

TWO VIRAL DISEASES OF *PSOPHOCARPUS TETRAGONOLOBUS*
IDENTIFIED IN IVORY-COAST

FAUQUET C, LAMY D., THOUVENEL J.-C.
Virology department
ORSTOM BP V51
Abidjan, Côte d'Ivoire

INTRODUCTION. The winged-bean is a backyard crop (*Psophocarpus tetragonolobus*) grown in Southeast Asia from Papua to Sri Lanka. It is a leguminous plant that is very rich in proteins either in the seeds (30/37 %), tubers (12/15 %), leaves (6/15 %) or flowers (5/6 %). The immature pods are very rich in vitamins and the seeds are a source of edible oil (15/20 % fat).

In 1975 a panel of the National Academy of Sciences in Washington, concerning underexploited tropical plants recognized its interest as a food crop through the humid tropical zone, where it can help solve the problem of malnutrition. The panel counselled testing the plant in experimental stations in different regions of the tropics. In Ivory-Coast, the *Psophocarpus* was introduced in 1976 in two regions where the annual rainfalls are respectively 1500 mm and 2300 mm. Two cultivars were used : one from Papua (New-Guinea) and one from Ghana (of Chinese origin).

From the beginning, viral diseases appeared on the first cultures. Two of them will be successively described here and the first results of their identification presented.

1° *Psophocarpus* Ringspot Mosaic Virus (Pso RSV)

- a) Symptoms : The disease was only observed in the less rainy region. Every plant of each culture was infected. The contamination seemed to be early as the symptoms could be observed on the very first leaves. The leaves of the diseased plants presented light-green ringspots. The spots can often join together to become a yellow mosaic. The exact reduction of yield caused by the diseased is not known but estimated to be about 20 %. As all the plants are infected, the total loss is rather severe.
- b) Transmission : The virus can easily be transmitted mechanically from *Psophocarpus* to *Psophocarpus*, and also to many other host-plants listed in table I. It must be noticed that the virus induces local lesions on *Chenopodium quinoa*, *Phaseolus mugo* and *Vigna unguiculata*. Many host-plants react symptomless and the virus often produces necrosis (*Nicotiana clelandii*, *N. glutinosa*, *Vigna sinensis*, *Capsicum annum*). The virus cannot infect *Cucumber*, *Tomato* or *Chenopodium amaranticolor*, among other negative host-plants (table II). In seeds harvested from naturally infected *Psophocarpus*, the transmission rate of the virus is about 1 %. *Aphis craccivora* transmit the disease in the non-persistent manner, producing the same symptoms.

- FAUQUET, C., LAMY, D. & THOUVENEL, J.-C. (1978). Two viral diseases isolated from Winged bean in the Ivory Coast. Communication au 7ème Meeting de l'International Working Group on Legume Viruses, Zürich, 24-25 août 1978.



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- c) Biological properties : The biological properties of the virus were determined in the crude sap of infected *Psophocarpus* and *Chenopodium quinoa*. Thermal inactivation was situated between 50° and 55°C. The longevity *in vitro* at, 20°C ended at 29 hours and the virus is still infectious after freezing.
- d) Purification : The crude sap of *Psophocarpus* infected leaves is clarified by chloroform and the virus is concentrated by alternance of high and low centrifugations. The virus is finally purified on a sucrose density gradient 10-40 % during 2 hours. The yield of virus is about 25 mg/kg. Purified preparations of the virus present ultraviolet absorption spectra typical of nucleoprotein and of isometric particles, with a A 260 nm/280 nm ratio of 1.45-1.5. The maximum absorption is at 260 nm and the minimum at 243 nm with a ratio of 1.18-1.25. In the electron microscope, virus preparations negatively stained with 2,5 % uranyl acetate appeared to be composed of spherical particles with a diameter of 24 nm. This purified suspension inoculated to young *Psophocarpus* induces ringspot symptoms.
- e) Serology : The virus is not a good antigen and the serum produced has only a titre of 1/32 in gel double diffusion test. The virus does not react with the following antisera : CMV to, CMV d, TAV, TAV 2, TAV ca, PSV 1 and PSV 2.
- f) Discussion : The disease is described on *Psophocarpus* for the first time. Many of its properties let us think the virus could be a cucumovirus. However, there are several differences in the host range and symptomatology between members of the cucumovirus group and the *Psophocarpus* Ringspot Virus (Table III) (MARROU et al. 1975) either those that are seed-transmitted in cowpea (ANDERSON 1975, BRANTLEY 1965, PHATAK et al. 1976, FISCHER 1976) or in bean (BOS and MAAT 1974, BIRD and al. 1974, MEINERS and al. 1977). None of the strains of CMV have been reported to produce the ringspotting symptoms in the primary leaves of cowpea characteristic of Pso RSV.

There is no serological relationship between Pso RSV and members of the CMV group but the cowpea and bean seed-transmitted strains were not tested, and the lack of serological relationships does not exclude the Pso RSV from the CMV group as for instance Cp RSV.

In order to test the hypothesis, it would be necessary to determine the molecular weight of the protein subunit and the number of nucleic acids.

For the time being, we propose to keep the name *Psophocarpus* Ringspot Virus for the causal agent of the disease.

2° *Psophocarpus* Necrotic Mosaic Virus (Pso NMV)

- a) Symptoms : The disease appeared only in the most rainy region. It affects 10 % of the plants, distributed in the whole field. The diseased leaves present necrotic patches producing distortions and sometimes we can observe a yellow mosaic on the young leaves. The surface of the leaves is very reduced and the plant is underdeveloped in comparison with the healthy plants. The severity of disease depends on the earliness of the attack. On young plants, strong apical necrosis develops and the winged-bean dies. On older plants, the number of flowers and consequently the yield are very reduced.

- b) Transmission : The disease is mechanically transmissible from *Psophocarpus* to *Psophocarpus* and also to a very limited host range, belonging to leguminosae and Solanaceae (see table IV). The only host-plant presenting symptoms out of *Psophocarpus* is *Canavalia ensiformis*, the others are symptomless.

The virus is not transmitted either by seed or aphid.

- c) Biological properties : The biological properties of the virus were determined in the crude sap of infected *Psophocarpus* and tested on seedlings of *Psophocarpus*. The thermal inactivation point is situated between 55° and 60°C, the longevity in vitro at 20°C reaches 26 hours and the virus is still infectious after 14 days congelation.
- d) Purification : The virus can be purified either from *Canavalia ensiformis* infected leaves or from *Nicotiana megalosifon* infected leaves. The crude sap is clarified with chloroform and concentrated with ammonium sulfate. The final purification is a sucrose density gradient 10 - 40 % during 3 hours. The yield of virus is about 10 mg/kg.

The ultraviolet spectra of purified preparations is typical of nucleoprotein with an helical symetry. The ratio $A_{260\text{ nm}}/A_{280\text{ nm}} = 1.09$; the maximum absorption is 260 nm and the minimum is 250 nm with a ratio of 1.03.

Virus preparations, negatively stained with 2,5 % uranyl acetate were observed in an electron microscope and revealed elongated particles with a width of 14 nm. The length of the virus is varying with the host-plant and is 614 nm long in *Nicotiana megalosifon* and 645 nm long in *Canavalia ensiformis*.

A purified suspension inoculated to young seedlings of *Psophocarpus* typical necrosis symptoms.

- e) Discussion : No such virus is known to infect the winged-bean and the disease is described for the first time. Comparing the host-range and symptoms of other filamentous viruses infecting legumes (table V) we can find no similarity with the *Psophocarpus* necrotic mosaic virus.

Besides there is no virus described with the same length and the same properties.

So we think that *Psophocarpus* Necrosis is caused by a filamentous virus previously undescribed, probably a carlavirus. We propose to name it *Psophocarpus* Necrotic Mosaic Virus.

CONCLUSION. Since the first cultures of winged-bean in 1976 in Ivory-Coast, at least two viral diseases appeared. The first one is a mosaic with ringspots and is caused by a spherical virus that is seed and aphid-transmitted. The *Psophocarpus* Ringspots and is caused Mosaic Virus (Pso RSV) is probably a cucumovirus, a particular strain of CMV or a different virus. The second disease affecting *Psophocarpus* is a necrotic mosaic the causal agent of which is a filamentous virus of 620-650 nm. It is certainly a new carlavirus infecting leguminosae and we suggest to name it *Psophocarpus* Necrotic Mosaic Virus (Pso NMV).

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TABLE I

Family	Plant host tested	Local	Systemic
Chenopodiaceae	<i>Chenopodium quinoa</i>	☆	—
Leguminosae	<i>Phaseolus lathyroides</i> <i>Phaseolus mungo</i> <i>Phaseolus vulgaris</i> <i>Pisum sativum</i> <i>Psophocarpus tetragonolobus</i> <i>Vicia faba</i> <i>Vigna sinensis</i>	 ☆ ☆ ☆	○ ● ● ○ ● ○ ●
Solanaceae	<i>Capsicum annum</i> <i>Nicotiana clevelandii</i> <i>Nicotiana glutinosa</i> <i>Nicotiana megalosiphon</i> <i>Nicotiana tabacum samsun</i> <i>Petunia hybrida</i>		● ● ● ○ ○ ○

Positive host-plants for *Psophocarpus* Ring-Spot Virus

☆ Chlorotic lesion

★ Necrotic lesion

○ Symptomless

● Mosaic

TABLE II

Family	Plant-host tested	Local	Systemic
Chenopodiaceae	<i>Beta vulgaris</i> <i>Chenopodium amaranticolor</i>		- -
Cucurbitaceae	<i>Cucumis sativus</i>		-
Leguminosae	<i>Arachis hypogea</i> <i>Canavalia ensiformis</i> <i>Cassia occidentalis</i> <i>Cassia oretusifolia</i> <i>Centrosema pubescens</i> <i>Clitoria rubiginosa</i> <i>Clitoria ternatea</i> <i>Crotalaria anagyroides</i> <i>Desmodium lasiocarpum</i> <i>Glycine max</i> <i>Phaseolus atropurpureus</i> <i>Trifolium repens</i>		- - - - - - - - - - - -
Malvaceae	<i>Hibiscus esculentus</i>		-
Solanaceae	<i>Datura stramonium</i> <i>Datura innoxia</i> <i>Lycopersicon esculentum</i> <i>Physalis alkekengi</i> <i>Physalis floridana</i> <i>Solanum melongena</i>		- - - - - -

Negative host-range for *Psophocarpus* Ring-Spot Virus

TABLE III

	CMV	TAV	PSV	CpRSV	CMV.PR	BVBMV	CpCMV	PsoRSV
<i>Arachis hypogea</i>	-		+	-	-		-	-
<i>Cucumis sativus</i>	+	-	+	-	+ -	+	+	-
<i>Datura stramonium</i>	+	-	+	-	-	+	-	-
<i>Lycopersicum esculentum</i>	+	+	+	(+)	-	+		-
<i>Phaseolus vulgaris</i>	+	-	+	+	+	+	+	(+)
<i>Vigna unguiculata</i>	+	-	+	+	+	+	+	+
<i>Chenopodium amaranticolor</i>	+			+	+	+	+	-
				Iran	PR	PR	Maroc	CI
Sero. CMV		+	+	-	+	+	+	-

Comparaison of host-range of different Cucumo-viruses

TABLE IV

Family	Plant-host tested	Systemic
Leguminosae	<i>Arachis hypogea</i>	○
	<i>Canavalia ensiformis</i>	●
	<i>Phaseolus vulgaris</i>	○
	<i>Phaseolus lathyroides</i>	○
	<i>Psophocarpus tetragonolobus</i>	●
	<i>Vicia faba</i>	○
Solanaceae	<i>Nicotiana megalosifon</i>	○

Positive host-range for *Psophocarpus* Necrotic Mosaic Virus

● mosaic

○ symptomless

TABLE V

Family	Plant-host tested	Systemic
Chenopodiaceae	<i>Beta vulgaris</i> <i>Chenopodium amaranticolor</i> <i>Chenopodium quinoa</i> <i>Chenopodium murale</i> . . .	- - - -
Cucurbitaceae	<i>Cucumis sativus</i>	-
Leguminosae	<i>Cassia occidentalis</i> <i>Cassia oretusifolia</i> <i>Centrosema pubescens</i> <i>Clitoria rubiginosa</i> <i>Clitoria ternatea</i> <i>Crotalaria anagyroides</i> <i>Desmodium lasiocarpum</i> <i>Glycine max</i> <i>Phaseolus atropurpurens</i> <i>Phaseolus mungo</i> <i>Trifolium repens</i> <i>Vigna sinensis</i> . . .	- - - - - - - - - - - -
Malvaceae	<i>Hibiscus esculentus</i>	-
Solanaceae	<i>Capsicum annum</i> <i>Datura innoxia</i> <i>Lycopersicon esculentum</i> <i>Physalis alkekengi</i> <i>Physalis floridana</i> <i>Nicotiana clevelandii</i> <i>Nicotiana glutinosa</i> <i>Nicotiana tabacum samsun</i> <i>Petunia hybrida</i> <i>Solanum melongena</i> <i>Solanum nigrum</i>	- - - - - - - - - - -

Negative host-range for *Psophocarpus* Necrotic Mosaic Virus

TABLE VI

	Length	Seed-Borne	Vector	Thermo-Inac.	Geo. distri.	<i>Arachis hypogea</i>	<i>Canavalia</i>	<i>Cassia</i>	<i>Chenopodium amaranticolor</i>	<i>Chenopodium quinoa</i>	<i>Cucumis sativus</i>	<i>Glycine max</i>	<i>Nicotiana clevelandii</i>	<i>Nicotiana tabacum</i>	<i>Phaseolus vulgaris</i>	<i>Pisum sativum</i>	<i>Vicia faba</i>	<i>Vigna sinensis</i>
Pea Early Browning V	105 215	+	Nem	74.78	Eur				★				★		★	★	★	★
Clover Yellow Vein V	760	-	Ap	55.60	USA				★	★			★	★	★	★	★	★
Pea Seed-borne Mos. V	770	+	Ap	55	USA Eur				★	★				★	★	★	★	★
Bean Yellow Mosaic V	750	-	Ap	50.62	WW					★		●		★	●	●	●	●
Bean Common Mosaic V	750	+	Ap	60	WW					★					★	●	●	●
✕ Peanut Mottle V	750	+	Ap	55.59	WW	●			—				★	★	★	●	●	●
✕ Cowpea Aphib-bo M.V	750	+	Ap	57.60	WW				★				★	★	★	●	●	●
Soybean Mosaic V	650 725	+	Ap	55.65	WW		●			★					★	●	●	●
✕ Cowpea Mild Mottle V	650	+	?	65.70	AF	●★				★					●			
Pea Streak V	620	-	Ap	60.80	USA	●			★							●		★
Red Clover Vein M.V	645		Ap	60.65	USA Eur				★	★						●	●	●
Clover Yellow Mos. V	540	+	Ap	58.62	USA				★	★						●	●	●
White Clover Mos. V.	480	+	?	60.80	WW					★	●				★	●	●	●
✕ Psopho. Necrotic M.V	620	-	?	50.55	AF	○	●	—	—	—	—	—	—	—	○	—	—	—

Comparison of filamentous viruses of legumes with *Psophocarpus* Necrotic Mosaic Virus
 Chlorotic lesion Necrotic lesion Symptomless Mosaic