Incidence of Potyviruses on Two Wild Yams Species, Potential Varietal Improvement Plant Genetic Resources, Involved in Farmers’ Domestication Process in Togo

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ABSTRACT

Introduction

Cultivated yams (Dioscorea spp.), especially yams of the complex Dioscorea cayenensis-rotoundata, represent the third food crop in Togo after maize and cassava, with an estimated annual production of 858,783 tones (FAOSTAT, 2020). However, yam cultivation in the country is hampered by viral diseases (Adjata, 1991), in particular those caused by Potyviruses, including Yam mosaic virus (YMV) and Yam mild mosaic virus (YMMV) (Ayisah et al., 2011, Ayisah, 2012). These Potyviruses have in fact been considered in West Africa to be the most prevalent viruses and the most economically damaging to yam plants (Hughes et al., 1998; Attiri et al., 2003).

To effectively control yam virus diseases, the appropriate solution will be to create resistant varieties of yams that can be exploited in a

Keywords

Potyvirus, Yam mosaic virus, Yam mild mosaic virus, Dioscorea abyssinica, Dioscorea praehensilis, Incidence rate

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Yams grown in Togo are severely infected by Yam mosaic virus (YMV) and Yam mild mosaic virus (YMMV), genus Potyvirus. The present work aims to study the susceptibility, to potyviruses, of wild species Dioscorea praehensilis and Dioscorea abyssinica, which can be exploited for the search for resistant genotypes. Thus, 107 leaf samples of D. abyssinica and 72 of D. praehensilis collected in the Maritime, Plateaux, Centrale and Kara regions were analyzed by ACP-ELISA to detect Potyviruses and then by RT-PCR to detect YMV and YMMV, using primers YMV1 & YMV2 and YMV-CP-2F & YMV-UTR-1R, respectively. Following ACP-ELISA analyzes, in Plateaux region, 20.69% of samples of D. abyssinica and 72 of D. praehensilis collected in the Maritime, Plateaux, Centrale and Kara regions were analyzed by ACP-ELISA to detect Potyviruses and then by RT-PCR to detect YMV and YMMV, using primers YMV1 & YMV2 and YMV-CP-2F & YMV-UTR-1R, respectively. Following ACP-ELISA analyzes, in Plateaux region, 20.69% of samples of D. abyssinica and 2.78% of D. praehensilis were positive for potyviruses. In Kara and Central regions, 33.33% and 1.41% respectively of D. abyssinica samples were positive. RT-PCR analyzes did not reveal any positive samples for YMMV. However, a leaf sample of D. abyssinica in domestication and another of D. praehensilis taken from near a yam plot were positive for YMV. The two yam species, less sensitive to Potyviruses, YMV, YMMV, could be potential sources of resistance.
sustainable manner using wild yams, more specifically, the species *Dioscorea praehensilis* and *Dioscorea abyssinica*, as basic plant genetic resources. Indeed, both species of yams are involved in the domestication process in rural communities in Togo and elsewhere in West Africa (Mignouna and Dansi, 2003). Moreover, according to Hamon (1987), yams of the complex *D.cayenensis-rotundata*, in particular the species *D. rotundata*, come from the wild yam species *D. praehensilis* and *D. abyssinica*. The susceptibility of these wild yams to viral diseases in their natural habitats has not yet been formally demonstrated. Nevertheless, studies have demonstrated spontaneous hybridizations between the two wild species *D. abyssinica* and *D. praehensilis* and the cultivated species *D. cayenensis-rotundata* (Scarcelli et al., 2006). Some of these wild interspecific hybrids can also be susceptible to viruses such as cultivated yams. Thus, for reliable varietal selection, it is essential to take an inventory of the Potyviruses which infect these wild yams in their habitats and to know the genetic links between the said Potyviruses and those which infect yams cultivated in the country.

The objective of the present work is to collect data on the virological environment of the wild yam species *D.praehensilis* and *D.abbyssinica* still in domestication in the country. Specifically, it was a question of identifying the Potyviruses in general, on wild yams *D. praehensilis* and *D. abyssinica* in different environments and to evaluate, in particular, their sensitivities to YMV and YMMV.

**Materials and Methods**

**Study areas**

The studies were carried out in four economic regions of Togo which are also major yam production areas of the country. These are the Maritime (6° 30'0"N, 1° 19'60"E), Plateaux (7° 30'0"N, 1° 10'0"E), Central (9° 15'0"N, 1° 0'0"E) and Kara (9° 33′ N, 1° 11′ E) regions. The Maritime and Plateaux regions have a subequatorial climate with two rainy seasons including a long rainy season (mid-March to late July) and a small rainy season (early September to mid-November). Annual rainfall varies from 800 mm to 1600 mm. The Central region has a Sudano-Guinean climate with only one rainy season (end-April to end-October). The average annual precipitation is 1300 mm. The Kara region has a Sudanese-type climate with a single rainy season (mid-May to end-October) and an average annual rainfall of between 900-1000 mm (ITRA, 2009).

**Plant material**

The work was carried out on leaves of wild yam species *D. praehensilis* and *D. abyssinica*. Some of the leaf samples were taken from farmers' fields on yam plants in domestication. In total, 179 leaf samples were collected for the detection of Potyviruses in laboratory (Table 1).

**Antibodies and primers used**

Universal anti-Potyvirus monoclonal antibodies (Agdia Inc.) were used to identify Potyviruses in yam leaves. In addition, two pairs of primers, YMV1 & YMV2 and YMV-CP-2F & YMV-UTR-1R, were used to detect YMV and YMMV respectively in leaf samples previously tested positive for Potyviruses (Table 2).

**Wild yam leaves collection**

Leaves of wild yams showing apparent symptoms of virus disease were collected from four of the five major yam production areas in Togo. The collection of yam leaf
samples covered 16 Districts, including 1 in Maritime region, 7 in Plateaux region, 5 in Central region and 3 in Kara region (Table 3). The yam leaf samples were taken in different environments including forests far from any crop, forests located near cultivated yam plots, but also in farmers' fields on wild yam plants in domestication. The leaf samples were stored on the fields in a cooler containing ice and then at -20 °C in the Laboratory of Virology and Plant Biotechnologies.

**Poty viruses immunological identification**

The leaf samples collected were analyzed by ACP-ELISA test (Antibody coating plat - Enzymelinked immunosorbent assay) according to the protocol provided by Agdia Inc as follows: fragments of 0.25 g yam leaves were ground in 5 ml of 0.05M carbonate buffer (pH9.6) supplemented with 2% PVP, then 100 μl of extract from each leaf sample were loaded in the wells of the microtiter plates (plates Nunc). The plates were then incubated at room temperature for 1 hour and after three rinses with PBST1X containing Tween 20 (0.05%), they were filled with the first anti-potyvirus monoclonal antibody diluted to 1/200. After incubation overnight at + 4 °C and after three successive rinses, the plates were filled with the "goat anti-mouse" conjugate antibody (dilution: 1/200) and then incubated for 1 hour at room temperature. At the end of the incubation followed by four washes, 200 μl of substrate extemporaneously prepared (1 mg of p-paranitrophenyl phosphate for 1 ml of buffer) were, dispensed into each well and then the plates were incubated in total darkness for 30 minutes at room temperature. The reactions were read on a spectrophotometer Multiskanat 405nm. On reading, the wells having an O.D. (optical density) greater than or equal to the double of the O.D. of the healthy control, were considered as positive.

**Molecular detection of YMV and YMMV**

Yam leaf samples positive at ACP-ELISA test, were analyzed by RT-PCR for the detection of YMV and YMMV. These two viruses were detected in duplex in the leaf samples according to the protocol which is as follows: PCR tubes (0.2ml, Starlab) were filled with extracts (25μl/tube) obtained by grinding 0.5mg of yam leaf in 5ml of carbonate buffer (pH9.6) and after centrifugation at + 4 °C and at 6000trs / min for 5mn. The tubes, after incubation overnight at + 4 °C, were rinsed twice with sterilized PBS 1X containing Tween 20 (0.05%) and once with sterile water-DEPC. Leaves taken from healthy yam plants in the greenhouse served as negative controls. The RT-PCR was carried out by adding to the contents of each PCR tube, 25 μl of RT-PCR reaction mixture (QiAgen one step RT-PCR kit) containing the detection primers for YMV (YMV1 & YMV2, 10 μM) and YMMV (YMVCP2F & YMVUTR1R, 1μM).

The RT-PCR reactions were carried out in thermocycler Biometra under the following conditions: one retrotranscription cycle (50 °C for 30 min then 95 °C for 15 min), 35 PCR cycles (denaturation: 94 ° C for 1 min, hybridization: 55 ° C for 1 minute; elongation: 72 ° C for 1 minute) and 10 minutes of elongation at 72 ° C to complete the cycles. At the end of the reactions, 12μl of PCR product (cDNA) from each tube mixed with 2μl of 6x loading buffer (2mM Tris-HCl pH 8, 10mM EDTA, 5% sucrose, 0.01% bromophenol blue), were loaded on agarose gel (1.5%) prepared in 0.5x TBE buffer (100 mM Tris Borate pH 8.3, 2 mM EDTA). A standard size marker of 1kb was also loaded on the gel. The electrophoresis was carried out in 0.5x TBE buffer for 35 minutes at a voltage of 100V. The agarose gel was coloured at the end of the electrophoresis in Ethidium Bromide for 15 min and washed for
5 min. The electrophoretic bands were visualized on a UV trans-illuminator.

**Results and Discussion**

**Incidence of Potyviruses**

The results of the ACP-ELISA, reported in Tables 4 and 5, indicated that, of the 179 leaf samples of *D. praehensilis* and *D. abyssinica* analyzed, only 12 were positive, i.e. an estimated incidence rate of Potyviruses at 6.70% for both species of yam. However, it should be noted that, depending on the yam species and the regions where the leaf samples were collected, the results obtained were variable. In *D. abyssinica* species, of the 107 leaf samples analyzed, 10 were positive, i.e. an estimated incidence rate of Potyviruses at 9.35%. Among these positive samples, 3 were collected from yam plants in domestication, 2 were taken from a forest near a plot of cultivated yams while 5 were collected from forests far from any cultivation. In *D. praehensilis* species, only 2 of the 72 samples analyzed were positive, for an estimated incidence rate of Potyvirus of 2.78%. One of the positive samples of this yam species was collected from a yam plant in domestication while the second came from a forest close to a cultivated yam plot.

At the level of the Districts, as shown in Table 4, in the Maritime and Plateaux regions, at least one leaf sample of *D. abyssinica* among those collected in the Districts of the Gulf, Ogou, Anié and Est-Mono, was positive for Potyviruses. On the other hand, in the Central region, among the five districts surveyed, only 1 leaf sample of *D. abyssinica* collected in the District of Blitta was positive. In the Kara region, of the three districts surveyed, 1 sample from Bassar District and 1 from the Kéran District were positive. In *D. praehensilis*, among the samples taken in seven Districts of Plateaux region, only 2 samples from the District of Agou were positive. No samples of *D. praehensilis* collected in Blitta District, in Central region, were positive.

The results concerning the regions of collection of the yam leaf samples analyzed, showed that in the Plateaux region, of the 29 samples of *D. abyssinica* analyzed, only 6 were positive, i.e. an incidence rate of potyviruses estimated at 20.69 % while in *D. praehensilis*, of the 63 leaf samples analyzed, only 2 were positive, i.e an estimated potyvirus incidence rate of 2.78%. On the other hand, in the other three other regions, only the samples of *D. abyssinica* were positive, including 1 sample from the Maritime region (incidence rate 100%), 2 samples from Kara region on a total of6samples analyzed (i.e. 33.33% of the viruses incidence rate) and 1 sample from the Central region, of the 71 samples analyzed, i.e. 1.41% Potyvirus incidence rate.

As shown by the results in Table 5, all the 3 leaf samples taken from plants of *D. abyssinica* in domestication, were positive. On the other hand, in *D. praehensilis*, only 1 of the 11 leaf samples taken from plants in domestication was positive.

**Incidence of YMV and YMMV**

The 12 leaf samples of both *D. praehensilis* and *D. abyssinica* species positive at ACP-ELISA test, were analyzed by RT-PCR. However, only 2 of these leaf samples were positive for YMV as indicated by the results reported in Table 6. This corresponds to an incidence rate of YMV estimated at 16.67% for the 12 leaf samples analyzed and at 1.12% for all the 179 leaf samples of the two yam species collected in the four regions of Togo. Among these 2 leaf samples positive for YMV, 1 was taken from a plant of *D. abyssinica* in domestication (i.e. an incidence
rate of 0.93%) and 1 sample was collected from a plant of *D. praehensilis* located near a cultivated yam plot in the District of Agou (Plateaux region), i.e., an incidence rate of YMV estimated at 1.37%. In contrast, for YMMV, no sample was positive.

**Table 1** Number of wild yam leaf sampled collected in the four yam producing areas of Togo

| Wild yam species | wild yam leaves collection areas and environments | Total |
|------------------|-------------------------------------------------|-------|
|                  | MR* | PR | CR | KR | Domestication | Forest |       |
| *D. abyssinica*  | 1** | 29 | 71 | 6  | 3              | 104    | **107**|
| *D. praehensilis*| 0   | 63 | 9  | 11 | 61             | 61     | **72** |
| **Total**        | 1   | 92 | 80 | 14 | 14             | 165    | **179**|

*MR = Maritime region, PR = Plateaux region, CR = Central region, KR = Kara region
**Yam plant in domestication at Agronomic Experimentation Station of the Higher School of Agronomy, University of Lomé

**Table 2** List of primers used for the detection of YMV and YMMV and their sources

| Primers | Sequences | Size of cDNA fragments | Viruses detected | Sources |
|---------|-----------|------------------------|------------------|---------|
| **YMV1** | 5'-TGCGGAACTCRAAAGAAGC-3’ | 196 pb | YMV | Bousalem *et al.*, (2000b) |
| **YMV2** | 5'-TGCCATCAAATCCAAACA-3’ | | | |
| **YMV-CP-2F** | 5’-GGCACACAGCAAATGAA AGC-3’ | 249 pb | YMMV | Mumford & Seal (1997) |
| **YMV-UTR-1R** | 5’-CACCAGTAGAGTGAACAT AG-3’ | | | |

**Table 3** List of the Districts of the wild yam leaf samples collection and the number of samples collected

| Regions | Districts | Wild yam species | D. abyssinica | D. praehensilis | Regions | Districts | Wild yam species | D. abyssinica | D. praehensilis |
|---------|-----------|-----------------|---------------|----------------|---------|-----------|-----------------|---------------|----------------|
| **MR**  | Gulfe*    |                 | 1             | 0              | **CR**  | Tchamba  |                | 16            | 0              |
|         | Agou**    |                 | 0             | 12             |         | Sotouboua|                | 27            | 0              |
|         | Kloto**   |                 | 0             | 14             |         | Tchaoudjo|                | 17            | 0              |
|         | Haho      |                 | 0             | 10             |         | Binah    |                | 2             | 0              |
|         | Badou**   |                 | 0             | 9              |         | Blitta*  |                | 9             | 9              |
|         | Ogou      |                 | 0             | 9              |         | Bassar   |                | 3             | 0              |
|         | Anié      |                 | 7             | 2              |         | Doufelgou|                | 1             | 0              |
|         | Est- mono*** |             | 22            | 7              |         | Keran    |                | 2             | 0              |

* Collecting localities of leaves of *D. abyssinica* domesticated
** Localities of collection of leaves samples of *D. praehensilis* in domestication
*** Localities of collection of leaves samples of *D. abyssinica* and *D. praehensilis* in domestication
Table 4 Number of yam leaf samples positive for Potyviruses by wild yam species and by District

| Regions | Districts | Wild yamspecies | Number of samples positive for Potyviruses |
|---------|-----------|-----------------|------------------------------------------|
|         |           | *D. abyssinica* |                                           |
| MR      | Gulfe*    | 1/1             |                                          |
| PR      | Agou**    | -               | 2/12                                     |
|         | Klotoo**  | -               | 0/14                                     |
|         | Haho      | -               | 0/10                                     |
|         | Badou**   | -               | 0/9                                      |
|         | Ogou      | -               | 0/9                                      |
|         | Anie      | 2/7             | 0/2                                      |
|         | Est-mono*** | 4/22         | 0/7                                      |
|         |           | *D. praehensilis* |                                             |
|         |           | 0/63            | 0/2                                      |
|         |           | 0               | 0                                        |
|         |           | 1/11            | 1/61                                     |
|         |           | 1/10            | 1/80                                     |
|         |           | 2/6             | 2/6                                      |
|         |           | 4/14            | 4/14                                     |
|         |           | 8/92            | 8/141                                    |

* MR = Maritime region, PR = Plateaux region, CR = Central region, KR = Kara region

* Collecting localities of leaves of *D. abyssinica* domesticated
** Localities of collection of leaves samples of *D. praehensilis* in domestication
*** Localities of collection of leaves samples of *D. abyssinica* and *D. praehensilis* in domestication

Table 5 Potyvirus incidence rates by wild yam species and number of positive yam leaf samples by region

| Wild yamspecies | Wild yam leaves collection areas and environments | Total | Incidence rates (%) |
|-----------------|-------------------------------------------------|-------|---------------------|
|                 | MR* | PR   | CR   | KR   | Domestication | Forest |                     |                     |
| *D. abyssinica* | 1/1 | 6/29 | 1/71 | 2/6  | 3/3          | 7/104  | 10/107               | 9,35                |
| *D. praehensilis* | 0  | 2/63 | 0/9  | 0    | 1/11         | 1/61   | 2/72                 | 2,78                |
| Total           | 1/1 | 8/92 | 1/80 | 2/6  | 4/14         | 8/141  | 12/179               | 6,70                |

*MR = Maritime region, PR = Plateaux region, CR = Central region, KR = Kara region

Table 6 Reactions of Potyviruses positive leaf samples of *D. praehensilis* and *D. abyssinica*, to YMV and YMMV detection analyzes

| Regions | Wild yam species | Positive wild yam leaves collection environments | Total |
|---------|-----------------|-------------------------------------------------|-------|
|         | *D. abyssinica* | Domestication | Forest |                     |       |
| MR      | 1/1             | 1/1          | -      |                     | 1/1   |
| PR      | 0/6             | 1/2          | 0/2    |                     | 1/8   |
| CR      | 0/1             | 0            | 0      |                     | 0/1   |
| KR      | 0/2             | 0            | 0      |                     | 0/2   |
| Total   | 1/10            | 1/2          | 0/2    |                     | 2/12  |

Potyviruses were detected in the two wild yam species studied, *D. abyssinica* and *D. praehensilis*, but virus incidence rates were highly variable depending on the yam species and the regions surveyed. In indeed, in the Maritime and Plateaux regions, with a soft and humid climate, Potyviruses were detected in both wild yam species, however, with an
incidence rate of the virus higher in *D. abyssinica*. In the Central and Kara regions, with a hot and dry climate, only *D. abyssinica* species was infected by Potyviruses but at very low rates. No sample of *D. praehensilis* collected in the Central Region was positive for Potyviruses despite the proximity of some wild yam plants to cultivated yam plots. These variations in the incidence rates of Potyviruses according to agroecological zones were also observed in Togo on yams of the complex *D. cayenensis-rotundata* (Ayisah et al., 2011) and this would be due to agroecological conditions, in particular to climate, and also, to the abundance of viruses and their vectors (Thresh et al., 2003; Astier et al., 2001).

Furthermore, depending on the development environment of the wild yam plants analyzed, the incidence rates of Potyviruses were also variable. Indeed, the results showed that 100% of the leaves taken from *D. abyssinica* plants in domestication were infected against only 6.73% of the samples collected in the forest. On *D. praehensilis*, 9.09% of leaves from plants in domestication were positive compared to 1.64% of those taken from wild plants. This suggests that in their natural habitats, both wild yam species are less prone to Potyvirus infections than in yam fields where infection conditions appear stronger; the importance of virus sources, the abundance and activity of vectors, and the susceptibility of plants being the main factors that promote virus expansion (Astier et al., 2001).

The low contamination rate of samples of *D. praehensilis* compared to those of *D. abyssinica*, suggests a greater sensitivity of the latter species. This has been observed in the laboratory, during mechanical inoculations using isolates of YMV, where *D. abyssinica* has been shown to be very susceptible to the virus unlike *D. praehensilis* (Ayisah, 2012; Ayisah et al., 2020). Nevertheless, it should be noted in general that the two species of yams were very weakly infected by Potyviruses.

In addition, of a total of 12 Potyviruses positive leaf samples analyzed, only 2 were infected by YMV, of which 1 sample taken from a plant of *D. abyssinica* in domestication and 1 harvested from *D. praehensilis* near a cultivated yam plot. But no sample was infected by YMMV. This suggests that, in their habitats, these wild yam species are not specifically susceptible to the two most important viruses that infect cultivated yams in West Africa (Brunt et al, 1996; Njukeng, 1998). The two YMV infections detected on *D. abyssinica* and on *D. praehensilis*, would have come from cultivated yams, in particular from yams of the complex *D. cayenensis-rotundata* very sensitive to virus infections (Hughes, 1997; Porth et al., 1987). It is also possible that some of the yam plants infected by the virus are the product of interspecific hybridizations. Indeed, many cases of interspecific hybridizations between different yam species, especially between wild yams, *D. abyssinica*, *D. praehensilis*, and cultivated yam species (*D. rotundata*, *D. cayenensis*) have been reported (Scarcelli et al., 2006; Zannou et al., 2009). This suggests the need to carry out a preliminary study to identify populations of wild yams resulting from spontaneous hybridization with cultivated species (potentially sensitive to viruses) with a view to better use in variety improvement programs. On the other hand, more than 83% of the wild yams leaf samples positive at ACP-ELISA test, were infected by Potyviruses other than YMV and YMMV. Similar results were obtained on yams of the complex *D. cayenensis-rotundata* cultivated in Togo where, more than 43% of leaf samples analyzed were contaminated by other species of Potyviruses (Ayisah, 2012). It is therefore possible that some of the
Potyviruses identified in the wild yams are the same as those which infect yams of the complex *D. cayenensis-rotundata*. This suggests the need to make an inventory of the Potyviruses which infect the two yam species *D. abyssinica* and *D. praehensilis* and to know their links with the viruses of cultivated yams.

In conclusion, it emerges from the present study that wild yams, *D. abyssinica* and *D. praehensilis*, regularly involved in the process of farmers’ domestication in Togo, are infected by Potyviruses. However, the incidence of the Potyviruses is generally very low, especially for *D. praehensilis*. Furthermore, these yams were not specifically susceptible to YMV and YMMV in their habitats. This suggests that the two species of spontaneous yams actually contain sources of resistance that can be exploited in variety improvement programs. But given the possibilities of infection by YMV and of interspecific hybridizations with cultivated yams which can lead to genotypes sensitive to viruses, it would be necessary to search beforehand for potentially sensitive interspecific hybrid populations among these wild yams before any exploitation as sources of resistance. It is also important to know the nature of the Potyviruses which infect the two spontaneous yam species as well as their links with the viruses of cultivated yams.

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