ISOLATION OF THE RICE YELLOW MOTTLE VIRUS IN IVORY COAST

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ABSTRACT

A sap-transmissible virus causing a mosaic disease was isolated in several different places in the Ivory Coast on 'IR 8' and 'Jaya' irrigated rices. This paper describes the biological properties, host range, purification, physico-chemical properties and serology of this 28-nm diameter isometric virus. These results and the reaction of the virus to the rice yellow mottle virus antiserum allow us to conclude that the virus isolated in the Ivory Coast is a strain of the rice yellow mottle virus (RYMV) isolated in Kenya.

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Rice culture is growing in importance in the Ivory Coast. Therefore, it appeared necessary to inventory the virus diseases affecting it. After many surveys, only one virus disease was observed, although it was present in different parts of the country: Gagnoa, Lam-To, and Yamoussoukro. Only irrigated rices were investigated, either IR 8 or Jaya varieties.

Scattered orange-yellow spots were observed either on recently planted, or on mature rice, in the contaminated fields. This disease appeared to be localized, but it is easily transmissible and could spread rapidly and become economically important. This paper describes the virus, its host range, and its biological and physico-chemical properties.

MATERIALS AND METHODS

Mechanical transmission: Young leaves of diseased rice were cut in half-centimeter pieces and crushed in a mortar with a 0.1 M sodium phosphate buffer at pH 7.1 containing 0.25% bentonite and 0.35% cysteine hydrochloride. The leaves to be inoculated were dusted with carborundum, then rubbed. Every inoculated seedling was reinoculated on a IR 8 rice seedling.

Seed transmission: Fifteen varieties of diseased rices were mechanically inoculated. Later, seeds were collected from each of the 15 varieties and germinated in sterilized soil.

Biological properties: All of the experiments were made from crude sap of 6-week-old IR 8 seedlings, mechanically inoculated 15 days earlier.

Purification: The purification was made from frozen leaves, according to Bakker's method for RYMV (1).

Spectro-photometric properties: A dilution of purified virus was used to determine the UV absorption spectrum of the virus on a Zeiss PMQ II spectrophotometer.

Determination of the iso-electric point: The iso-electric point of the virus was determined by electro-focusing. An LKB column, 110 ml ampholytes gradient, pH 3.5-10 was filled with 5-mg virus, then put under a 300-V tension for 72 hours. The column content was later divided into 1-ml fractions. The optical density of these fractions was measured at 280 nm with a Uvikord LKB flow cell. The pH of each fraction was determined with a tacussel ISIS 4,000 pH meter. The iso-electric point of the virus is indicated by superposing the two curves.

Determination of the density: The density of the virus was determined by ultracentrifugation in a cesium chloride gradient, at a speed of 38,000 rpm in a SW 50 rotor for 20 hours. The content of the tubes was bottom-collected and divided into parts to determine alternately the optical density and the index of refraction with an Abbe refractometer.

Electron microscopy: A suspension of purified virus at 0.1 $OD_{260\ nm}$/ml was fixed on carbon grids, stained with uranyl acetate at 1% for 1 min, and observed on a Siemens Elmiskop 1 A electron microscope.

Determination of the sedimentation coefficient: The sedimentation coefficient was determined with a Spinco model E analytical ultra-centrifuge. The virus was suspended in 0.01 M sodium phosphate buffer pH 7.0, and spun at 35,600 rpm at 18° C. Four concentrations of purified virus were analyzed: 1, 2, 3 and 4 mg/ml. Shifting of the peaks on the photographs, taken with Schlieren optics, was measured and the S value of each concentration was determined by the method of Markham (3). The coefficient at infinite dilution S° was obtained by extrapolation to a virus concentration of zero. The absorption technique was also used to determine the S value.

Serology: Antiserum to the purified virus was prepared by injecting rabbits intra-muscularly with 3-mg samples of freshly purified virus emulsified with Freund's incomplete adjuvant. The rabbits' left and right thigh muscles were injected alternately, once a week for 3 weeks. Serum was collected 10 days after the last inoculation and stored at 4° C, with an equal volume of glycerol. One percent agar in 0.85% sodium chloride and 0.02% sodium azide were used in the Ouchterlony agar double diffusion tests. The tests were performed at 4° C over 72 hours. Dilutions of sap and antisera were made with 0.85% sodium chloride.

RESULTS

Symptoms: The diseased rice plants in the fields showed the following symptoms: the oldest leaves were yellow at the lower part of the plant, and orange at the top; the young leaves showed a very thin mottle ranging from yellow to dark-green (Fig. 1).

Mechanical transmission: Symptoms observed on mechanically inoculated rice were exactly the same as those described on rice in the fields, plus a dwarfing of the plant, more or less emphasized according to the date of inoculation (Fig. 2). No further symptoms developed.

All of the 15 IRAT varieties tested were somewhat susceptible to the virus (Table 1). This virus was lethal to young seedlings and systemic for older plants. Yield could be lowered by 10 to 40% according to the cultivar tested, if inoculated early -- for instance, at 21 days of age. No sterility caused by this virus disease was observed on the tested rices, however.

Table 2 lists the plants mechanically inoculated. Only the Gramineae are hosts for this virus.

FIGURE 1. Symptoms of virus disease on leaf of rice 'IR 8' in the field.
FIGURE 2. Dwarfing of inoculated rice 'IR 8' on the right, and healthy plant on the left.

Table 1. Reaction of rice cultivars inoculated with the rice virus from Ivory Coast.

Cultivar	Symptoms	Virus recovered	Cultivar	Symptoms	Virus recovered
LSX 104 x 144 B9	mottle	+	Iguape Cateto	mottle	+
1,487/9/5	do.	+	C 463 A	do.	+
Mutant 50	do.	+	Jaya	do.	+
IR 8	do.	+	Tepep	do.	+
IR 5	do.	+	OS 6	do.	+
Carréon	do.	+	CICA 4	do.	+
Zenith	do.	+	OS 5	do.	+
Moroberekan	do.	+			

Table 2. List of plants inoculated with the rice virus, and symptoms observed.

Plant tested	Symptoms observed
Oryza sativa cv. FK 135	Mosaic and strong mottle
O. sativa cv. Pacita	Light mottle and yellowing
O. sativa cv. Taichung native 1	Apical necrosis
O. rufipogon balunga	Chlorosis
O. rufipogon cubensis	Chlorosis
O. rufipogon Taiwan	Strong mottle
O. spontanea	Necrosis
O. glaberrima	Yellowing
O. nivarra	Chlorosis
O. barthii	Mottle
O. australiensis	Chlorosis
O. latifolia	Light mottle
O. alta	Mottle on young leaves
Eleusine indica	Nonhost
Eleusine coracana	Symptomless host
Zea mays	Nonhost
Chenopodium amaranticolor	Nonhost
Datura innoxia	Nonhost
Datura stramonium	Nonhost
Nicotiana glutinosa	Nonhost
Nicotiana tabacum cv. Samsun	Nonhost
Physalis alkekengii	Nonhost
Physalis floribunda	Nonhost

Seed transmission: No seedling of the 6,000 grown from the seeds collected on contaminated rice showed any symptom of the virus disease. Consequently, seed transmission is considered negative.

Biological properties: The dilution end-point varied with the origin of the inoculum. It fluctuated from 10^{-6} to 10^{-9}. The virus concentration seemed to reach a maximum 21 days after the inoculation. The inoculum progressively loses its capacity for infection from 55° up to 70° C. In crude extract, the virus can remain viable for at least 34 days at 27° C, but not for 41 days. At a temperature of 4° C, it remained infectious for more than 84 days. It remained infectious in dry leaves for more than 56 days, and for several months in frozen leaves. Consequently, it is a very stable virus that is easily preserved.

Electron microscopy: The electron microscope observations displayed spherical virus particles of 28 ± 3 nm in diameter.

Purification: The production of virus fluctuated from 400 to 600 mg per kg of extracted leaves, assuming an extinction coefficient of $E^{0.1\%}_{1\ cm,\ 260\ nm} = 6.5$ (1).

Spectrophotometric properties: The absorption reached a maximum at 260 nm and a minimum at 243 nm, which gave the following ratios:

$$E_{260}/E_{280} = 1.46 \pm 0.02; \text{ and } E_{Max}/E_{Min} = 1.29 \pm 0.03.$$

This is distinctive of a paraspherical virus having at least 15% RNA (4). Correction for light-scattering was not possible, probably because of impurities in the virus preparations.

Determination of the iso-electric point: The iso-electric point of the virus was 6.0 ± 0.2. It was observed that the virus, concentrated after this experiment, was always contaminated with impurities that prevented the light-scattering correction.

The virus was infectious after passage on the ampholyte column.

Determination of virus density: Only one band was visible in the tube, corresponding to a density of 1.359. The virus was always infectious after passage on cesium chloride.

Determination of the sedimentation coefficient: The Schlieren optics method, as well as the absorption technique, gave $S°_{20} = 116.5 \pm 1$.

Serology: The antiserum produced against this virus reacted specifically with the crude sap up to a dilution of 1/1,024. It also reacted against RYMV isolated in Kenya up to a dilution of 1/512. Respectively, RYMV antiserum from Kenya (titer 1/1,024) reacted against the virus from Ivory Coast up to a dilution of 1/512. The presence of a spur between the two viruses (Fig. 3) proves that the strains are different.

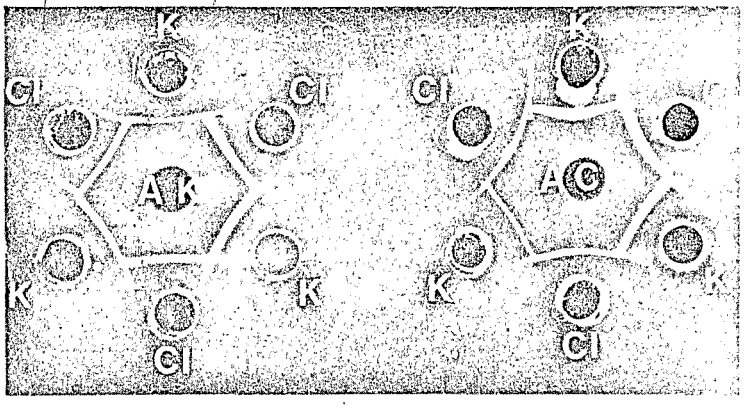

FIGURE 3. Serological reactions of crude sap from diseased rice against rice yellow mottle virus (RYMV) antisera. AK = Antiserum RYMV strain Keyna; ACI = Antiserum RYMV strain Ivory Coast; K = Crude sap from rice inoculated with RYMV strain Kenya; CI = Crude sap from rice inoculated with RYMV strain Ivory Coast.

DISCUSSION

In the rice cultures of Ivory Coast, a virus disease characterized by a mottle of the young leaves, a yellowing of the older leaves, and a more or less emphasized dwarfing of the plant was identified. This mechanically transmissible disease is caused by a spherical virus of 28 ± 3 nm in diameter. Only the Gramineae, mainly the genus Oryza, served as hosts of this virus in our tests. The virus was highly stable in crude sap as well as in dry leaves. Its thermo-inactivation occurs at 70-71° C. The concentration of virus in the plant was very high (400 to 600 mg per kg of extracted leaves). The E_{260} / E_{280} is particularly low for an isometric virus. The iso-electric point of the virus is pI = 6.0, and its density is 1.359.

When these results are compared with the results obtained on RYMV by Bakker (1,2), the almost perfect identity between the two viruses appears obvious.

The reaction of the virus described in this paper with the antisera of RYMV from Kenya proves beyond doubt the identity between the two viruses, but shows that the strains are different. It must be pointed out that the virus has been isolated only in Africa; and, up to now, it is not classified in any virus group.

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