

Aetiology and ecology of a yam mosaic disease in Burkina Faso

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Abstract Yam virus diseases were surveyed for 3 years in Burkina Faso. Mosaic and green vein banding symptoms were observed on two species (*Dioscorea cayenensis rotundata* and *Dioscorea alata*) in the south-west and southern areas, and on the Pilimpikou yam cultivar in the central area. Using electron microscopy, ELISA and host range studies, it was found that plants were infected with a potyvirus similar to yam mosaic virus in host range, morphology and serology. This virus was not detected in the southern area but occurred at a low rate (13%) in the south-west and more frequently (71.5%) in the central area. Healthy yam plants were exposed to natural infection for 5 months in these two areas and checked for virus contamination by ELISA. Tubers from markets were also checked. During crop growth, in natural conditions, disease contamination was relatively slight compared with that in tubers from markets. The reliability of virus detection methods is discussed.

Keywords: yam mosaic virus, yam, Burkina Faso, aetiology, ELISA.

Introduction

Yams (*Dioscorea* spp., Dioscoreaceae) are staple food crops widely cultivated in tropical areas. The major virus disease is the yam mosaic virus (YMV) described by Thouvenel and Fauquet (1979). This potyvirus is transmitted by aphids in a non-persistent manner and by vegetative propagation (tubers). *Nicotiana benthamiana* can be infected with YMV by mechanical inoculation and is a suitable laboratory host.

In Burkina Faso yams are produced in a small area about 100 km north of Ouagadougou and in a broad band along the borders with Côte d'Ivoire and Ghana. In the southern and south-western areas, species such as *Dioscorea cayenensis* Lam.-*rotundata* Poir. and *Dioscorea alata* L. are widely produced. A cultivar grown only in the central area is Pilimpikou yam, which differs from the other species cultivated in Burkina Faso as it is a possible hybrid of *Dioscorea abyssinica* and an unknown perennial species of *Dioscorea* (Dumont and Hamon, personal communication).

In West Africa and in the Caribbean, Terry (1976), Thouvenel and Fauquet (1979), Reckhaus and Nienhaus (1981) and Marchoux (1980) have recorded and described

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potyviruses which infect yam and are serologically related to YMV. As YMV occurs in neighbouring countries and as tuber seeds are exchanged between Burkina Faso and these countries, it was supposed that YMV also occurs in Burkina Faso. This hypothesis has now been investigated.

Materials and methods

Survey and sampling

An intensive survey was carried out between 1991 and 1993 in three yam-producing areas. Between 6 and 15 fields were surveyed per area and 50% of the yam plants of each field were examined. Samples were collected from all yam plants showing typical yam mosaic symptoms (mosaic, green vein banding, blistering), and from one yam plant out of 10 without these symptoms. Double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) and infectivity tests were used to detect the virus.

To estimate the importance of tuber seeds in virus spread, 410 tubers (276 *D. cayenensis-rotundata* and 134 *D. alata*) were collected respectively from three, four and five markets in the central area, the southern area and the south west area; 30-35 tubers were collected per market. These tubers, and the plants grown from them, were also tested for virus infection.

Serology

Wells of polystyrene microtitre plates (NUNC microwell) were coated by incubation for 3 h at 37°C with YMV polyclonal antibodies (100 ml/well) at 0.5 mg/ml in coating buffer (0.05 M sodium carbonate buffer, pH 9.6). This antiserum was produced by LPRC/ORSTOM and was shown to detect a typical Côte d'Ivoire YMV isolate but none of the following African potyviruses: groundnut eyespot virus, sugarcane mosaic virus, guinea grass mosaic virus, pepper veinal mottle virus, turnip mosaic virus, datura shoestring virus, peanut mottle virus.

Plates were washed (three washes, each for 3 min) with 0.01 M phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20 (PBST) then 3% non-fat milk powder (Regilait) in PBST was added for 1 h at 37°C (200 ml/well). Wells were washed as above. Antigen samples were obtained by grinding 1 g (leaf bled or tuber flesh) in 20 ml PBST. After 5 min centrifugation at 10 000g, 100 ml of crude extract was added. After washing, YMV antibody globulin/alkaline phosphatase conjugate (1:4000) in PBST was added to wells (100 ml/well) and incubated 3 h at 37°C or overnight at 4°C. The washing was repeated and bound alkaline phosphatase was detected using *p*-nitrophenylphosphate (Sigma) at 5 mg/ml in 10% (v/v) diethanolamine, pH 9.8 (100 ml/well). Absorbance at 405 nm was recorded after 1 h of enzymatic reaction at 37°C and after incubation overnight at 4°C. Controls consisted of virus-free yam leaf extract. YMV infected *N. benthamiana* were used as positive controls. All samples with absorbance at 405 nm greater than 0.100 and twice the control value were held to be ELISA positive.

Infectivity test

For each tested sample, 1 g yam leaf or tuber flesh was ground in 10 ml 0.03 M sodium

phosphate buffer, pH 8.3, containing 0.2% sodium diethyldithiocarbamate. Each crude extract containing 600 mesh carborundum was used to inoculate five *N. benthamiana* plants. Infection of inoculated plants was checked by recording the eventual symptoms and by DAS-ELISA 1–2 weeks after inoculation. The infected plants were used for light and electron microscopy and for host range studies.

Host range

Host range studies were conducted using the following species: *Nicotiana benthamiana*, *Nicotiana clelandii*, *Nicotiana glutinosa*, *Nicotiana megalosiphon*, *Nicotiana sylvestris*, *Nicotiana tabacum* cv Samsun, *N. tabacum* cv Samsun NN, *N. tabacum* cv Xanthi nc, *Petunia hybrida*, *Datura stramonium*, *Chenopodium amaranticolor*, *Chenopodium quinoa*, *Gomphrena globosa*. Five to ten plants of each species were inoculated with sap from infected yam first, and then in another experiment with sap from infected *N. benthamiana*. All inoculated plants were kept in an insect-proof greenhouse at 25–28°C, 70% relative humidity, and at 16 h photoperiod with a light intensity of 4–5000 lux. Infection of inoculated plants was checked as above.

Electron microscopy

Carbon-coated collodion grids were floated on drops containing samples for 2 min, rinsed in water then negatively stained with 2% uranyl acetate in distilled water. Grids were observed with an electron microscope (Jeol JEM-100 CX II) and pictures were taken at a magnification of 10 000 and 14 000.

Observation of viral inclusions

Cylindrical inclusions were observed by light microscopy in epidermal strips of infected *N. benthamiana*, stained as described by Christie and Edwardson (1977).

Virus spread in natural conditions

Virus spread through yam fields was estimated during the 1993 cropping season. Seeds were collected in southern and south-western areas and tested for YMV by ELISA. The non-infected checked tuber seeds were planted at random at the beginning of July (when aphid population is known to be important) in infected yam fields, 115 tuber seeds in a field in the south-western area and 230 in a field in the central area. The contamination of the plants grown from these tuber seeds was checked 1, 2, 3 and 5 months after planting, using leaf observation and DAS-ELISA. The tubers were harvested at the fifth month and checked by DAS-ELISA.

Results

Aetiology

The typical symptoms of yam mosaic were observed on the main species of yam in the three areas. Other virus-like symptoms (leaf mottling, vein yellowing or leaf deformation) were

present on some plants. Two groups of samples were distinguished by ELISA. Those giving A_{405} not significantly different from healthy control (0.050); were classified ELISA negative; those with A_{405} significantly higher than control samples were classified ELISA positive. Positive samples were found in central and south-western areas but not in the south.

The virus could be detected in 86 of the 97 tubers from the central area and in 25 of the 172 tubers from the south-west, but it was not detected in any from the south. In the south-west, the virus was detected only in the seedlings growing from the 25 ELISA-positive tubers but all the seedlings grown from the 97 tubers of the central area were ELISA positive. No infection was recorded in the seedlings grown from the tubers of the southern area.

Flexuous filaments were observed on negatively stained grids coated with purified virus suspensions. By measuring 165 particles from different electron micrographs, the mean particle was $753 (\pm 27)$ nm long and 13 nm wide. Pinwheels were observed by light microscopy in epidermal cells of inoculated *N. benthamiana*.

N. benthamiana, *N. clevelandii* and *N. megalosiphon* were easily infected by mechanical rubbing with leaf crude extract from diseased *D. cayenensis-rotundata*, *D. alata*, Pilimpikou Yam plants or infected *N. benthamiana* plants. They showed, after an incubation time of 1–2 weeks, systemic symptoms (mosaic, mottling). When inoculated with infected *N. benthamiana* extracts, *C. amaranticolor* developed chlorotic local lesions on inoculated leaves after 10 days; it developed the same symptoms when inoculated with the Côte d'Ivoire YMV isolate. The other inoculated species showed no symptoms and were ELISA negative.

Serology

Results of serological tests indicated that four types of samples were distinguished by ELISA and foliage observation: (1) typical YMV symptoms and ELISA positive (S+E+); (2) typical YMV symptoms and ELISA negative (S+E-); (3) without typical YMV symptoms but ELISA positive (S-E+); (4) without typical YMV symptom and ELISA negative (S-E-). The percentage of ELISA-positive samples was higher in samples showing typical YMV symptoms than in samples without these symptoms (Table 1). Applying the infectivity test to 10 S+E- samples of each area, infected and ELISA-positive *N. benthamiana* was obtained with all samples from the central area but with none from other origins.

Table 1. Detection of virus in yams in three yam-producing areas of Burkina Faso

Area	Plants with typical YMV symptoms		Plants without typical YMVsymptoms		% Total infected plants
	Number tested	% ELISA positive	Number tested	% ELISA positive	
South-west					
Comoe	228	30.2	391	3.58	13.4
Gaoua	18	11.1	240	0.41	1.2
South	41	0	654	0	0
Central	143	90.9	78	16.6	71.5

The geographical distribution of the virus indicates that the rate of infection is higher in the central area (71.5%) than in the south-west (1.2–13.4%). Although mosaic was observed on foliage in the southern area no virus was detected there.

Virus spread

Only 92 and 173 tubers sprouted in the trials of the south-western and central areas respectively. No plants were infected in the two areas during the first 3 months of the study. The absence of positive detection 1 month after planting is evidence that all the tubers planted were YMV-free. After 5 months' exposure to natural infection, YMV could be detected in two yam plants from the south-west. ELISA tests on harvested tubers revealed four infected tubers (including the two infections previously identified in the field). No plants or tubers from the trial of the central area became infected although they were planted in a 100% contaminated field.

Discussion

Yam plants from the central and south-western areas of Burkina Faso are infected with a filamentous virus 750 nm long and 13 nm in diameter. Pinwheels were observed in *N. benthamiana* leaves infected strains from these areas. Consequently, this virus belongs to the potyvirus group. It is tuber transmitted and induces typical symptoms of mosaic and green vein banding on yam leaves. The host range is similar to that reported for YMV (Thouvenel and Fauquet 1979) and it reacts strongly with anti-YMV polyclonal antibodies. It was found that *C. amaranticolor* could be infected with this potyvirus and YMV, although this has not previously been reported for YMV. The conclusion is that the potyvirus infecting yam plants in Burkina Faso is very similar to that described by Thouvenel and Fauquet (1986). Several potyviruses infecting yam were considered to be related to YMV as strains: Yam virus in Nigeria; Dioscorea green banding mosaic virus in Togo, *D. trifida* virus in Guadeloupe (Porth *et al.* 1987; Brunt *et al.* 1990).

The percentage of infected plants from the central area seems to be an underestimate as the virus could be detected in some ELISA-negative samples by infectivity test. The use of only the cultivar Pilimpikou yam, which is not grown elsewhere in Burkina Faso, and the use of infected tuber seeds year after year, has probably lead to general contamination of this cultivar in this area.

The south-west of Burkina Faso adjoins the yam-producing area of the north of Côte d'Ivoire and tubers are often exchanged across the border; this could explain the occurrence of YMV here.

Although serological tests were applied to 659 samples collected over 2 years, the virus could not be detected in any sample from the southern area. This suggests either that YMV did not occur in this area or that it was infected with a strain of YMV that was not recognized by the polyclonal antiserum used in our tests.

Results of the assay of virus dissemination in natural conditions should be discussed cautiously as only one cropping season was studied. The low incidence of virus in the southwest

and the absence of detectable contamination in the central trial after 5 months could have many causes. The insect vector population may not have been efficient during the period of the assays. Thouvenel and Dumont (1990) reported that exterior infection during the crop cycle was relatively low in comparison with the infection rate of the planting material in the north of Côte d'Ivoire. In the central area, YMV strain adapted to the Pilimpikou Yam cultivar may not be able to infect the varieties of *D. cayenensis-rotundata* and *D. alata* used in the experiments. This is supported by the difference of absorbance at 405 nm obtained in samples from the south-west and central area and it seems that two strains of the virus are occurring in Burkina Faso. Porth *et al.* (1987) recorded close relations between some yam potyviruses in West Africa but also differences in serology.

From the symptoms and the results of DAS-ELISA on yam plants that it would be unwise to link the expression of typical YMV symptoms to the virus presence. The absence of typical symptoms on YMV-infected yam leaves has often been reported in Nigeria (Thottappilly 1981). On the other hand, typical YMV symptoms on ELISA-negative samples could be attributed to other pathogens as other viruses have been isolated from yam crops: the cucumber mosaic virus was identified on edible yam in Guadeloupe (Migliori 1977) and was responsible of symptom of yellowing on yam leaves; beet mosaic virus infected *D. alata* and induced a leaf mottling on yam leaves (Porth and Nienhaus 1983). This virus was found in association with a potyvirus named *D. alata* virus (Porth *et al.*, 1987). Double infection also occurred in *D. alata* with internal brown spot badnavirus and an uncharacterized potyvirus which induced symptoms of mosaic (Harrison and Roberts 1973) and resembled YMV in Côte d'Ivoire (Thouvenel *et al.* 1988); *Dioscorea* latent potexvirus was identified on medicinal yams in association with a potyvirus inducing green vein banding (Hearon *et al.* 1978; Phillips *et al.* 1986); Chinese yam necrotic mosaic carlavirus was also cited on *D. batatas* in Japan (Fukumoto and Tochiyama 1978). In addition, symptoms of chlorosis, shoestring, leaf curl and vein yellowing have been observed on edible yam leaves but the causal agents were not identified (Thouvenel and Fauquet 1982; Thottappilly 1983).

Some failures of ELISA to detect the virus in tubers or leaves, although the infectivity test was positive, indicate that in our conditions the serological test was less sensitive than the infectivity test. It is suggested that the two methods are used together for more accurate diagnosis.

Conclusions

YMV infects yam crops in Burkina Faso. The disease incidence varies strongly, with no contamination in the southern area. Using traditional agriculture practices, it should be possible to keep large areas free of virus disease. This is probably related to the low efficiency of virus dissemination by vectors observed in our field experiments. Infected tuber seeds seem to be the main source of virus spread. These results need to be confirmed by further field work, but justify the development of a sanitary selection program to reduce or suppress the virus in defined areas.

References

- Brunt A., Crabtree K. and Gibbs A. (1990) *Viruses of tropical plants*. Wallingford: Commonwealth Agricultural Bureau.
- Christie R.G. and Edwardson J.R. (1977) *Light and electron microscopy of plant virus inclusions*. Florida Agricultural Experimental Station Monograph 9.
- Fukumoto F. and Tochihara H. (1978) Chinese yam necrotic mosaic virus. *Annals of the Phytopathological Society of Japan* **44**, 1-5.
- Harrison B.D. and Roberts I.M. (1973) Association of virus-like particles with internal brown spot of Yam (*Dioscorea alata*). *Tropical Agriculture (Trinidad)* **50**, 335-340.
- Hearon S.S., Corbett M.K., Lawson R.H., Gillaspie Jr. A.G. and Waterworth H.E. (1978) Two flexuous-rod viruses in *Dioscorea floribunda*: symptoms, identification, and ultrastructure. *Phytopathology* **68**, 1137-1146.
- Marchoux G. (1980) Pathologie des ignames en Guadeloupe; maladies virales. In: *Les Colloques de l'I.N.R.A., l'Igname. Séminaire International de Pointe-à-Pitre*, pp.93-105.
- Migliori A. (1977) Maladie à virus de l'igname (*Dioscorea* spp.). *Nouvelles agronomiques Antilles-Guyane* **3**, 428-435.
- Phillips S., Piggott J.D'A. and Brunt A.A. (1986) Further evidence that *Dioscorea* latent virus is a potyvirus. *Annals of Applied Biology* **109**, 137-145.
- Porth A. and Nienhaus F. (1983) *Dioscorea alata* ring mottle virus, a new potyvirus on yam in Togo. *Zeitschrift Pflanzenkrankheiten und Pflanzenschutz* **90**, 352-362.
- Porth A., Lesemann D.E. and Vetten H.J. (1987) Characterization of potyvirus isolates from West African Yams (*Dioscorea* spp.). *Journal of Phytopathology* **120**, 166-183.
- Reckhaus P. and Nienhaus F. (1981) Etiology of a virus disease of white yam *D. rotundata* in Togo. *Journal of Plant Disease and Protection* **88**, 492-509.
- Terry E.R. (1976) Incidence, symptomatology and transmission of a yam virus in Nigeria. *Proceedings 4th Symposium, International Society of Tropical Roots Crops*, pp.170-173. Cali: Colombia.
- Thottappilly G. (1981) *Annual Report*. Ibadan, Nigeria: International Institute of Tropical Agriculture.
- Thottappilly G. (1983) *Annual Report*. Ibadan, Nigeria: International Institute of Tropical Agriculture.
- Thouvenel J.C. and Fauquet C. (1979) Yam mosaic, a new potyvirus infecting *Dioscorea cayenensis* in the Ivory Coast. *Annals of Applied Biology* **93**, 279-283.
- Thouvenel J.C. and Fauquet C. (1980) Utilisation de la technique ELISA dans le diagnostic de la mosaïque de l'igname. *Compte-rendu de la Deuxième Conférence Internationale sur l'Impact des Maladies à Virus sur le Développement des Pays Africains et du Moyen Orient. Nairobi, Kenya, 02-06 Décembre 1980*.
- Thouvenel J.C. and Fauquet C. (1982) Les viroses de l'igname en Côte d'Ivoire. In *Yams, ignames*, pp. 245-252 (Miege J. and Lyonga S.N., eds). Oxford: Clarendon Press.
- Thouvenel J.C. and Fauquet C. (1986) Yam mosaic virus. In *CMI/AAB Description of Plant Viruses* No. 314.
- Thouvenel J.C., Fargette D. and Fauquet C. (1988) La maladie des taches brunes du tubercule d'igname en Côte d'Ivoire. *Proceedings, 7th Symposium, International Society for Tropical Roots Crops, Gosier, Guadeloupe, 1985*.
- Thouvenel J.C. and Dumont R. (1990) Perte de rendement de l'igname infectée par le virus de la mosaïque en Côte d'Ivoire. *L'Agronomie Tropicale* **45**, 125-129.