

Screening Ten Yam (*Dioscorea* spp.) Varieties for Resistance to Yam mosaic virus and Cucumber mosaic virus in Côte d'Ivoire

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ABSTRACT

Yam (*Dioscorea* spp.) production in Côte d'Ivoire is threatened by viral diseases. In order to provide high-yielding yam varieties to rural populations, the Swiss Center for Scientific Research (CSRS) obtained improved varieties from the IITA breeding program in Nigeria. Seven of these improved varieties (including *D. alata* and *D. cayenensis-rotundata* complex), selected for their good organoleptic characteristics and agronomic performances, as well as three locally grown varieties (Krenglè, Bètèt-bètè and Florido) were evaluated after mechanical inoculation with Yam mosaic virus (YMV) and Cucumber mosaic virus (CMV) separately. The parameters measured included disease severity (scores and indexes) and virus accumulation (overtime and in different plant parts) as determined by the enzyme-linked immunosorbent assay (ELISA) test. The results showed that all varieties became infected with YMV and all but Krenglè became infected with CMV. That variety seemed immune to CMV infection. Varieties TDr 89/02665, TDr 96/02629 and TDa 00/00010 were resistant to YMV. These varieties, in addition to TDr 95/18544, were also found to be resistant to CMV. Regardless of the virus, its accumulation depended on the variety, the plant parts tested and the physiological stage of the plants.

Keywords: serology, severity index, symptom, virus accumulation

INTRODUCTION

Yam (*Dioscorea* spp.) is a tuber plant consumed by thousands of people living in tropical and subtropical regions (Coursey 1967). Approximately 603 *Dioscorea* species are grown throughout the world for consumption, trade or therapeutic purposes (Degras 1993a; Kenyon *et al.* 2001). Yams generate enough income and are a significant source of carbohydrates, protein and vitamins. In addition, they play a defining role in social activities of people (Degras 1993b). The West and Central African countries such as Nigeria, Côte d'Ivoire, Ghana, Benin, Togo and Cameroon grow 95% of the world production estimated at 51.95 million tons (FAO 2007). Nigeria is the largest grower with 71.5% of the global production, followed by Côte d'Ivoire with 4.9 million tons (FAO 2007). In Côte d'Ivoire, this crop is consumed by more than 2/3 of the population. Yam breeding is mainly focused on the two mostly cultivated species, *D. alata* and *D. cayenensis-rotundata*. Unfortunately, the susceptibility of these two yam species to various viral diseases constitutes a major constraint in yam production. Viruses threaten yam cultivation by affecting the production of susceptible varieties (Thouvenel *et al.* 1990; Goudou-Urbino *et al.* 1995; Asiedu *et al.* 1998). Viral diseases cause substantial yield losses in Côte d'Ivoire. Yam mosaic virus (YMV) was reported to cause 27% of yield losses on Florido, a variety of *D. alata*, which was considered to be resistant to YMV (Thouvenel and Dumont 1990). Disease symptoms are characterized by chlorosis, mosaic, mottle, shoestring and blisters on the leaves (Thouvenel *et al.* 1990; Odu *et al.* 2004). Infected plants are sometimes stunted. Several viruses are known to infect yam. Among them, YMV (*Potyvirus*), CMV (*Cucumber mosaic virus*, *Cucumovirus*), DMoV (*Dioscorea mottle virus*, a possible member of *Comovirus* genus), DAV (*Dioscorea alata virus*, *Potyvirus*), DaBV (*Dioscorea alata bacilliform virus*, *Badnavirus*) and DDV (*Dioscorea dumetorum virus*,

Potyvirus) have been identified from yam plants and tubers in Sub-Saharan Africa (Thouvenel *et al.* 1979; Odu *et al.* 1999; Kenyon *et al.* 2001). YMV is one of the most important viruses infecting yams in sub-Saharan Africa. In Côte d'Ivoire, YMV and CMV are the two viruses known to occur, with YMV being the most widespread and well studied (Kenyon 2001). YMV was characterized and reported for the first time by Thouvenel and Fauquet (1979) and subsequently detected in all yam-growing regions in Africa, the Caribbean and Pacific (Goudou-Urbino *et al.* 1996). YMV particles are composed of a long flexuous rod of about 650 to 900 nm long containing a single-stranded, positive sense RNA molecule. The virus is transmitted mechanically from one *Dioscorea* species to another and also to susceptible plants such as *Nicotiana benthamiana* and *N. megalosiphon*. YMV is transmitted by several insect vectors such as *Aphis gossypii*, *A. craccivora*, *Rhopalosiphum maidis* and *Toxoptera citricidus* (Thouvenel *et al.* 1978) and can spread through tuber seeds.

CMV, an isometric virus particle of 29 nm in diameter, encapsidating an RNA molecule, belongs to the *Cucumovirus* genus (Brunt *et al.* 1996). It was first identified in Côte d'Ivoire on *D. alata* by Thottappilly (1992). The virus is known to infect more than 800 plant species. CMV is transmissible mechanically and also by aphids in a non-persistent mode. CMV infection is characterized by severe chlorosis and mosaic on the leaves as well as leaf deformation. These viruses cause important yield losses (Thouvenel and Dumont 1990; Thottappilly 1992; Odu *et al.* 1999). They are transmitted by insect vectors, infected tubers and also by wind-mediated contact between infected and healthy leaves (Thouvenel *et al.* 1989, 1990; Goudou-Urbino 1995).

The use of resistant varieties represents one of the most effective ways to control viral disease infections. Some improved varieties from the breeding program of the International Institute of Tropical Agriculture (IITA, Nigeria) were

made available to the Swiss Center for Scientific Research (SCSR, Côte d'Ivoire). Previous studies conducted by Kouamé *et al.* (2003) and Ettien (2004) confirmed the agronomic and organoleptic qualities of these varieties which were found ready for wide dissemination to rural production areas. However, during testing in the field, the presence of viral disease symptoms was reported. It was therefore important to study the behavior of these yam varieties under high disease presence, by inoculating them with YMV and CMV.

MATERIALS AND METHODS

Study area

The study was conducted for 2 years (2006 and 2007) in the Experimental Station of the SCSR located in the village of Bringakro in the Department of Toumodi (Côte d'Ivoire). That area is a transition zone, located between forest and savanna. The semi-deciduous forest vegetation consists of *Ceiba pentadra* and *Chromolena odorata*, while the savanna is mainly dominated by palm trees (*Borassus aethiopicum*) and several Poaceae species (Comoé 2001). The forest soils have a clay-sandy texture, while those of the savannah have a sandy-clay texture. The climate is equatorial, with a big rainy season starting from March to July and a short one starting from August to October. The average annual rainfall is 1,200 mm of rain spread over 5 to 6 months.

Plant and viruses sources

The plant material was composed of two yam species: *D. alata* and the complex *D. cayenensis-rotundata*. Seven improved IITA varieties (TDr 89/02565, TDr 95/18544, TDr 96/00664, TDr 89/02665 and TDr 96/02629 of *D. cayenensis-rotundata* species and TDa 98/01176 with TDa 00/00010 of *D. alata* species) and three other varieties (Krenglè of *cayenensis-rotundata*; Bètè-bètè and Florido of *D. alata* species) grown locally were all provided by the SCSR. The IITA improved varieties were selected for their good organoleptic characteristics and agronomic performance.

The viruses included YMV and CMV extracted from leaves of *Dioscorea* spp. presenting symptoms of each virus and tested previously. Polyclonal antibodies, the monoclonal and conjugated monoclonal antibodies (AS-0176-0435/10 and AS-0475-0491/1) produced in rabbits against YMV and CMV, respectively, were used for the ELISA test. These antibodies and the positive control of the antigen were produced by DSMZ (Germany).

Planting

The tubers of each variety, previously tested by TAS-ELISA for the presence of either CMV or YMV, were cut into minisets of 50 g each and planted individually in 20 cm diameter plastic pots containing fertile soil. The experimental design was a randomized block of Fisher with 4 repetitions. Each repetition included all 10 varieties of *Dioscorea* spp. A total of 42 plants per variety (including 2 checks) were planted for each of the viruses tested.

Virus detection and accumulation

The serological test enzyme-linked immunosorbent assay (ELISA) was conducted according to method described by Clark and Adams (1977). 100 mg of virus-infected leaves or tubers sampled in the fields were ground with fontainbleau sand (Carlo Erba, France) and recovered in 5 mL of PBS-T buffer (0.5% Tween-20) containing 2% polyvinylpyrrolidone (PVP 360). The supernatants obtained after centrifugation at 4000 rpm for 8 min were collected and kept at -20°C. The supernatants were used as antigen for the ELISA test. The purified IgG antibodies were first diluted at 1/1000 in a coating buffer pH 9.6 (0.15% Na₂CO₃, 0.29% NaHCO₃, 0.02% NaN₃) and 200 µL of this diluted buffer was put in each of the 60 middle wells of certified Nunc-Immuno Maxi-Sorb F96 ELISA plate (Nunc, Roskilde, Denmark). The plates were then incubated at 37°C for at least 2 h, then washed in PBS-T buffer (PBS + 0.05% Tween-20) with plate washer (Wellwash 4, Finland) before being carefully dried. All the wells of the plates

were blocked with 200 µL of 2% skimmed milk diluted in PBS-T. After 30 min incubation at 37°C, the plates were washed and dried. 200 µL of antigens were put in each of the 60 middle wells of the plates. The plates were then incubated at 4°C overnight. The plates were washed and dried. 200 µL of monoclonal antibody (mAb) diluted at 1/1000 in conjugate buffer containing PBS-T + 2% PVP 360 + 0.2% egg albumin (Sigma A-5253) were added in the 60 middle wells of the plates. These plates were then incubated at 37°C for 2 h, washed and dried. Then, 200 µL of mAb conjugated with alkaline phosphatase (Mab-AP) diluted at 1/1000 in conjugate buffer. The plates were then incubated at 37°C for 2 h, washed and then dried. 200 µL of the substrate buffer (1 M diethanolamine pH 9.8 containing 0.02% NaN₃) in which 1 mg/mL *p*-nitrophenyl phosphate substrate was dissolved, were poured into individual wells of the plates. After an incubation of at least 1 h, at room temperature in the dark, the absorbance was read at 405 nm using an ELISA microplate reader (Multiskan Ex. LabSystems inc. 10.3.1999, Finland). The sample which absorbance was higher or equal to twice that of the negativity threshold was thus considered positive (viruses-infected). The negativity threshold was the average absorbance of the wells which did not receive the antibodies.

Mechanical inoculation

The plants of each variety previously tested negative to each of the two viruses using TAS-ELISA were mechanically inoculated at the two-leaf stage with YMV and CMV. The plants already naturally infected by one of the two viruses, had only one of the two sets inoculated with the other virus. The virus inocula were prepared using 100 mg of yam leaf presenting characteristic symptoms of each virus previously tested positive by ELISA. These leaf extracts were diluted in the inoculation buffer (10 mM phosphate buffer pH 7.7 containing 1 mM ethylene diamine tetra-acetic acid (EDTA) and cystein 0.1 mM). The inoculation was made in a greenhouse with cheesecloth and sterilized fine sand.

Varietal responses and symptom severity

The inoculated plants were kept in an insect-proof screen house. No special treatment was done to the plants. The number of infected plants was determined at 2 weeks after inoculation and the different percentages were calculated. The percentage of leaves showing virus-like symptoms compared to the total number of leaves of the plant was calculated to determine symptom severity. Symptoms severity was assessed starting at 2 weeks after inoculation. Four measurements were made at a rate of one measurement per week and an average severity was calculated for each variety. Disease was scored using a rating scale from 1 to 5 (Mignouna *et al.* 2001), where 1 = no visible symptom, 2 = 1-25% of leaves showing symptoms, 3 = 26-50% of leaves showing symptoms, 4 = 51-75% of leaves showing symptoms and 5 = over 75% of leaves showing symptoms. The severity index (SI, %) of the virus symptoms was calculated using the following formula proposed by Rempel and Hall (1996):

$$\text{Severity Index (\%)} = \frac{(\sum \text{score} \times \text{number of infected plants}) \times 100}{\text{Score} \times \text{highest total number of plants}}$$

Varieties with the same rating were grouped into the same class. Those which scored less than or equal to 2, with a SI < 50% were considered resistant (Mignouna *et al.* 2001).

Dynamics of virus accumulation in different plant parts

To determine virus accumulation, plants grown in pots were transported to the field 6 weeks after inoculation. The plants were taken out of pots and planted directly in the soil. Mounds were made around each plant tuber in the soil. The experimental design was a randomized block of Fisher. Virus accumulation was determined in leaf, stem and tuber samples at the ages of 3, 5 and 7 months after inoculation for the 10 varieties studied. Varieties having scored ≤ 2, with a low viral accumulation in the organs were considered resistant. Those with a score > 2 and a high virus accumulation in the organs were considered susceptible (Mignouna *et al.* (2001).

Table 1 Serological responses of yam varieties before and after inoculation with YMV using TAS-ELISA.

| Varieties | Response before inoculation | Response after inoculation | Infected plants (%) ± SE | Total number of infected plants | Number of infected but symptomless plants |
|--------------|-----------------------------|----------------------------|--------------------------|---------------------------------|---|
| TDr 89/02665 | - | + | 12.50 ± 2.13 | 6 | 2 |
| TDr 96/02629 | - | + | 25.00 ± 4.28 | 7 | 1 |
| TDr 95/18544 | - | + | 37.50 ± 6.42 | 20 | 2 |
| TDr 89/02565 | - | + | 50.00 ± 2.95 | 21 | 1 |
| TDr 96/00664 | - | + | 50.00 ± 5.20 | 20 | 0 |
| Krenglè | - | - | 62.50 ± 4.41 | 25 | 0 |
| TDa 00/00010 | - | + | 25.00 ± 3.02 | 10 | 3 |
| Florido | - | + | 87.50 ± 6.58 | 35 | 0 |
| Bètè-bètè | - | + | 100.00 ± 8.30 | 40 | 0 |
| TDa 98/01176 | + | + | 97.50 ± 6.04 | 39 | 0 |

42 plants per variety used.

Table 2 Serological responses of yam varieties before and after inoculation with CMV using TAS-ELISA.

| Varieties | Response before inoculation | Response after inoculation | Infected plants (%) ± SE | Total number of infected plants | Number of infected but symptomless plants |
|--------------|-----------------------------|----------------------------|--------------------------|---------------------------------|---|
| TDr 89/02665 | - | + | 12.50 ± 3.26 | 6 | 1 |
| TDr 96/02629 | - | + | 12.50 ± 4.42 | 8 | 2 |
| TDr 95/18544 | - | + | 25.00 ± 5.33 | 9 | 1 |
| TDr 89/02565 | - | + | 50.00 ± 6.52 | 20 | 0 |
| TDr 96/00664 | - | + | 37.50 ± 5.03 | 19 | 0 |
| Krenglè | - | - | 0 | 0 | 0 |
| TDa 00/00010 | - | + | 37.50 ± 7.24 | 11 | 2 |
| Florido | - | + | 62.50 ± 8.36 | 23 | 0 |
| Bètè-bètè | - | + | 100.00 ± 9.73 | 40 | 0 |
| TDa 98/01176 | - | + | 100.00 ± 7.51 | 40 | 0 |

42 plants per variety used.

Statistical analysis

The Generalized Linear Model (GLM) of SAS (1999) was used. Data were analyzed using analysis of variance (ANOVA) and Fisher's Least Significant Difference test (LSD) at a significance level of $\alpha = 0.05$.

RESULTS

Response of yam varieties to virus inoculation

The TAS-ELISA test conducted on the first two leaves of each variety just before inoculation showed that var. TDa 98/01176 was already infected with YMV (**Table 1**). Thereafter, YMV was mechanically inoculated only to all other yam varieties. Two weeks after inoculation, all the varieties were tested positive for YMV by ELISA. Vars. TDr 89/02665, TDr 96/02629, TDr 95/18544 and TDa 00/00010 had < 50% of their plants infected by YMV as determined by ELISA (**Table 1**). Plants of vars. Bètè-bètè and TDa 98/01176 were infected with YMV at 100 and approximately 98%, respectively (**Table 1**).

The TAS-ELISA test used to detect CMV showed that none of the yam varieties were infected with the virus before inoculation (**Table 2**). After inoculation, interestingly, none of the plants of var. Krenglè was infected with CMV.

Vars. TDr 89/02665, TDr 96/02629, TDr 95/18544, TDr 96/00664 and TDa 00/00010 had a percentage of infected plants < 50% of the total number of inoculated plants per variety. Plants of vars. Bètè-bètè and TDa 98/01176 were all infected with CMV after inoculation (**Table 2**).

Symptom severity

Symptoms caused by YMV consisted of mosaic on the leaves (**Fig. 1**) and stunting of the whole plant. YMV infections were less severe on improved vars. TDr 89/02665, TDr 96/02629 and TDa 00/00010. For these varieties, the highest number of infected plants had a disease rating score of 2. The SIs regarding YMV were very low for these three varieties (**Table 3**). Var. TDa 00/00010 had the lowest SI among the *D. alata* varieties. Vars. Bètè-bètè and TDa 98/01176, with the majority of plants having the highest disease score of 5, displayed the highest disease SI (**Table 3**). Some varieties with a score of 1, meaning that they did not show any symptoms, were positive for YMV by ELISA (**Tables 1, 3**).

Plants infected with CMV showed strong chlorosis of the leaves (**Fig. 2**) and sometimes of the stems. CMV infections were less severe on improved vars. TDr 89/02665, TDr 96/02629, TDr 95/18544 and TDa 00/00010 (**Table 4**). All these varieties had most of their infected plants with a dis-

Table 3 Symptom severity caused by YMV on yam varieties

| Yam varieties | Disease score | | | | | Severity index |
|---------------|---------------|---|----|----|----|-----------------|
| | 1 | 2 | 3 | 4 | 5 | |
| TDr 89/02665 | 2 | 4 | 0 | 0 | 0 | 5.00 ± 1.20 a |
| TDr 96/02629 | 1 | 5 | 1 | 0 | 0 | 7.00 ± 2.31 a |
| TDr 95/18544 | 2 | 6 | 9 | 3 | 0 | 26.50 ± 4.62 b |
| TDr 89/02565 | 1 | 5 | 13 | 2 | 0 | 29.00 ± 5.97 b |
| TDr 96/00664 | 0 | 3 | 4 | 12 | 1 | 35.50 ± 6.75 b |
| Krenglè | 0 | 2 | 16 | 7 | 0 | 60.00 ± 7.62 c |
| TDa 00/00010 | 3 | 6 | 1 | 0 | 0 | 9.00 ± 3.02 a |
| Florido | 0 | 1 | 6 | 20 | 8 | 75.00 ± 8.19 cd |
| Bètè-bètè | 0 | 2 | 5 | 10 | 23 | 87.00 ± 9.31 d |
| TDa 98/01176 | 0 | 0 | 1 | 9 | 29 | 92.00 ± 7.66 d |

These values represent the differences between the sample absorbance and the negativity threshold. On the same column, values with the same letters are not statistically different ($\alpha = 0.05$). 42 plants per variety used.

Table 4 Symptom severity caused by CMV on yam varieties.

| Yam varieties | Disease score | | | | | Severity index |
|---------------|---------------|---|----|----|----|----------------|
| | 1 | 2 | 3 | 4 | 5 | |
| TDr 89/02665 | 1 | 5 | 0 | 0 | 0 | 5.50 ± 1.02 a |
| TDr 96/02629 | 2 | 5 | 1 | 0 | 0 | 0.50 ± 3.14 a |
| TDr 95/18544 | 1 | 6 | 2 | 0 | 0 | 8.00 ± 2.05 a |
| TDr 89/02565 | 0 | 1 | 5 | 12 | 2 | 32.50 ± 6.23 b |
| TDr 96/00664 | 0 | 3 | 9 | 6 | 1 | 26.00 ± 5.42 b |
| Krenglè | 0 | 0 | 0 | 0 | 0 | 0 |
| TDa 00/00010 | 2 | 6 | 2 | 1 | 0 | 10.00 ± 4.01 a |
| Florido | 0 | 3 | 13 | 5 | 2 | 37.50 ± 6.48 b |
| Bètè-bètè | 0 | 2 | 4 | 9 | 25 | 88.50 ± 8.51 c |
| TDa 98/01176 | 0 | 0 | 1 | 9 | 30 | 97.50 ± 7.20 c |

These values represent the differences between the sample absorbance and the negativity threshold. On the same column, values with the same letters are not statistically different ($\alpha = 0.05$). 42 plants per variety used.



Fig. 1 Plant of variety Bètè-bètè infected with YMV showing a severe mosaic and leaf puckering.



Fig. 2 Plant of variety Bètè-bètè infected with CMV showing leaf chlorosis.

ease score of 2 in a scale of 1 to 5. For vars. Bètè-bètè and TDa 98/01176, > 50% of infected plants from these two varieties scored 5. They showed the highest SIs. Interestingly, none of the plants of var. Krenglè (*D. cayenensis-rotundata*) was infected with CMV (Table 4). In almost half of the varieties, a few were tested positive to CMV but remained symptomless.

Virus accumulation overtime in different plant parts

1. YMV accumulation

At 3 months after inoculation, YMV accumulated more in the leaves than in both stems and tubers. In those two plant parts virus accumulation was similar. If no difference was found in YMV accumulation in the stems and tubers, the difference was statistically significant when compared to YMV accumulation in the leaves (Table 5). This result was the same regardless of the yam variety tested (Table 5).

At 5 months after inoculation, for all the yam varieties, YMV accumulation was about the same in the leaves and the stems, and higher in the tubers. The difference was statistically significant (Table 6).

At 7 months after inoculation, regardless the variety tested, the results indicated that YMV accumulation was the highest in the tubers, less in the stems, and the lowest in the leaves (Table 7). The differences were statistically significant.

Table 5 YMV accumulation in different plant parts of yam varieties 3 months after inoculation.

| Yam varieties | Plant parts | | |
|---------------|---------------|---------------|---------------|
| | Leaves | Stems | Tubers |
| TDr 89/02665 | 0.24 ± 0.02 a | 0.13 ± 0.01 b | 0.11 ± 0.02 b |
| TDr 96/02629 | 0.26 ± 0.01 a | 0.15 ± 0.02 b | 0.12 ± 0.01 b |
| TDr 95/18544 | 0.33 ± 0.03 a | 0.25 ± 0.02 b | 0.22 ± 0.04 b |
| TDr 89/02565 | 0.35 ± 0.05 a | 0.26 ± 0.03 b | 0.24 ± 0.01 b |
| TDr 96/00664 | 0.37 ± 0.06 a | 0.27 ± 0.02 b | 0.23 ± 0.05 b |
| Krenglè | 0.43 ± 0.01 a | 0.28 ± 0.02 b | 0.29 ± 0.02 b |
| TDa 00/00010 | 0.29 ± 0.02 a | 0.24 ± 0.04 b | 0.17 ± 0.03 b |
| Florido | 0.47 ± 0.01 a | 0.28 ± 0.01 b | 0.27 ± 0.03 b |
| Bètè-bètè | 0.49 ± 0.07 a | 0.30 ± 0.05 b | 0.30 ± 0.02 b |
| TDa 98/01176 | 0.55 ± 0.04 a | 0.40 ± 0.03 b | 0.38 ± 0.03 b |

These values represent the differences between the sample absorbance and the negativity threshold. On the same line, values with the same letters are not statistically different ($\alpha = 0.05$). For each variety and each plant part, 40 samples were used for each virus tested.

Table 6 YMV accumulation in different plant parts of yam varieties 5 months after inoculation.

| Yam varieties | Plant parts | | |
|---------------|---------------|---------------|---------------|
| | Leaves | Stems | Tubers |
| TDr 89/02665 | 0.13 ± 0.01 b | 0.15 ± 0.02 b | 0.20 ± 0.04 a |
| TDr 96/02629 | 0.16 ± 0.03 b | 0.18 ± 0.07 b | 0.25 ± 0.08 a |
| TDr 95/18544 | 0.24 ± 0.08 b | 0.25 ± 0.01 b | 0.34 ± 0.01 a |
| TDr 89/02565 | 0.26 ± 0.07 b | 0.28 ± 0.02 b | 0.35 ± 0.04 a |
| TDr 96/00664 | 0.31 ± 0.04 b | 0.31 ± 0.01 b | 0.37 ± 0.03 a |
| Krenglè | 0.27 ± 0.05 b | 0.29 ± 0.03 b | 0.38 ± 0.04 a |
| TDa 00/00010 | 0.19 ± 0.05 b | 0.20 ± 0.02 b | 0.26 ± 0.02 a |
| Florido | 0.30 ± 0.03 b | 0.31 ± 0.04 b | 0.37 ± 0.02 a |
| Bètè-bètè | 0.31 ± 0.02 b | 0.33 ± 0.03 b | 0.43 ± 0.01 a |
| TDa 98/01176 | 0.38 ± 0.01 b | 0.40 ± 0.02 b | 0.50 ± 0.01 a |

These values represent the differences between the sample absorbance and the negativity threshold. On the same line, values with the same letters are not statistically different ($\alpha = 0.05$). For each variety and each plant part, 40 samples were used for each virus tested.

Table 7 YMV accumulation in the different plant parts of yam varieties 7 months after inoculation.

| Varieties | Plant parts | | |
|--------------|---------------|---------------|---------------|
| | Leaves | Stems | Tubers |
| TDr 89/02665 | 0.09 ± 0.03 c | 0.18 ± 0.03 b | 0.25 ± 0.05 a |
| TDr 96/02629 | 0.13 ± 0.01 c | 0.21 ± 0.05 b | 0.29 ± 0.04 a |
| TDr 95/18544 | 0.18 ± 0.03 c | 0.28 ± 0.03 b | 0.37 ± 0.03 a |
| TDr 89/02565 | 0.23 ± 0.02 c | 0.30 ± 0.04 b | 0.38 ± 0.01 a |
| TDr 96/00664 | 0.24 ± 0.06 c | 0.33 ± 0.02 b | 0.39 ± 0.04 a |
| Krenglè | 0.20 ± 0.03 c | 0.34 ± 0.01 b | 0.43 ± 0.01 a |
| TDa 00/00010 | 0.14 ± 0.03 c | 0.23 ± 0.01 b | 0.30 ± 0.05 a |
| Florido | 0.24 ± 0.05 c | 0.36 ± 0.06 b | 0.40 ± 0.06 a |
| Bètè-bètè | 0.26 ± 0.03 c | 0.38 ± 0.04 b | 0.48 ± 0.07 a |
| TDa 98/01176 | 0.30 ± 0.02 c | 0.45 ± 0.01 b | 0.58 ± 0.06 a |

These values represent the differences between the sample absorbance and the negativity threshold. On the same line, values with the same letters are not statistically different ($\alpha = 0.05$). For each variety and each plant part, 40 samples were used for each virus tested.

2. CMV accumulation

At 3 months after inoculation, CMV did not accumulate in any of the plant parts (leaves, stems and tubers) of var. Krenglè (Table 8). For all other varieties, the CMV accumulation pattern was similar to that of YMV at the same physiological stage of the plants. CMV accumulated more in the leaves than in both the stems and tubers. That difference was statistically significant (Table 8).

At 5 months after inoculation, the accumulation pattern of CMV in the different plant parts was again similar to that of YMV at the same stage. CMV accumulated to the same level in both leaves and stems (Table 9). However, virus accumulation was higher in the tubers compared to the two other plant parts. The difference was statistically significant (Table 9).

At 7 months after inoculation with CMV, virus accumulation was the highest in tubers and the lowest in leaves. In

Table 8 CMV accumulation in different plant parts of yam varieties 3 months after inoculation.

| Varieties | Plant organs | | |
|--------------|---------------|---------------|---------------|
| | Leaves | Stems | Tubers |
| TDr 89/02665 | 0.15 ± 0.02 a | 0.08 ± 0.01 b | 0.05 ± 0 b |
| TDr 96/02629 | 0.17 ± 0.01 a | 0.10 ± 0.02 b | 0.07 ± 0.01 b |
| TDr 95/18544 | 0.19 ± 0.04 a | 0.11 ± 0.01 b | 0.09 ± 0.03 b |
| TDr 89/02565 | 0.25 ± 0.07 a | 0.16 ± 0.02 b | 0.12 ± 0.01 b |
| TDr 96/00664 | 0.26 ± 0.07 a | 0.18 ± 0.02 b | 0.14 ± 0.01 b |
| Krenglè | - | - | - |
| TDa 00/00010 | 0.20 ± 0.01 a | 0.10 ± 0.05 b | 0.11 ± 0.03 b |
| Florido | 0.37 ± 0.01 a | 0.24 ± 0.03 b | 0.20 ± 0.04 b |
| Bètè-bètè | 0.38 ± 0.09 a | 0.26 ± 0.04 b | 0.22 ± 0.01 b |
| TDa 98/01176 | 0.44 ± 0.01 a | 0.29 ± 0.05 b | 0.27 ± 0.04 b |

These values represent the differences between the sample absorbance and the negativity threshold. On the same line, values with the same letters are not statistically different ($\alpha = 0.05$). For each variety and each plant part, 40 samples were used for each virus tested.

Table 9 CMV accumulation in different plant parts of yam varieties 5 months after inoculation

| Varieties | Plant parts | | |
|--------------|---------------|---------------|---------------|
| | Leaves | Stems | Tubers |
| TDr 89/02665 | 0.07 ± 0.01 b | 0.06 ± 0.02 b | 0.12 ± 0.04 a |
| TDr 96/02629 | 0.09 ± 0.03 b | 0.10 ± 0.07 b | 0.15 ± 0.08 a |
| TDr 95/18544 | 0.13 ± 0.07 b | 0.12 ± 0.02 b | 0.17 ± 0.03 a |
| TDr 89/02565 | 0.14 ± 0.08 b | 0.13 ± 0.04 b | 0.19 ± 0.01 a |
| TDr 96/00664 | 0.16 ± 0.08 b | 0.16 ± 0.04 b | 0.22 ± 0.01 a |
| Krenglè | - | - | - |
| TDa 00/00010 | 0.12 ± 0.04 b | 0.14 ± 0.04 b | 0.18 ± 0.01 a |
| Florido | 0.19 ± 0.05 b | 0.20 ± 0.01 b | 0.25 ± 0.01 a |
| Bètè-bètè | 0.21 ± 0.06 b | 0.22 ± 0.02 b | 0.28 ± 0.02 a |
| TDa 98/01176 | 0.26 ± 0.03 b | 0.28 ± 0.06 b | 0.37 ± 0.02 a |

These values represent the differences between the sample absorbance and the negativity threshold. On the same line, values with the same letters are not statistically different ($\alpha = 0.05$). For each variety and each plant part, 40 samples were used for each virus tested.

Table 10 CMV accumulation in different plant parts in yam varieties 7 months after inoculation.

| Yam varieties | Plant parts | | |
|---------------|---------------|---------------|---------------|
| | Leaves | Stems | Tubers |
| TDr 89/02665 | 0.01 ± 0 c | 0.09 ± 0.03 b | 0.18 ± 0.05 a |
| TDr 96/02629 | 0.04 ± 0.01 c | 0.12 ± 0.05 b | 0.21 ± 0.04 a |
| TDr 95/18544 | 0.06 ± 0.04 c | 0.16 ± 0.07 b | 0.24 ± 0.04 a |
| TDr 89/02565 | 0.09 ± 0.03c | 0.18 ± 0.02 b | 0.26 ± 0.04 a |
| TDr 96/00664 | 0.10 ± 0.03c | 0.19 ± 0.02 b | 0.30 ± 0.04 a |
| Krenglè | - | - | - |
| TDa 00/00010 | 0.07 ± 0.02 c | 0.18 ± 0.02 b | 0.29 ± 0.07 a |
| Florido | 0.12 ± 0.02 c | 0.21 ± 0.06 b | 0.33 ± 0.02 a |
| Bètè-bètè | 0.13 ± 0.04 c | 0.24 ± 0.07 b | 0.35 ± 0.05 a |
| TDa 98/01176 | 0.21 ± 0.02 c | 0.30 ± 0.01 b | 0.42 ± 0.08 a |

These values represent the differences between the sample absorbance and the negativity threshold. On the same line, values with the same letters are not statistically different ($\alpha=0.05$). For each variety and each plant part, 40 samples were used for each virus tested.

the stem, CMV accumulation was intermediate between the two other plant parts (**Table 10**). The differences in CMV accumulation in all three plant parts were statistically significant (**Table 10**).

Relationship between virus accumulation and disease symptoms severity

1. YMV accumulation

Based on severity scores, the yam variety were classified into 4 groups (**Table 11**): group 1 constituted of varieties having a score of 2 (TDr 89/02665, TDr 96/02629 and TDa 00/00010); a score of 3 (TDr 95/18544 TDr 89/02565 and Krenglè); a score of 4 (TDr 96/00664 and Florido); and a score of 5 (Bètè-bètè and TDa 98/01176). The differences observed were statistically significant.

Based on the severity indexes, the yam varieties fell

Table 11 Relationship between YMV accumulation and severity index : varietal reaction.

| Variety | Severity score | Severity index | Virus accumulation* | Reaction |
|--------------|----------------|-----------------|---------------------|----------|
| TDr 89/02665 | 2 | 5.00 ± 1.20 a | 0.15 ± 0.02 a | R |
| TDr 96/02629 | 2 | 7.00 ± 2.31 a | 0.18 ± 0.01 a | R |
| TDr 95/18544 | 3 | 26.50 ± 4.62 b | 0.27 ± 0.03 ab | S |
| TDr 89/02565 | 3 | 29.00 ± 5.97 b | 0.29 ± 0.05 ab | S |
| TDr 96/00664 | 4 | 35.50 ± 6.75 b | 0.31 ± 0.06 ab | S |
| Krenglè | 3 | 60.00 ± 7.62 c | 0.32 ± 0.01 ab | S |
| TDa 00/00010 | 2 | 9.00 ± 3.02 a | 0.20 ± 0.02 a | R |
| Florido | 4 | 75.0 ± 8.19 cd | 0.33 ± 0.01 ab | S |
| Bètè-bètè | 5 | 87.00 ± 9.31 d | 0.40 ± 0.07 b | S |
| TDa 98/01176 | 5 | 92.00 ± 07.66 d | 0.43 ± 0.04 b | S |

R: resistant; S: susceptible. In the same column, values with the same letters are not statistically different ($\alpha = 0.05$). Values represent mean virus accumulation in leaves, stems and tubers. For each variety and each plant part, 40 samples were used.

into 5 groups (**Table 11**). From the lowest severity index to the highest, there were: group 1 (TDr 89/02665, TDr 96/02629, TDa 00/00010); group 2 (TDr 95/18544, TDr 89/02565 and TDr 96/00664); group 3 (Krenglè); group 4, intermediary between groups 3 and 5 (Florido); and group 5 (Bètè-bètè and TDa 98/01176). This classification was made on the basis of the results of the statistical analysis.

Based on the YMV accumulation, only 3 groups were found (**Table 11**). From the lowest to the highest accumulation values the groups were: group 1 (TDr 89/02665, TDr 96/02629, TDa 00/00010); group 2, intermediary between groups 1 and 3 (TDr 98/18544, TDr 89/02565, TDr 96/00664 and Krenglè); and group 3 (Bètè-bètè and TDa 98/01176).

Based on the method used by Mignouna *et al.* (2001), taking into account the disease score, severity index and virus accumulation, three varieties were considered resistant to YMV (TDr 89/02665, TDr 96/02629 and TDa 00/00010) and the others susceptible (**Table 11**).

2. CMV accumulation

Based on severity scores, the yam varieties were put into 5 groups (**Table 12**). The groups were made off: group 1, with severity score of zero (Krenglè); group 2 with a score of 2 (TDr 89/02665, TDr 96/02629, TDr 95/18544 and TDa 00/00010); group 3 with a score of 3 (TDr 96/00664 and Florido); group 4 with a score of 4 (TDr 89/02565); and group 5 (Bètè-bètè and TDa 98/01176).

On the basis of severity indexed for CMV (going from the lowest to the highest), the varieties of yam tested were divided into 4 groups (**Table 12**): group 1 with nil index severity (Krenglè); group 2 (TDr 89/02665, TDr 96/02629, TDr 95/18544 and TDa 00/00010); group 3 (TDr 89/02565, TDr 96/00664 and Florido); and group 4 (Bètè-bètè and TDa 98/01176). These classes were determined based on

Table 12 Relationship between CMV accumulation and severity index: varietal reaction.

| Variety | Severity score | Severity index | Virus accumulation* | Reaction |
|--------------|----------------|----------------|---------------------|----------|
| TDr 89/02665 | 2 | 5.50 ± 1.02 a | 0.09 ± 0.02 a | R |
| TDr 96/02629 | 2 | 7.50 ± 3.14 a | 0.11 ± 0.01 a | R |
| TDr 95/18544 | 2 | 8.00 ± 2.05 a | 0.13 ± 0.04 a | R |
| TDr 89/02565 | 4 | 32.50 ± 6.23 b | 0.20 ± 0.07 ab | S |
| TDr 96/00664 | 3 | 26.00 ± 5.42 b | 0.23 ± 0.07 ab | S |
| Krenglè | 0 | 0 | - | I |
| TDa 00/00010 | 2 | 10.00 ± 4.01 a | 0.14 ± 0.01 a | R |
| Florido | 3 | 37.50 ± 6.48 b | 0.32 ± 0.01 b | S |
| Bètè-bètè | 5 | 88.50 ± 8.51 a | 0.38 ± 0.09 b | S |
| TDa 98/01176 | 5 | 97.50 ± 7.20 b | 0.40 ± 0.01 b | S |

R: resistant; S: susceptible; I: immune. In the same column, values with the same letters are not statistically different ($\alpha = 0.05$). Values represent mean virus accumulation in leaves, stems and tubers. For each variety and each plant part, 40 samples were used.

the results of the statistically analysis.

Based on CMV accumulation, the varieties were classified (from the lowest to the highest accumulation) into 4 groups (Table 12) as follows: group 1 (Krenglé); group 2 (same varieties as in the above classification); group 3, intermediary between groups 2 and 3 (TDr 89/02565 and TDr 96/00664); and group 4 (Florido, Bètè-bètè anf TDa 98/01176). The differences observed between the different groups were statistically significant.

With the method used by Mignouna *et al.* (2001), taking into account disease score, severity index and virus accumulation, four varieties were considered resistant to CMV (TDr 89/02665, TDr 96/02629, TDr 95/18544 and TDa 00/00010) and the others susceptible (Table 12).

Interaction between virus accumulation, varieties, plant parts and physiological stages

There were statistically significant virus accumulation, variety, plant part and physiological stage effects (Table 13). Three positive interactions were also found: an interaction between virus accumulation and physiological stage, an interaction between virus accumulation and variety, and an interaction between virus accumulation and the different plant parts tested.

Table 13 Analysis of variance: effects of viral accumulation, variety, plant parts and physiological stage on yam virus diseases.

| Source | DF | Type III SS | F | Pr > F | R ² |
|----------------------------|----|-------------|--------|--------|----------------|
| Effects | | | | | |
| Viral accumulation | 1 | 0.052 | 109.68 | <.0001 | 0.95 |
| Varieties | 9 | 0.040 | 96.24 | <.0001 | |
| Plant parts | 2 | 0.032 | 102.37 | <.0001 | |
| Physiological stage | 2 | 0.070 | 115.25 | <.0001 | |
| Accumulation X | 2 | 0.002 | 6.30 | 0.0085 | |
| physiological stage | 9 | 0.003 | 10.48 | <.0001 | |
| Accumulation X variety | 2 | 0.004 | 17.32 | <.0001 | |
| Accumulation X plant parts | | | | | |

Sample size = 1200

Time course accumulation of viruses in different plant parts

1. YMV accumulation

Regardless the plant variety tested, in the leaves, YMV accumulated the most at 3 months and the least at 7 months (Fig. 3). Accumulation at 5 months was intermediary between those obtained at 3 and 7 months. The differences observed were statistically significant.

In the stems, YMV accumulation was about the same at 3, 5 and 7 months after inoculation. The same result was obtained for all the varieties. The statistical analysis showed that there were no significant differences between virus accumulations at these three physiological stages (Fig. 4).

In yam tubers, for each of the yam varieties, YMV accumulated the most at 7 months after inoculation and the least at 3 months after inoculation (Fig. 5). Virus accumulation at 5 months was intermediary between those obtained at 3 and 7 months. Statistical analysis showed that the differences between YMV accumulations at these three physiological stages were significant (Fig. 5).

2. CMV accumulation

In the leaves, regardless the variety, CMV accumulated more at 3 months after inoculation, less at 5 months, and even less at 7 months after inoculation (Fig. 6). The differences were statistically significant between those three periods.

For all the varieties, CMV accumulation in the stems was the same at 3, 5 and 7 months after inoculation (Fig. 7). No statistical significant differences were observed.

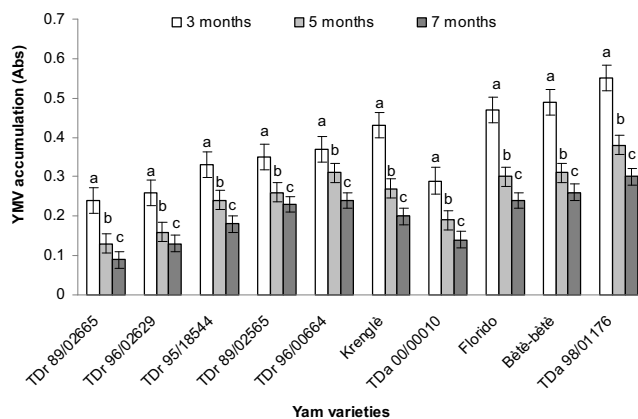


Fig. 3 Time course accumulation of YMV in yam leaves. The values represent the differences between the sample absorbance and the negativity threshold. On the same variety, values with the same letters are not statistically different ($\alpha = 0.05$).

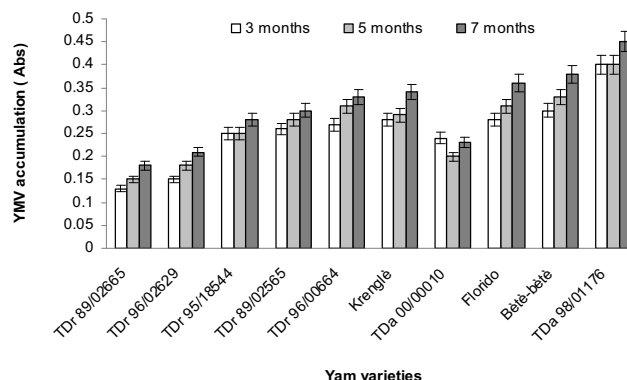


Fig. 4 Time course accumulation of YMV in yam stems. The values represent the differences between the sample absorbance and the negativity threshold. On the same variety, values are not statistically different ($\alpha = 0.05$).

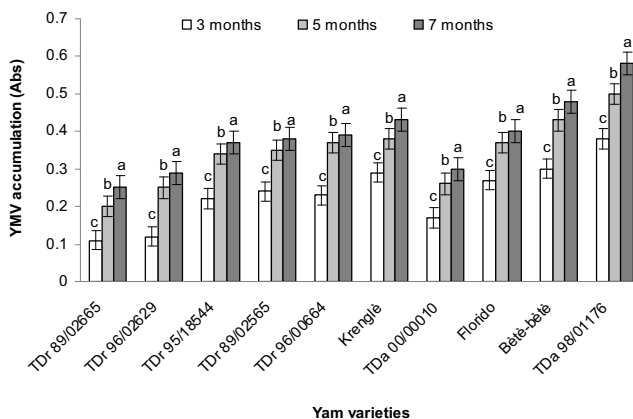


Fig. 5 Time course accumulation of YMV in yam tubers. The values represent the differences between the sample absorbance and the negativity threshold. On the same variety, values with the same letters are not statistically different ($\alpha = 0.05$).

In the tubers, CMV accumulated the most at 7 months and the least at 3 months, with the accumulation at 5 months being intermediate (Fig. 8). The differences in virus accumulations were statistically significant.

DISCUSSION

Improved varieties from the IITA breeding program were used during 3 years by the CSRS to carry out varietal trials in rural areas. The serological test, TAS-ELISA test performed on the leaves and tubers before inoculation showed the absence of YMV on all varieties except TDa 98/01176.

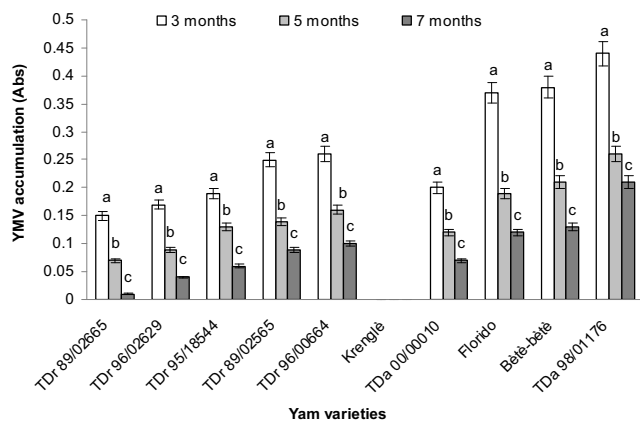


Fig. 6 Time course accumulation of CMV in yam leaves. The values represent the differences between the sample absorbance and the negativity threshold. On the same variety, values with the same letters are not statistically different ($\alpha = 0.05$).

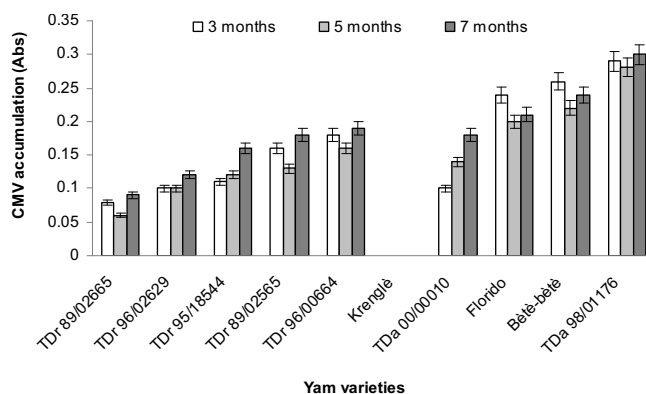


Fig. 7 Time course accumulation of CMV in stems. The values represent the differences between the sample absorbance and the negativity threshold. On the same variety, values are not statistically different ($\alpha = 0.05$).

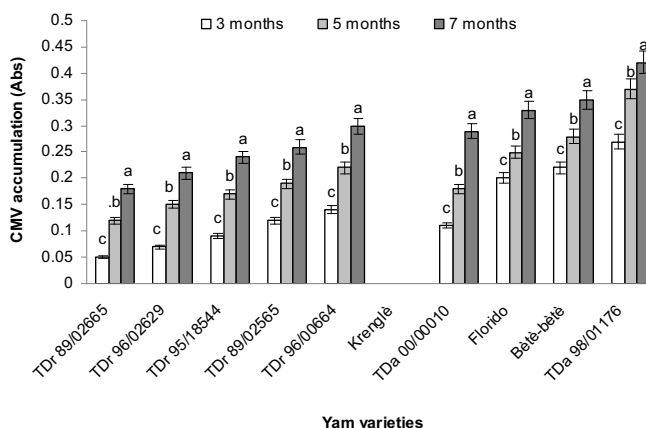


Fig. 8 Time course accumulation of CMV in tubers. The values represent the differences between the sample absorbance and the negativity threshold. On the same variety, values with the same letters are not statistically different ($\alpha = 0.05$).

This variety was already infected with YMV was probably infected during the varietal trials conducted by the SCSR in rural areas.

All varieties inoculated with YMV became infected. This reveals higher virulence of the virus strains used and the susceptibility of the 10 yam varieties tested. These findings are consistent with the work of Thouvenel and Fauquet (1979) who reported that YMV is a real obstacle to the cultivation of yams in Côte d'Ivoire. All plants of the local Bètè-bètè variety and TDa 98/01176 were infected with YMV. This reflects the great susceptibility of these varieties

to the virus. Thouvenel and Dumont (1990) also reported that the variety Bètè-bètè (*D. alata*) is one of the local varieties, the most susceptible to YMV. Some plants of the varieties TDr 89/02665, TDr 96/02629, TDr 95/18544 and TDr 00/00010 were tested positive to YMV by ELISA after inoculation while they did not showed any symptom. Some plants of the first three varieties, even though they remained symptomless after inoculation CMV, were tested positive to the virus by ELISA. Similar results were observed by Odu *et al.* (2004) on infected plants with no apparent symptoms of viral infection. This shows that evaluation of varieties based on visual symptoms observations alone is not reliable. YMV was less severe on varieties TDr 89/02665, TDr 96/02629 and TDr 00/00010. These varieties displayed some level of resistance to YMV. Whatever the variety, YMV accumulation varied according to the plant parts tested and also with the physiological stage of the plants. These findings comply with those of Lebas (2002). He showed that the relative virus concentration of virus in leaved depended on the age of the leaf and the age of the plant. The virus accumulated in the tubers at the expense of leaves as the development of the plant continues. The viruses are transported through the plant vessels for its distribution in the different parts of the plant. The characteristic symptoms of the virus infection are observed initially on the level of the leaves. During the development of the dry matter, the viral particles multiply and accumulate in the storage structures of the plant to ensure its survival (Astier *et al.* 2001; Mazié *et al.* 2008).

Before CMV inoculation, none of the yam varieties was tested positive to CMV by TAS-ELISA. Then, except for Krenglè, all inoculated varieties became infected by the virus. The plants of the variety Krenglè did not present any symptoms of CMV and reacted negatively to test ELISA. This variety could be immune to CMV like most of the varieties of the species *D. cayenensis*, *D. dumetorum* and *D. bulbifera* (Eni *et al.* 2008). Indeed, Krenglè belongs to the species *D. cayenensis*. However, CMV was less severe on inoculated varieties especially on varieties TDr 89/02665, TDr 96/02629, TDr 95/18544 and TDr 00/00010. The percentages of infected plants per varieties were lower than those obtained with YMV. These findings agree with those of Eni *et al.* (2008) which showed that the rate of CMV prevalence was $< 10\%$ in the neighboring countries such as Ghana, Benin and Togo. CMV accumulation was lower than that of YMV in all varieties. CMV accumulation was lower in varieties TDr 89/02665, TDr 96/02629, TDr 95/18544 and TDr 00/00010. These varieties were considered resistant to CMV. Mixed infection with both YMV and CMV occurred in plants of the variety TDa 98/01176. However, even though CMV accumulation was high, it is impossible to conclude a synergistic interaction between both viruses. Indeed, in synergistic interactions involving a *Potyvirus*, the accumulation of the non-*Potyvirus* is greater than in the single infection (Diallo *et al.* 2004, 2008). All plants of this variety TDa 98/01176 were infected with higher severity indexes and viruses accumulations. For the ten yam varieties studied and whatever the virus, virus accumulation was higher for the plant showing more severe symptoms. Varieties TDr 89/02665, TDr 96/02629 and TDr 00/00010 had some individuals plants not infected with either virus after inoculation. This could be due to a vertical or high resistance of these varieties *vis-à-vis* these pathogens. Similar behavior was reported by Sorho (2007) with *Rice yellow mottle virus*. This author obtained in partially resistant varieties, a good correlation between symptom severity and number of infected plants. But among highly resistant varieties, naturally virulent isolates did not infect a high proportion of individuals plant tested. This work also reports on the accumulations patterns of CMV and YMV in different yam plant parts and over time. The different parameters measured in this study are important to include in any variety screening trials.

CONCLUSIONS

The behavior of 10 varieties of *Dioscorea* spp. mechanically inoculated with CMV and YMV revealed that the varieties TDr 89/02665, TDr 96/02629 and TDr 00/00010 were resistant to YMV. These three varieties in addition to variety TDr 95/18544 were also found to be resistant to CMV. None of the plants of the local variety Krenglè (*Dioscorea rotundata-cayenensis*) was infected with CMV after mechanical inoculation. This variety could be immune to CMV. Other additional virus transmission tests using insect vectors could be carried on the local variety Krenglè to better assess the response of this variety CMV infection. Viral accumulations vary according to the varieties, the plant parts and the physiological stages of the plants. In infected plants, at a later stage, the virus accumulated more in the tubers as opposed to the leaves while it was contrary during the early stage. CMV and YMV accumulation patterns in the same plant part were similar at each physiological stage, regardless the yam variety.

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