrophenyl phosphate (Sigma 104-105) were dissolved in 50 ml of the substrate buffer (97 ml diethanolamine, 600 ml H_2O, 0.2g sodium azide and adjusted to pH 9.8 with hydrochloric acid) after which 100l of the solution was added to each well of the microtitre plates. Finally, the plates were incubated at room temperature for 30 minutes.. Assay was by visual inspection of the resulting yellow p- nitrophenol hydrolysis products of the well contents (Clark and Adams, 1977).

RESULTS AND DISCUSSION

The results of the TAS ELISA are presented in Table 2. Of the 30 isolates, only six tested positive. The Bakori, Gangara and Wurno isolates were detected by the MAb AS 0478/11 and

herein termed isolates of serotype A (SA) while the Gwargwaji, Jaji and Tudun Iya isolates tested positive to the MAb AS 0478/22 and these were referred to as isolates of serotype B (SB).The presence of only one serotype (SA) in Katsina and Sokoto States, in the Sudan Savanna agro ecological zone of Nigeria does not imply that this serotype is ecologically adapted to the zone alone since both serotypes were found co-existing in Kaduna State, which is situated in northern Guinea Savanna agro-ecological zone . This was supported by the detection of both serotypes under lowland and upland ecologies.

The presence of two serotypes within the same State (Kaduna) indicates the possibility of this State becoming a hot spot of the virus in the nearest future. Besides, the preliminary surveys carried out in the State in 2005 showed that RYMV incidence in most fields was as high as 100 % (data not shown). The detection of SA and SB at Gangara and Tudun Iya, respectively, which are less than 2 km apart corroborates the findings of N'Guessan *et al.*

(2001) who discovered two different serotypes of the virus co-existing in the nearby fields in Cote d'Ivoire. However, the implication of this is that subsequent mutation and replication of the virus could result in several serotypes with varying degree of pathogenicity on the one hand, and double infection of rice plants on the other hand. It also indicates that two individual or group of genes would be involved in developing resistant rice cultivars to the virus (Konate *et al.*, 1997).

The negative reaction of the Adamawa, Niger and Zamfara isolates to all the MAbs implies that they belonged to entirely different serotype(s) of the virus. Similarly, the negative serological reaction of the Cameroon and Nigér isolates to all the MAbs indicates that they were of another serotype(s) of the pathogen. However, future research should aim at molecular characterization of the isolates of the two identified serotypes. Also, pathogenicity and virulence of these serotypes should be investigated in view of their importance in epidemiological surveys and development of resistant rice cultivars.

CONCLUSION

The results of this work indicate that two serotypes of RYMV (SA and SB) exist in northern Nigeria. However, more isolates of the virus should be investigated. Also, molecular characterization of the isolates of these serotypes is important. Meanwhile, inocula could be obtained from the isolates of these serotypes and used in rice breeding programme to develop resistant cultivars.

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REFERENCES

Abo, M.E., Ukwugwu, M.N. and Onasanya, A. (2002): The distribution, incidence, natural reservoir hosts and insect vectors of Rice yellow mottle virus (RYMV), Genus Sobemovirus in northern Nigeria. *Tropic.* 20 (4): 198-202.

Alegbejo, M.D., Raji, B.A., Abubakar, I.U. and Banwo, O.O. (2006): Rice yellow mottle virus disease, a new disease of rice in Zamfara, Nigeria. *Int. Rice Res. Notes*, 31:1

Awoderu, V. A. (1991): Rice yellow mottle virus in West Africa. *Trop. Pest Manage.*, 37 (4): 356-362

Bakker, W. (1970): Rice yellow mottle virus, a mechanically transmissible virus disease of rice in Kenya. *Neth. J. Plant Pathol.*, 76:53-63.

Bakker, W. (1971): Three new beetle vectors of Rice yellow mottle virus in Kenya. *Neth. J. Plant Pathol.*, 77:201-206.

Bakker, W. (1974): Characterization and ecological aspects of Rice yellow mottle virus in Kenya. Ph.D thesis, Agricultural University, Wageningen, The Netherlands, 152pp

Banwo, O.O., Winter, S., Koerbler, M., Abdullahi, I., Abdallah, R.S. and Makundi, R.H.(2002): Molecular Characterisation of Rice yellow mottle virus isolates from Tanzania. In: Eight International Plant Virus Epidemiology Symposium, held at Aschersleben, Germany, 12-17 May, 2002.

Clark, M.F. and Adams, A.N. (1977): Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.*, 34: 475-483.

Fargette, D., Pinel, A., Halimi, H., Brugidou, C., Fauquet, C. and Van Regenmortel, M. (2002): Comparing of molecular and immunological typing of isolates of Rice yellow mottle virus. *Arch. Virol.*, 147:583-586.

Konate, G., Traore, O. and Coulibaly, M. (1997) : Characterization of Rice yellow mottle virus isolates in Sudano-Sahelian areas. *Arch. Virol.*, 142:1117-1124.

Luzi-Kihupi, A., Mlozi, M.R.S., Mabagala, R.B., Mushobozy, D.M.K. and Nchimbi-Msolla, S. (2000): Occurrence of Rice yellow mottle virus in *Tanzania. Tanzania J. Agric. Sci.*. 3 (2): 87-96.

Mansour,A. N. and Baillis, K. W. (1994): Serological relationships among Rice yellow mottle virus isolates. *Ann. Appl. Biol.*, 125:133-140

Ndjiondjop, M.N., Albar, L., Fargette, D., Fauquet, C. and Ghesquiere, A. (1999): The genetic basis of high resistance to Rice yellow mottle virus (RYMV) in cultivars of two cultivated rice species. *Plant Dis.* 83 (10): 931-935.

N' Guessan,P.N., Pinel, A., Sy, A. A., Ghesquiére, A. and Fargette, D. (2001): Distribution, pathogenicity, and interactions of two strains of Rice yellow mottle virus in forested and savanna zones of West Africa. *Plant Dis.*, 85 (1): 59-64.

Noordam, D. (1973): Identification of plant viruses: Methods and experiments. Centre for Agricultural Publishing and

Documentation (Pudoc), Wageningen, pp 19-22

Pinel-Galzi, A. and Fargette, D. (2006): First report of Rice yellow mottle virus in rice in uganda. *Plant Dis.*, 90:683.

Pinel, A., N'Guessan, P., Bousalem, M. and Fargette, D. (2006): Molecular Variability of geographically distinct isolates of Rice yellow mottle virus in Africa. *Arch. Virol.*, 145:1621-1638

Raymundo, S.A. and Buddenhagen, I.W. (1976): A virus disease in West Africa. *Int. Rice Commis. Newslett.*, 25:58.

Rossel, H. W., Thottappilly, G., Adeoti ,A.A. and Alam, K.Z. (1982): A new record of Rice yellow mottle virus disease in Nigeria. *Int. Rice Commis. Newslett.*, 29: 51-53.

Sarra, S., Oevering, P. Guindo, S. and Peters, D. (2004): Wind- mediated spread of Rice yellow mottle virus (RYMV) in irrigated rice crops. *Plant Pathol.* 53: 149-157

Singh, B. N., Fagade, S., Ukwungwu, M.N., Williams, C., Jagtap, S.S., Oladimeji, O., Efisue, A. and Okhidievbie, O. (1997): Rice growing environments and biophysical constraints in different agroecological zones of Nigeria. *Met. J.*, 2(1): 35-44.

Table 1: Number of isolates of Rice yellow mottle Sobemovirus used and their ecologies

Country	State	Location	Number of isolate	Ecology
Nigeria	Adamawa	Yola	1	Lowland
Nigeria	Kaduna	Bomo	1	Lowland
Nigeria	Kaduna	Gangara	1	Lowland
Nigeria	Kaduna	Gwargwaji	3	Lowland
Nigeria	Kaduna	Jaji	1	Lowland
Nigeria	Kaduna	Kwangila	1	Lowland
Nigeria	Kaduna	Mawai	1	Lowland
Nigeria	Kaduna	Samaru	3	Lowland
Nigeria	Kaduna	SayenGobirawa	2	Lowland
Nigeria	Kaduna	Shika	1	Upland
Nigeria	Kaduna	Tudun Iya	1	Upland
Nigeria	Katsina	Bakori	2	Upland
Nigeria	Niger	Edozhigi	1	Ni
Nigeria	Niger	Minna	2	Lowland
Nigeria	Sokoto	Wurno	3	Lowland
Nigeria	Zamfara	Kotorkoshi	1	Lowland
Nigeria	Zamfara	Yargeda	2	Lowland
Cameroon	ni	ni	1	ni
Niger Republic	ni	ni	2	ni

Table 2: Results of triple antibody sandwich enzyme-linked immunosorbent assay tests showing reaction profiles of isolates of Rice yellow mottle Sobemovirus

	Monoclonal Antibody (Mab)			
Isolate	AS-0478/11	AS-0478/12	AS-0478/21	AS-0478/22
Yola	-	-	-	-
Bomo	-	-	-	-
Gangara	+	-	-	-
Gwargwaji	-	-	-	- - +
Jaji	-	-	-	+ - +
Kwangila	-	-	-	-
Mawai	-	-	-	-
Samaru	-	-	-	-
SayenGobirawa	-	-	-	-
Shika	-	-	-	-
Tudun Iya	-	-	-	- - +
Bakori	+	-	-	-
Edozhigi	-	-	-	-
Minna	-	-	-	-
Wurno	+	+	-	-
Kotorkoshi	-	-	-	-
Yargeda	-	-	-	-
Cameroon	-	-	-	-
Niger Republic	-	-	-	-