

Epidemiology of Cassava Mosaic Disease and Control Methods in the Central African Republic

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Abstract Cassava is the leading crop in the Central African Republic. Since 2000, severe mosaic symptoms have been observed on cassava in this country. Thus, surveys carried out between 2005 and 2011 showed an incidence of mosaic of 85% with the presence of ACMV and the severe Ugandan strain of EACMV-UG diagnosed. The surveys showed more than 50% of the plants were co-infected by the two species and the viral synergy increased the severity of the disease and made it possible to estimate 50% of harvest losses and to demonstrate that the main factor in the spread of the disease was the deplorable phytosanitary state of the cuttings used by farmers for replanting plots with 79% of cuttings contaminated. Subsequently, several studies were conducted to identify disease control measures. This is how; A varietal selection was carried out between 2008 and 2011 and resulted in the identification of 14 mosaic-resistant varieties with high yield potential and better meeting the population's culinary habits. Our work revealed through Farmer Field Schools that conventional technical itineraries double cassava production unlike traditional practices. In the strategy of sanitation of plant material by the therapy technique, contaminated cuttings of a local variety susceptible to mosaic were heat-treated in hot water baths between 41°C and 51°C for 30 minutes and then evaluated in the open field over a period of 12 months until harvest. Our results show that 49°C represents the optimal treatment temperature, without negative impact on the regeneration capacity of the cuttings, and for which we observed the highest tuber yield (4.7 kg / plant), equivalent to untreated cuttings from asymptomatic plants (4.6 kg / plant). Our studies have shown that regeneration of infected cuttings in a high-temperature room (37°C to 40°C) for 15 days followed by meristem culture effectively disinfects the contaminated plant material. The combination of thermotherapy and meristem culture techniques is an effective way to disinfect infected plant material. The popularization of resistant varieties and the sanitation techniques tested in this study constitute an effective means of disease control.

Keywords: Mosaic, epidemic, begomovirus, control, sanitation, Central African Republic

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1. Introduction

Cassava Mosaic Disease (CMD), first described in East Africa in 1894 [1], represents one of the first viral constraints of cassava in Africa [2,3,4,5]. The viruses responsible for this disease belong to the *Geminivirus* family and more specifically to the genus *Begomovirus*. transmitted by the whitefly *Bemisia tabaci*. The presence of mosaic has been reported in the vast majority of sub-Saharan African countries [6,7,8,9,10] where it causes

serious yield losses [3,10,11,12] [13,14,15,16] [17,18,19]. These losses are estimated at 15-24% of production, equivalent to 15-27 million tonnes per year in Africa [20]. In the 1990s, a severe epidemic of cassava mosaic disease occurred in Uganda. It was initially associated with the sole presence of a new recombinant strain (called Ugandan strain or Ugandan variant) resulting from genetic recombination between ACMV and EACMV (EACMV-UG) [13,14,21,22,23]. Research subsequently showed that this severe form of CMD was mainly caused by co-infection and viral synergy between ACMV and EACMV-UG [16,24,25,26]. In the early 2000s, this severe CMD

epidemic spread rapidly across Africa [27]. The viruses responsible for the disease have been identified in Cameroon, Congo, the Democratic Republic of Congo, Gabon and recently in Burkina Faso [15,16,27,28,29]. Cassava represents a fundamental crop for African subsistence agriculture and particularly for the Central African Republic. Indeed, cassava consumption per capita was 115 kg in 2010 in African ACP countries compared to 18 kg in the rest of the world. Over the last decade, human consumption has increased by 39% in Africa. In 6 African countries (Zaire, Mozambique, Congo, Angola, Ghana and the Central African Republic), cassava roots provide more than 25% of the population's energy intake and therefore constitute the basis of the diet [30];[20]. The annual production of cassava roots in the Central African Republic is ten times that of maize [31], which illustrates the importance of this foodstuff.

In the Central African Republic, despite the description of CMD and its impact on cassava crops [31], the work of identifying pathogenic agents has never been carried out, although it represents an essential step in the implementation of integrated control strategies. The description of severe symptoms of CMD in the Central African Republic in the early 2000s raised fears of a severe pandemic in this country [32]. The description of both very severe symptoms of CMD (4 or 5 according to the Cours scale) and a drop in yields seemed to confirm this fear, despite the absence of diagnosis and molecular characterization of the causal agents. Faced with this worrying health situation, the use of improved cassava varieties presumed to be resistant to mosaic disease was urgent. These, introduced by the Central African Institute of Agricultural Research (ICRA) from the *International Institute of Tropical Agriculture* (IITA) in Ibadan, Nigeria between 1990 and 2000, have not been entirely satisfactory for several reasons: insufficient or varying levels of resistance, inadequacy with the expected organoleptic qualities and the culinary habits of the populations, distrust of the populations towards the new material and difficulty of access for farmers to basic plant material (cuttings). It is in this socio-economic context and agronomic and parasitic constraints that this work is situated. Its challenge is to contribute significantly to improving cassava production in the Central African Republic and thus preserve the food security of rural populations.

The objective of this work was to achieve:

- an epidemiological study with emphasis on the identification of the pathogens responsible for mosaic disease
- a varietal screening
- an evaluation of the effectiveness of techniques for sanitizing infected plant material by thermotherapy and tissue culture

2. Cassava Mosaic Epidemic Situation

2.1. Expression and Origin of the Disease

Faced with this worrying situation, a survey was conducted to identify the main biotic constraints responsible for cassava production losses [18]. The survey

conducted between 2005 and 2006 in a limited number of localities around Bangui made it possible to assess the importance of cassava mosaic disease, with a high incidence and severity likely to halve cassava production, and to hypothesize that Central African cassava crops are affected by the severe CMD pandemic. To verify this hypothesis, a large-scale epidemiological survey was conducted between 2007 and 2008, using standardized protocols by IITA [33] for the assessment of the main cassava pests and diseases. This survey confirmed that CMD represents the most important constraint of cassava in the Central African Republic, with an average incidence of 85% (Figure 1). Importantly, 79% of farmers appear to use infected cuttings when replanting plots, highlighting the poor sanitary quality of the material [34].

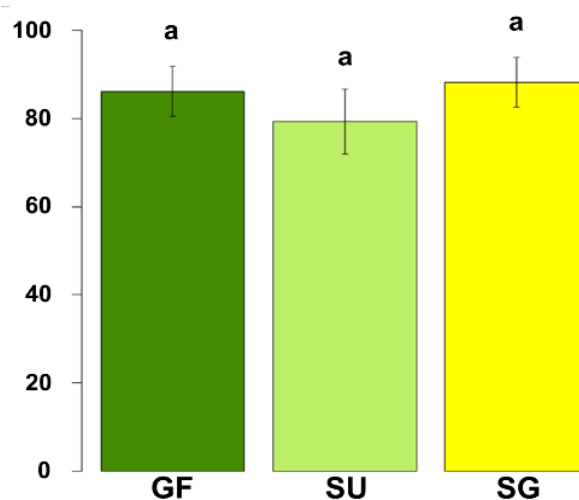


Figure 1. Average incidence of CMD (85%) (Ecological zones, GF: Forest Guinea zone, SU: Sudano Oubanguienne, SG: Sudano Guinean)

CMD has indeed proven to be the first biotic constraint of cassava in Central Africa with a high incidence and severity, respectively 85% and 2.7 severity. Similar incidences, recorded during the severe CMD pandemic in the Democratic Republic of Congo (86%, [32]), in Uganda [14] and in Tanzania [8], confirmed the severe epidemic situation of CMD in the Central African Republic (CAR), even if slightly higher average severities were recorded in Uganda [33] and in the DRC [28]. On the other hand, our work has demonstrated that this epidemic situation in the Central African Republic was mainly due to the poor phytosanitary quality of the cuttings used by farmers [34]. Indeed, 79% of the cuttings used for replanting are contaminated by CMD, confirming the results of similar work carried out elsewhere in Africa [4,28,33]. The use of cuttings of poor sanitary quality is explained by the unavailability of healthy material and/or by ignorance of the disease.

2.2. Diagnosis and Molecular Diversity of Begomoviruses Involved in Mosaicism in CAR

PCR diagnosis and direct sequencing of amplification products revealed that the causative agents of CMD in CAR are *African cassava mosaic virus* (ACMV) and the Ugandan strain of *East African cassava mosaic virus*

(EACMV-UG). We also demonstrated that 58% of samples had mixed infections (ACMV and EACMV-UG) and that these samples had significantly more severe CMD symptoms than single infections. Our results confirm that mixed infection and synergy between CMGs could be a key element in reducing cassava plant yield in CAR, similar to other severe CMD epidemics reported in East Africa [34].

To validate the taxonomic position of CMGs involved in CMD in the Central African Republic and southern Chad, we cloned and sequenced complete DNA-A sequences of CMGs [35] (Figure 2). Nucleotide sequence analysis (pairwise nucleotide identity rate) confirmed that these sequences correspond to isolates of the species ACMV (96-97% nucleotide identity) and EACMV-UG (99% nucleotide identity). Phylogenetic analysis showed that the DNA-A and DNA-B sequences of EACMV-UG form very low-diversity clades, suggesting a recent and unique introduction of EACMV-UG into the Central African Republic. In contrast, the identification of three distinct phylogenetic groups of Central African ACMV isolates suggests several introduction events and a more ancient and complex evolutionary history [35]. While a first phylogenetic group of Central African ACMV isolates is related to isolates from Cameroon, Nigeria and Ghana (West Africa), a second is related to isolates from Kenya (East Africa). Also, using regression analyses, we were able to show that the spatial distance between isolates was a good indicator of genetic distance, which could indicate a generally regular and short-distance spread of CMGs in CAR.

Our PCR diagnostic work with specific primers made it possible to detect the presence of ACMV and EACMV-UG, in single or mixed infection, in symptomatic samples of Central African cassava leaves [35]. The molecular characterization of their bipartite genome (DNA-A and DNA-B) and the obtaining of complete nucleotide sequences made it possible to confirm their taxonomic status [35]. We were thus able to demonstrate the involvement of ACMV and EACMV-UG in the severe CMD epidemic in the Central African Republic, in a very similar way to what had been observed in Uganda in the 1990s, and in neighboring countries affected by the pandemic, notably the Democratic Republic of Congo (DRC), Congo, Gabon and Cameroon [10,16,15,27,36,37]. Our results seem to confirm the hypothesis put forward by Legg et al. [32] that the Central African Republic was likely affected by the severe CMD pandemic in the early 2000s and that it represented a pivotal area between East and West Africa. The higher proportion of plants co-infected with ACMV and EACMV-UG in the forest zone bordering the two Congos, where the severe pandemic has already been described [27,28,37], suggests that this severe pandemic entered the CAR from the south of the country and progressed northward. This hypothesis is supported by the fact that the percentage of co-infected plants in the CAR decreases along a south-north gradient. It is also consistent with recent descriptions of EACMV-UG in Cameroon, in a border area with the Central African Republic [36], and in Burkina Faso [29].

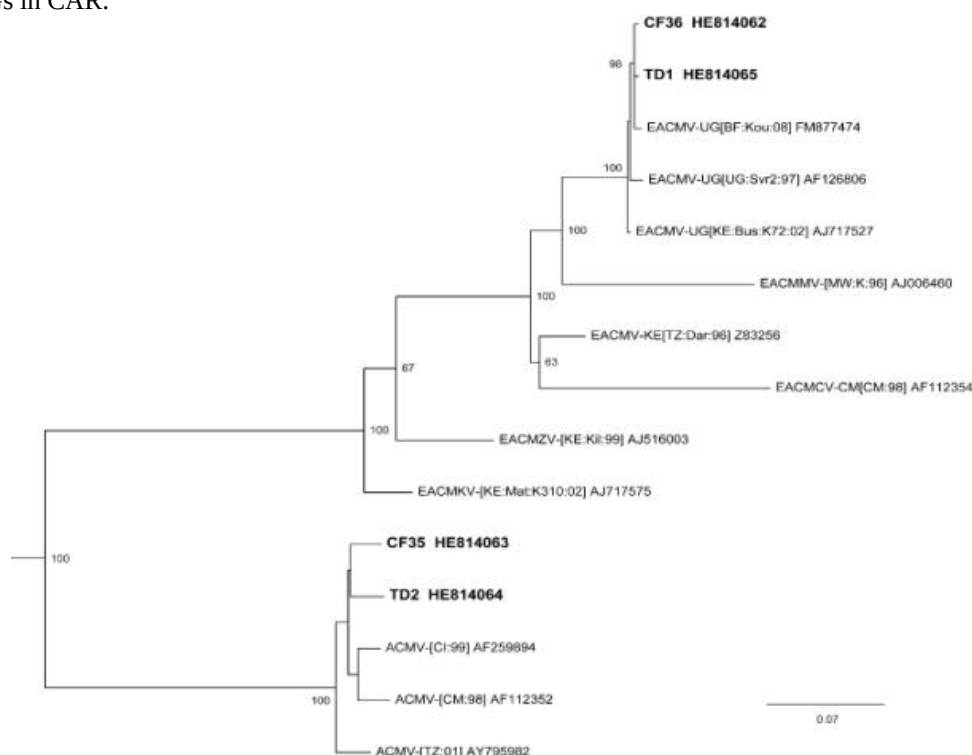


Figure 2. Phylogenetic tree based on the complete DNA-A sequences of the Central African Republic (CF) and Chad (TD) isolates of African cassava mosaic virus and East African cassava mosaic virus - Uganda reported here (in bold), as well as other African cassava mosaic geminivirus sequences. The sequences were aligned using the MUSCLE alignment tool implemented in MEGA5. Phylogenetic reconstruction was performed using PHYML and GTR+G4 selected as the best model of sequence evolution. Numbers associated with nodes indicate the percentage of bootstrap support (1000 repeats). EMBL- GenBank -DDJP accession numbers are indicated. The tree was rooted using Sri Lankan cassava mosaic virus-Sri Lanka [Sri Lanka: Colombo: 1998] (SLMCV-SL [SL:Col:98])

Phylogenetic analysis showed that EACMV-UG DNA-A and DNA-B sequences form very low-diversity clades, suggesting a recent and unique introduction of EACMV-UG into the Central African Republic. In contrast, phylogenetic analysis of ACMV DNA-A and DNA-B sequences shows the existence of three phylogenetic groups of Central African ACMV isolates, suggesting several introduction events and a more ancient and complex evolutionary history for this species [38]. While the first phylogenetic group of Central African ACMV isolates is related to isolates from Cameroon, Nigeria and Ghana (West Africa), the second phylogenetic group is related to isolates from Kenya (East Africa).

3. Control Method

3.1. Participatory Selection of Elite Varieties

Several research programs have been developed in the past to (1) identify sources of CMD resistance among local or introduced varieties of *Manihot esculenta* and (2) increase resistance by crossbreeding with other *Manihot* species, particularly *Manihot glaziovii*. Thus, a major program was initiated by Storey and his colleagues in East Africa from 1937 to 1957 [39] and then continued by the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria [32,40,41,42]. This program largely used germplasm from East Africa, but also incorporated accessions from South America and India to improve the level of resistance, yield, and taste and nutritional quality [40,41]. The resistant lines created in Nigeria by IITA were classified into three groups according to needs: industrial, human food and livestock feed [43]. They were distributed in 20 African countries but were not always satisfactory, for several reasons: circumvention of resistance, unsuitable organoleptic qualities, distrust of the population towards the new material, difficult accessibility [32,40,41]. These CMD-resistant lines were introduced in the Central African Republic between 1990 and 2000 (Inola and Kafara, unpublished). Unfortunately, the distribution of this material was probably done without taking into account their organoleptic specificities and the culinary habits of the populations and resulted in the rejection of the lines. Today, faced with the parasitic constraints associated with cassava cultivation, it appears essential to provide farmers with improved plant material that meets the demands of the population.

Our epidemiological study [34,44,45] showed that cassava mosaic disease is the main constraint of cassava in the Central African Republic with an average yield reduction estimated at more than 50%. In order to improve cassava productivity in the Central African Republic, we evaluated Central African cultivars in the context of a participatory, multi-site and multi-criteria varietal selection [46], based on resistance to CMD, yield, organoleptic qualities of tubers and their suitability with the culinary habits of the Central African populations. A participatory selection was carried out between 2010 and 2011 on two ecologically distinct sites: Damara (savannah zone) and Pissa (forest zone). It was preceded by a pre-selection of elite cultivars over a period of two years (2008/2009 and 2009/2010), from 163 cassava cultivars

tested in parallel on the two sites. At the end of the pre-selection work, 36 cassava cultivars were identified as resistant to CMD and the main cassava pests on the Pissa site and 17 in Damara where the natural pressure of the disease is higher. The organoleptic qualities of these pre-selected cultivars were evaluated by the producers. Ultimately, eleven cultivars were selected and subjected to a varietal evaluation in the open field at the Damara and Pissa sites between 2010 and 2011. These cultivars were selected on the basis of the following criteria: the incidence and severity of CMD, the incidence of the main pests, yield, and organoleptic qualities.

This study showed that CMD expression was much higher in Damara than in Pissa. A clear correlation between the evolution of whitefly populations on plants and the evolution of disease incidence and symptoms was observed, similar to other epidemic situations in Africa [41,42,47,48]. Of the eleven cultivars tested, eight were highly resistant with an average annual incidence of less than 10% at both sites, while three were partially resistant with incidences appearing to depend on the environment and disease intensity. Nevertheless, our study did not show any significant effect of the disease on yield in these partially resistant cultivars or on the local control, the latter ultimately proving highly tolerant to the disease. Our study shows an average yield potential of 13-14 t/ha of the cultivars tested in our conditions compared to the estimated average yield of 10 t/ha in CAR [30] and 7-8 t/ha on the African continent [49]. As has been observed in other countries, the local control remains the preference of farmers, but does not exclude the popularization of more resistant clones with comparable organoleptic qualities and equivalent yield potential. The question that arises is what will be the impact of highly resistant varieties on the evolution of viral populations in the more or less long term. The distribution and popularization of these clones require prior sanitation work to avoid the spread of CMGs involved in CMD in the Central African Republic through infected but asymptomatic plants. The forest area, less affected by the disease, could be chosen for the installation of multiplication plots.

3.2. Comparative Study of Traditional and Conventional Practice of Cassava Production

The majority of Central African producers do not use conventional cultivation techniques for cassava production. The objective of our study is to conduct a comparative assessment in an approach between farmers' agricultural practices and conventional practices in order to have convincing arguments to raise awareness among producers on good cultural practices. Three adapted cassava varieties (Rendre, Togo blanc and Gabon) were tested on two plots of the same size (38 m X 26 m (988 m²)) in a peasant environment, one by researchers using conventional methods and the other by producers with traditional practices. 242 cuttings per variety were planted on each experimental plot. After twelve months of experimentation, the following parameters were measured in each plot: incidence and severity of mosaic, stem height, number of tubers and yield in weight of fresh tubers. This study shows that the incidence and severity of the disease

and the height of the stems in the two cultivation systems are almost homogeneous with a non-significant difference. On the other hand, the study revealed that the plot of conventional techniques of all varieties combined gave on average 46.9 t/ha of fresh tuber against 23.15 t/ha for traditional cultivation techniques with a very significant difference. Number of tuber produced by the conventional plot was 5.7 and on the traditional plot was 4.4 with a significant difference. These results show that the variety Rerend is very susceptible to mosaic (80% incidence) and is rather a tolerant variety, as its yield potential was 50t/ha under conventional cultivation practices. The study revealed that the variety Togo Blanc developed 100% resistance on both plots and showed a high yield of 50t/ha on the conventional plot [45].

It can be concluded that conventional cultivation techniques improve the double production of cassava compared to those practiced by farmers. The phenomenon is observed in all the varieties studied with a very significant difference.

3.3. Sanitation by Thermotherapy

The main control methods used to control CMD in Africa are the use of resistant varieties and the sanitation of plant material [41,50]. In the case of sanitation of plant material, two techniques are mainly used with the sanitary selection of asymptomatic cuttings and the elimination of symptomatic plants (viral sources) within the plot [19]. The use of healthy cuttings quickly reduces the impact of the disease on the field, improves plant recovery and growth, and ultimately improves yield [19,41]. Other sanitation techniques such as thermotherapy [51,52], associated or not with tissue culture [53,54], have made it

possible to sanitize sensitive cassava cultivars [52,53,54]

Surveys conducted in 2007 and 2008 in the Central African Republic showed that 79% of cuttings used for planting new cassava plots were contaminated by CMD [38]. However, there is no doubt that the sanitary quality of cuttings represents a key element in avoiding early CMD epidemics and limiting the impact on yield [41]. Given the population's attachment to local cassava varieties and the deplorable sanitary state of cuttings, we undertook to develop and evaluate the effectiveness of thermotherapy [55]. Thermotherapy has the advantage of being a simple and inexpensive technique. Previous work has demonstrated its value in the fight against CMD [51,52]. However, it remained to test this technique in the epidemiological conditions of CAR and to develop a method for its routine use in open fields.

Contaminated cuttings of a CMD-susceptible local variety were heat-treated in hot water baths between 41°C and 51°C for 30 minutes and then evaluated in the field over a period of 12 months until harvest. Overall, although none of the treatment temperatures tested eliminated the virus and resulted in 100% CMD-free seedlings, heat-treated cassava cuttings produced plants with significantly lower incidence and severity of CMD symptoms than untreated plants. Our results show that 49°C represents the optimal treatment temperature, with no negative impact on the regenerative capacity of the cuttings, and for which we observed the highest tuber yield (4.7 kg/plant), equivalent to untreated cuttings from asymptomatic plants (4.6 kg/plant) [55] (Figure 3). Our data confirm the value of thermotherapy in maintaining a high level of performance of local cultivars under severe CMD epidemic conditions. The feasibility study of deploying this sanitation method in rural areas is currently underway.

Average yield (Kg/plant)

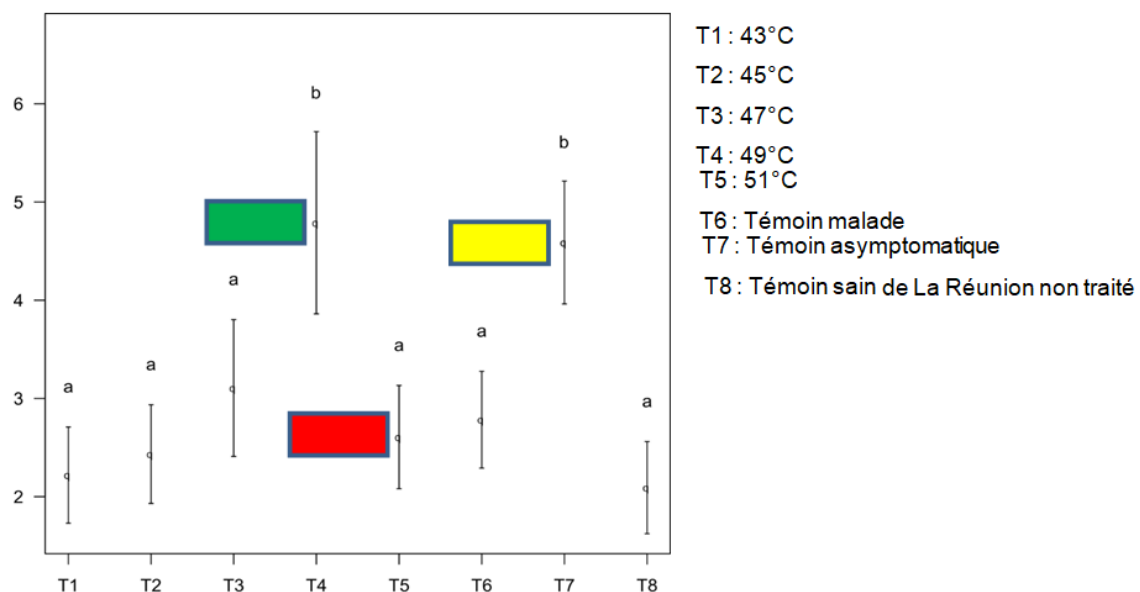


Figure 3. Yield of the variety Rerend having undergone the different treatments

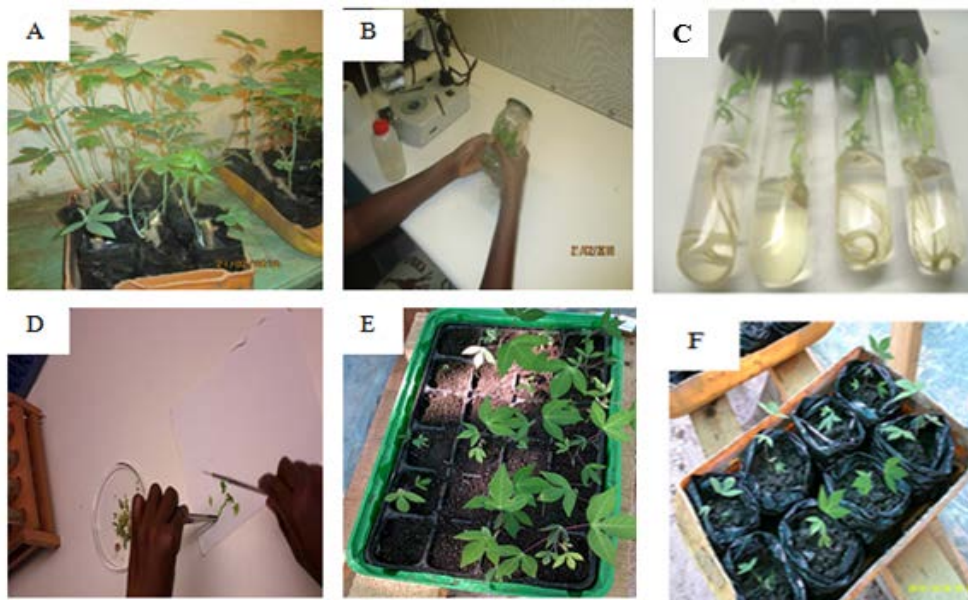
3.4. Plant Sanitation by Tissue Culture and Explants

A recent survey in the Central African Republic shows that 90% of consumers have culinary preferences for leaves showing mosaic symptoms [44]. This attitude is worrying because it could encourage the spread of the disease. This strongly justifies the need for work on cleaning up plant material.

The study was carried out at the In Vitro Culture Laboratory using thermotherapy and tissue and explant culture techniques in a specific medium. The study was conducted at the Laboratory of Biological and Agronomic Sciences for Development of the University of Bangui, Central African Republic from December 2017 to June 2018. A cassava variety named Six-Mois which is very susceptible to mosaic disease was used for this work. The cuttings used were infected with mosaic disease with high severity. They were transplanted into a heated room of 37°C to 40°C for about fifteen days. Explants and meristems were taken from the stems and apices respectively. These collected materials were treated and inoculated on appropriate culture media. Then, the in vitro

plants produced were acclimatized and the leaves were harvested to verify their phytosanitary status by the PCR technique. The emergence rate of acclimated seedlings and disease expression on microplants were evaluated. The results show that 75% of weaned seedlings recovered under acclimatization. In addition, the acclimated plants were left to grow in the greenhouse for four months and remained asymptomatic. Molecular analysis by PCR showed that begomoviruses were not detected in meristem samples unlike in stem fragment samples. The combination of thermotherapy and meristem culture techniques is an effective way to disinfect infected plant material [56] (Figure 4).

These results corroborate previous studies on thermotherapy which showed that it had this capacity to inhibit the multiplication of the virus when applied in many plants [52,54,57,58,59,60,61]. The regeneration of the explants evaluated after one week of culture in the acclimatization room showed that 48% of the meristems and 50% of the stem fragments had recovered. Infections due to microorganisms were also noted with losses of 52% and 50% respectively for the meristems and stem fragments.



A: Regeneration of cassava cuttings in a thermotherapy room.
B: Sanitation of explants under hood. C: Production of tissue culture plants.
D: In vitro plant multiplication. E: Development of vitroplants two weeks after weaning. F: Hardening of plants in vitro.

Figure 4. Steps of in vitro culture and weaning

4. Conclusion

Our study showed that CMD is the most important biotic constraint of cassava in the Central African Republic and that producers mainly use infected cuttings. This could be explained by the reduced availability of healthy material or the inability of producers to select cuttings. Without symptoms due to the lack of supervision and certification structures. Two species of begomovirus: ACMV and EACMV-UG in all surveyed areas. Phylogenetic data showed a multiple and ancient introduction of ACMV vs and a single and recent introduction of EACMV-UG. The results show a predominance of ACMV with a decreasing North-South

gradient of the presence of EACMV-UG. Co-infection in more than 50% of cases was revealed. Mixed infections are responsible for a higher severity of the disease by viral synergy. A greater expression of the disease is noted in the savannah zone. Partially resistant to very resistant cultivars with a yield potential >15 t/ha have been identified. The Togo Blanc variety (very resistant) and the Rendre variety (very susceptible) can reach 50 t/ha in optimal conditions. Considering its yield potential, the Rendre variety can be considered a tolerant variety.

Thermotherapy combined with tissue culture improves phytosanitary status. It delays the onset of the disease and reduces its impact on yield. Treatment at 49°C / 30 min seems the most suitable. The use of technical itineraries doubles production compared to traditional practices.

Continuation of epidemiological surveys throughout the country is necessary. The constitution of a national collection of cultivars and the launch of a varietal creation program seem to be promoting, as well as the establishment of a certification system for healthy plant material. Raising awareness among producers about CMD on the choice of healthy cuttings and the large-scale popularization of resistant cultivars are essential.

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