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Yield loss caused by *yam mosaic virus* (YMV) and *cucumber mosaic virus* (CMV) on the varieties of *Dioscorea* spp.

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Abstract

The main objective of this work was to evaluate the effect of the yam (YMV) and cucumber (CMV) mosaic viruses on the yield of ten yam varieties. Forty plants per variety in a greenhouse inoculated with each of the two viruses were compared to healthy plants. The DAS-ELISA and TAS-ELISA tests have confirmed the infection of the experimental plants. Our findings showed a reduction in the mass of tubers in the plants infected by these viruses. A viral concentration decreasing from the apical portion to the base portion of the infected tuber was recorded. The results showed that YMV and CMV cause yield losses of yams. The use of the apical portion of the tuber of which serology is not known, could be a source of infection and viral propagation. The harvest of the infected tuber when the stems are still fresh decreases by 50% the viral concentration in infected tubers.

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Introduction

Yam (*Dioscorea* species of Dioscoreaceae family) is a multi-species, polyploid and vegetatively propagating tuber crop widely cultivated in the tropics and subtropics (Mignouna *et al*., 2003). Yams are important foodstuff (Anonymous, 2010) and play a major role in sociocultural activities in Côte d'Ivoire (Degras, 1993). They are also a significant source of income for thousands of people in Africa and around the world (Odu *et al.,* 2004). Over 90 % of world yam production occurs in the yam belt of West and Central Africa (FAO, 2002; Mignouna *et al*., 2003) with Nigeria alone accounting for 71.5 percent of the world total production estimated at 52 million tons, followed by Ghana with 6 million tons, and Côte d'Ivoire with 5.4 million tons (Anonymous, 2010).

In Cote d'Ivoire, yam is classified as the first food crop in terms of the tonnage of production (Anonymous, 2010) and is generally grown in the beginning of April in rainy season each year (Ettien *et al*., 2014).

Indeed, the high sensitivity of the *Dioscorea* species to Yam Mosaic Virus (YMV) of the genus *Potyvirus* and Cucumber Mosaic Virus (CMV) of the genus *Cucumovirus* is a source of major concerns for yams production in Côte d'Ivoire (Thouvenel and Fauquet, 1979; Seka *et al*., 2009a) and elsewhere in Africa (Odu *et al*., 2004). Indeed, CMV and YMV limit significantly reduce yam production resulting in significant yield losses (Eni *et al*., 2008; Jones *et al.,* 2008. Séka *et al*., 2009a).The incidence of the viruses depends on the variety, the parts of the infected plant and the phenological stage of the host plant (Séka *et al*., 2009a and 2009b).The viral activity result either in the reduction of the dry matter, the number of tubers (Hughes *et al*., 2004), the size and/or the weight of the tubers depending on the pathosystem (Jones *et al*. 2008).The use of seeds genetically resistant to virus is one major step to reduce or eliminate the viral infections thereof (Mignouna *et al*., 2003 ; Asiedu *et al*., 2003; Odu *et al.,* 2011).

In these studies (Kouamé *et al*., 2003; Ettien, 2004) selected ten yam varieties based on their organoleptic and agronomic qualities. In a recent work, the resistance of each of these varieties to YMV and CMV was tested (Seka *et al*., 2009b). The presence of one or several viroses did not allow for yam to be grown in some production areas in Cote d'Ivoire. Many farmers had to abandon their farms to create others in new areas and even start growing other crops. In addition no preventive control means was adopted in the farming areas to limit the yield losses.

Thus, the present work has been undertaken to determine the levels of impact of these two viruses on the yield of the 10 *Dioscorea* spp varieties selected.

The overall objective is to improve yam productivity by controling the YMV and CMV environment of the orchards in the producing areas of Toumodi, Dimbokro and Bouaké.

Material and methodology

Biological material

The present study was undertaken in the locality of Bringakro (6°55' N; 5°03'W) (S/P of Toumodi, Côte d'Ivoire) from 2006 to 2007. The agronomic characteristics of the area have been previously highlighted in a recent work (Séka *et al*., 2009a).

Seven (7) improved varieties of yam *D. cayenensis-rotundata* (TDr 89/02565, TDr 95/18544, TDr 96/00664, TDr 89/02665, TDr 96/02629 et TDa 98/01176, TDa 00/00010 of the species *D. alata*) were selected based on their organoleptic and agronomic qualities and three (3) local varieties (Krenglè, Bètè-bètè et Florido) as controls are used in the present study as planting material.

The viral material comprises YMV and CMV being extracted from leaves and tubers of the infected yam (*Dioscorea* spp.). The polyclonal and monoclonal antibodies directed against these isolates and conjugated antibodies (AS-0176-0435/10 and AS-0475-0491/1) produced by rabbits, respectively against YMV and CMV were used for the ELISA test. These antibodies and the positive antigen control

were provided by a commercial company (DSMZ, Germany).

Methodology

Planting

Healthy tubers of the ten (10) yam varieties were cut into small pieces of 50 gram placed in polyethylene pots of 20 cm diameter containing fertile soil.

Experimental design

The experimental design applied is the randomized block (Fischer) with 4 replications. Each replication consisted of 10 varieties of 40 plants per variety. A total of 400 plants infected with virus and 400 healthy plants (control) were used to evaluate yield losses caused by the YMV or CMV.

Mechanical inoculation in yam plants

Varieties of yam previously tested negative for DAS-ELISA and ELISA for each of the two virus using Clark and Adams method (1977), were mechanically inoculated with either with the YMV or CMV (viral) suspensions (Odu *et al.,* 2004). These inocula were prepared from 100 mg of yam leaves showing the characteristic symptoms of each viral infection. The viral suspensions were diluted in an inoculation buffer (10 mM phosphate buffer pH = 7.7 containing 1 mM ethylene diamine tetra acetic acid (EDTA) and 0.1 mM cysteine). The inoculation, of 350 ng/µl, was performed in an anti-insect shelter by friction with a cloth and fine sterilized sand.

Effect of CMV and YMV on the mass of tubers

At harvest, the mass of the infected tubers was compared to the mass of the healthy ones in order to evaluate the effect of the virus. For varieties with 2 crops like TDr 89/02565, TDr 96/02629, TDr 95/18544, TDr 89/02665, TDr 96/00664, the mass of tubers from the two crops were aggregated. Tubers from the infected plants were grouped into 3 lots according to their mass (Thouvenel and Dumont, 1990). Tubers which mass does not exceed 400 g are classified as small tubers. Those which weight ranged between 401 g and 799 g were considered mean tubers while large tubers are those having a mass greater than 800 g. In addition, the DAS-ELISA and TAS-ELISA tests were carried out on tubers of infected plants to determine their level of contamination.

Effect of the phenological stage of plant at harvest on the YMV and CMV concentration levels

Infected mature tubers were harvested when the stems are dried while others are collected with stems still fresh. The DAS-ELISA and TAS-ELISA serological tests were run on tubers of infected plants to assess their level of infection. This cropping practice is important since it can allow us to determine the cropping stage prone for high or low concentrations of viral particles.

Concentration gradient of virus in yam tubers

A longitudinal section /straight cut was made in the tuber and each of the 2 parts obtained was cut transversely into 5 equal parts (Fig. 1). Each sample in one level of the infected tuber was ground in liquid nitrogen and the extract collected in 5ml of phosphate buffer saline (PBS) containing 0.5 % Tween. The viral concentration was determined in each of the different parts of the tuber using the ELISA test.

Statistical analysis

A two -way variance analysis (ANOVA) was made to determine the effects of virus, variety, parts of tubers and stage of the plant at harvest with the generalized linear model (GLM) of SAS software (1999). The correlation between virus, varieties, parts of infected tuber and stage of the plant at harvest was also studied. When, a significant difference of parameters was noted, a multiple comparison of means was realized using the Newmann-Keuls at 5 % threshold.

Results

Effect of YMV and CMV on the mass of the tubers and yield

The mass of tubers from healthy plants ranged from 2775 ± 1150 g to 3404 ± 1237 g both for local and improved varieties (Table 1). Statistical analysis showed significant differences between the masses of tubers of the varieties tested ($P < 0.001$). For the YMV

and CMV-infected plants, the mass ranged from 1506 ± 1027 g to 2560 ± 1154 g in the different varieties tested (Table 1).Yield losses caused by YMV and CMV ranged from 20.20 ± 04.52% to 48 ± 09.95% for both local and improved varieties. Significant differences (P <0.001) between the varieties tested were observed in yield losses caused by YMV and CMV. Speaking about yield losses, three groups of varieties can be observed. thus, we have group 1 composed of Krenglè, group 2 consisted of varieties TDr 89/02565, TDr 96/02629, TDa 00/00010, TDr 95/18544, and group 3 composed of TDr 96/00664, TDr 89/02665, Florido, TDa 98/01176, Bètè-Bètè (Table 2).

Table 1. Effect of YMV and CMV on the mass (g) of tubers produced per plant and yam variety.

Varieties	Healthy plants	Plants infected with YMV	Plants infected with CMV	Statistical analysis	
				F	P
TDr 89/02665	3404 ±1237 ^{ab}	2560 ± 1154 ^{b}	2716 ± 1074 ^{b}		
TDr 96/02629	3516 ±1386 ^{ab}	2517 ± 1165 ^{b}	2720 ± 1143 ^{b}		
TDr 95/18544	3920 ± 1464 ^{a}	2545 ± 1186 ^{b}	2869 ± 1236 ^{b}		
TDr 89/02565	3254 ±1134 ^{ab}	2407 ± 1105 ^{b}	2196 ± 1042 ^{c}		
TDr 96/00664	2982 ± 1173 ^{b}	1895 ± 1125 ^{c}	1970 ± 1013 ^{c}	25.97	< 0.001
Krenglè	2650 ± 1156 ^{b}	1652 ± 1012 ^{c}	2650 ± 1156 ^{b}		
TDa 00/00010	3420 ±1253 ^{ab}	2494 ± 1135 ^{b}	2607 ± 1145 ^{b}		
Florido	2967 ± 1105 ^{b}	1713 ± 1095 ^{c}	1865 ± 1010 ^{c}		
Bètè-bètè	2810 ± 1084 ^{b}	1461 ± 1008 ^{c}	1700 ± 1008 ^{c}		
TDa 98/01176	2775 ± 1150 ^{b}	1506 ± 1027 ^{c}	1769 ± 1011 ^{c}		

YMV: Yam mosaic virus, CMV: Cucumber mosaic virus, F: Fischer Value, P: Probability. In rows and columns, the values with the same letters are equal according to the Newmann-Keuls test (α = 0.05), N = 2,100 tubers.

Table 2. Yield losses caused by YMV and CMV on yam varieties

Varieties	Yield losses caused by viruses (%)		Statistical analysis	
	YMV	CMV	F	P
TDr 89/02665	24.80 ± 05.57 ^{b}	20.20 ± 04.52 ^{b}		
TDr 96/02629	28.40 ± 04.68 ^{b}	22.63 ± 05.67 ^{b}		
TDr 95/18544	35.08 ± 06.35 ^{c}	26.82 ± 06.34 ^{b}		
TDr 89/02565	36.03 ± 06.75 ^{c}	32.51 ± 07.36 ^{c}		
TDr 96/00664	36.46 ± 06.86 ^{c}	33.95 ± 07.79 ^{c}	138.47	< 0.001
Krenglè	37.65 ± 07.32 ^{c}	00 ^{a}		
TDa 00/00010	27.08 ± 06.02 ^{b}	23.77 ± 06.65 ^{b}		
Florido	42.26 ± 08.95 ^{d}	37.13 ± 08.23 ^{c}		
Bètè-bètè	48.00 ± 09.95 ^{d}	39.50 ± 09.06 ^{c}		
TDa 98/01176	45.73 ± 08.64 ^{d}	36.26 ± 07.94 ^{c}		

F: Fischer Value, P: Probability. In rows and columns, the values with the same letters are equal according to the Newmann-Keuls test (α = 0.05), N = 2,100 tubers .

Effect of plant stage at harvest on YMV and CMV concentration levels

The absorbance values used to measure the concentrations of virus in tubers harvested with undried stems varied from 0.09 ± 0.04 Abs à 0.27 ± 0.03 Abs for local and improved varieties. Significant difference (P<0.001) are observed in viral concentrations between varieties analyzed (Tables 3 and 4). As for tubers harvested with dried stems, viral concentrations ranged from 0.18 ± 0.05 Abs to 0.58 ± 0.06 Abs (Tables 3 and 4) showing significant (P<0.001) differences in viral concentrations for local as well as improved varieties (Tables 3 and 4). The tubers sampled with undried stems reduced average viral concentration of 48.7 % for YMV and 46.7 % for CMV in tubers compared with those harvested with dried stems. Statistical analysis showed significant differences (P<0.001) between viral concentrations in tubers harvested with fresh stems and those harvested with dried stems.

Table 3. YMV concentration (Abs) in yam tubers depending on the plant stage at harvest.

Varieties	YMV Concentration in tubers harvested with fresh stem	YMV Concentration in tubers harvested with dried stem	Reduction of YMV concentration (%)
TDr 89/02665	0.13 ± 0.04 ^{d}	0.25 ± 0.05 ^{c}	52 ^{A}
TDr 96/02629	0.15 ± 0.02 ^{d}	0.29 ± 0.04 ^{c}	51 ^{A}
TDr 95/18544	0.18 ± 0.05 ^{d}	0.37 ± 0.03 ^{ab}	49 ^{A}
TDr 89/02565	0.17 ± 0.04 ^{d}	0.38 ± 0.01 ^{ab}	44 ^{A}
TDr 96/00664	0.18 ± 0.07 ^{d}	0.39 ± 0.04 ^{ab}	46 ^{A}
Krenglè	0.21 ± 0.02 ^{cd}	0.44 ± 0.01 ^{a}	48 ^{A}
TDa 00/00010	0.17 ± 0.03 ^{d}	0.30 ± 0.05 ^{c}	53 ^{A}
Florido	0.18 ± 0.08 ^{d}	0.40 ± 0.06 ^{ab}	45 ^{A}
Bètè-bètè	0.22 ± 0.05 ^{cd}	0.48 ± 0.07 ^{a}	46 ^{A}
TDa 98/01176	0.27 ± 0.03 ^{c}	0.58 ± 0.06 ^{a}	47 ^{A}

YMV : Yam mosaic virus. These values represent the absorbance difference between samples and positive threshold. In rows and columns, those with the same letters identical in character are equal according to the Newmann-Keuls test (α = 0.05), N = 300 tubers.

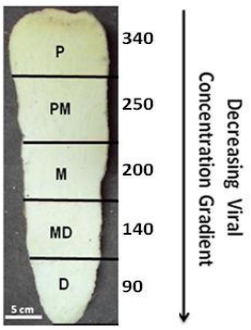
Table 4. CMV Concentration in yam tubers depending on the plant stage at harvest.

Varieties	CMV Concentration in tubers harvested with fresh stem	CMV Concentration in tubers harvested with dried stem	Reduction of CMV concentration (%)
TDr 89/02665	0.09 ± 0.04 ^{d}	0.18 ± 0.05 ^{c}	50 ^{A}
TDr 96/02629	0.11 ± 0.03 ^{d}	0.21 ± 0.04 ^{c}	52 ^{A}
TDr 95/18544	0.13 ± 0.02 ^{d}	0.24 ± 0.04 ^{c}	54 ^{A}
TDr 89/02565	0.12 ± 0.04 ^{d}	0.26 ± 0.04 ^{c}	46 ^{A}
TDr 96/00664	0.13 ± 0.06 ^{d}	0.30 ± 0.04 ^{bc}	43 ^{A}
Krenglè	00 ^{e}	00 ^{e}	-
TDa 00/00010	0.13 ± 0.05 ^{c}	0.29 ± 0.07^{bc}	53 ^{A}
Florido	0.16 ± 0.07 ^{c}	0.33 ± 0.02 ^{b}	48 ^{A}
Bètè-bètè	0.16 ± 0.08 ^{c}	0.35 ± 0.05 ^{b}	45 ^{A}
TDa 98/01176	0.18 ± 0.04 ^{c}	0.42 ± 0.08 ^{a}	43 ^{A}

CMV: Yam mosaic virus. These values represent the difference in absorbance between samples and positive threshold. In rows and columns, those with the same letters identical in character are equal according to the Newmann-Keuls test (α = 0.05), N = 300 tubers.

Concentration gradient of YMV and CMV in the tubers

YMV and CMV distribution in the tuber is measured as a gradient of viral concentration. A concentration gradient of YMV and CMV decreasing from 0.34 to 0.09 Abs starting from the apical portion to the basal portion of the tuber was observed. Significant differences (P<0.001) are detected between the apical, middle and basal tuber (Fig. 1). The same values have been obtained for both viruses (YMV and CMV)



P : Proximal, PM : Proxi-median, M : median, MD : Medial-distal, D : distal

Viral concentration : ng / µl , These values are viral concentration differences between infected samples and the positive threshold in parts of the tuber within the same variety, N = 300 tubers

Fig. 1. YMV Concentration gradient in the infected yam tuber.

Interactions between virus, varieties, parts of infected tubers and plant stage at harvest

Significant differences (P<0.001) in the concentration of virus was found between varieties, parts of infected tuber and the stage of the plant at harvest. The stage of the plant at harvest had no effect on the mass and size of the tuber. However, three interactions were identified: interaction between virus concentration and the stage of the plant at harvest, an interaction between the virus concentration and variety and finally, interaction between virus concentration and different parts of infected tubers (Table 5).

Table 5. Variance analysis : effect of the virus, the variety, the part of the infected tuber and the plant stage at harvest and different interactions.

Source Effects	DF	Type III SS	F	P
virus	1	0.043	91.06	<.0001
Varieties	9	0.00	83.70	<.0001
Parts of infected tuber	4	0.012	102.37	<.0001
Plant stage at harvest	1	0.694	2.58	0.0723
virus x harvest stage	1	0.059	11.67	0.0090
virus x varieties	9	0.006	21.89	<.0001
virus x parts of infected tuber	4	0.004	18.02	<.0001

DF : Degree of freedom, F: Fischer value, P : Probability.

Discussion

Our findings showed that yam mosaic virus (YMV) and cucumber mosaic virus (CMV) reduced the mass of tubers of the infected plants. These results are consistent with previous studies (Thouvenel *et al*., 1990; Thresh, 2003; Amusa *et al*., 2003; Jones *et al*., 2008) that showed that YMV and CMV reduced the mass of yam tubers.

Varieties of group 2 (tolerant), infected by YMV, with yield losses below 30% had a resistance level higher than the varieties of Group 3. The varieties of group 3 producing the lowest masses of tubers and having the highest rates of yield loss contained the varieties susceptible to YMV. The gene that has been conferring a resistance of Florido to YMV was circumvented by the virus. Human pressure in the selection of varieties has certainly caused an evolution in the YMV. More virulent pathotypes or bypassing isolates may exist. This obligate parasite has lost some recognition factors vis-à-vis the Florido variety. Similar findings were obtained by Karasawa *et al.* (1999) with CMV and Iskra-Caruana *et al.* (2003) with pararetroviruses (EPRV). No yield loss was recorded with the Krenglè variety (group 1, non-host reaction) in the presence of CMV thus confirming the work of Séka *et al.* (2009b) on a probable immunity of Krenglè variety in the presence of CMV. The selection within the Krenglè variety was recommended by Tokpa and Dumont (1998) to reduce viral infections. Tubers harvested when the stem was dried had YMV and CMV concentration higher than the tubers harvested with undried stems. The state of the stem at the time of harvest influenced the YMV and CMV concentration in the tuber. The movement of viral particles was achieved from the first infected cells (usually leaves) to the vegetative reproduction cells (tubers). The survival of these viruses is not at threat since stems are fresh; as result, they persisted and were eliminated at harvest (Astier *et al*., 2001).The movement of YMV and CMV in the tuber is done from the proximal portion to the distal portion. This colonization of neighboring cells from the infected cell necessitated the crossing of different tissue barriers. It has been further reported that the cells of the distal portion of the tuber multiply rapidly and the invasion of newly formed virus cells could be delayed (Astier *et al.,* 2001; Njukeng *et al*., 2006).

Conclusion

YMV and CMV significantly reduced the mass of yam tubers. The viral concentration was done following a decreasing gradient from the proximal portion to the distal one of the infected tubers. The average yield losses of 30 % and 40 % were caused by CMV and YMV respectively. Cropping (cultural) control

technique using tuber harvested when the stem is still fresh eliminates about 50 % YMV and CMV particles. The original results of the present work are relevant as they can allow yams producers to reduce viral concentrations in tubers and to have high yield and production.

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