Original Research Article

Phenotypic evaluation of cassava (*Manihot esculen*ta Crantz) genotypes to cassava mosaic virus by mechanical methods of transmission

ABSTRACT:

Aims: Cassava (*Manihot esculen*ta Crantz), subsistence crop in sub-Saharan Africa was threatened by cassava mosaic virus that caused a devastated disease. This study aims to test thermotherapy as sanitation method in mechanical transmission study of cassava mosaic virus.

Methodology: Cuttings of different cultivars were treated by heating during two hours and twenty four hours at 50 °C before potting. Four mechanical techniques of virus transmission were used. Contact technique consisting to put an infected plant with a healthy plant together by one junction point and the graft technique involved the grafting of axillary buds of diseased plants to healthy plants. Latex technique consisted of passing the latex from the infected plants on the injured healthy plants several times and sap technique involved the injection of inoculum prepared from the infected leaves to the healthy plants.

Results: Four cultivars had a sprouted rate superior to 80% by two hours of heating and less

than 50% by 24 hours of heating. The bands related to ACMV or EACMV were not observed in the samples of different cultivars. Seventy five per cent (75 %) of plants inoculated by contact technique had a perfect adhesion with 65 % of plants expressing the mosaic symptoms. Six plants out of fourteen (6/14) of the plants of Hombete inoculated with latex expressed the disease symptoms and three plants out of ten (3/10) of the plants of Ornania expressed the disease symptoms. Hundred per cent (100 %) of the plants of Djadjakor inoculated by grafting expressed the disease symptoms. Any plants of Atinwewe and Adjatindaho inoculated by Sap technique did not express the disease symptoms.

Conclusion: These results suggest that heat is effective for virus elimination and grafting constitutes the mechanical transmission technique which can be used to screen cassava germplasm for virus resistance.

Keywords*: Manihot esculenta*, mechanical transmission, virus, inoculation, thermotherapy

1. INTRODUCTION

Cassava mosaic virus (CMV) is one the most important threat affecting food security in the tropics **[1]**. It is manifested by a bright yellow mosaic on the leaf, severe leaf curling and malformations of leaflets which can appear some weeks after planting of infected cuttings and persist in mature leaves [**2, 3**]. Cassava mosaic viruses is a *Geminiviridae* which is a large family of plant viruses with circular, single-stranded DNA (ssDNA) genomes packaged within geminate particles. The family of *Geminiviridae* is divided into four genus (*Begomovirus, Curtovirus*, *Mastrevirus* and *Topocuvirus*) according to their biological properties and genome constitution [**4, 5**]. Their genomes were devised by two components namely bipartite, with genome components called DNA-A and DNA-B. A successful infection of cassava plant need necessary the both components [**6**]. The whiteflies (*Bemisia tabaci*) are the vectors of mosaic disease and presented in all areas where cassava is produced. Therefore, the mechanism of transmission of the disease need to be understood in order to find a sufficient control measure against the disease. Many greenhouse studies were carried out and it was investigated that using whiteflies as vectors to transmit the causal viruses under greenhouse conditions was not efficient, very labor, costly because of the low transmission rates [**7, 8**]. It is requested to use mechanical methods to understand what's happening in the plants. Therefore, sap inoculation, grafting,

seeds and the cutting tools were reported as mechanical transmissions techniques [**9**]. Sap technique was revealed to be efficient for some pants and inefficient to other such as cassava plants [**10, 7**]. Moreover, the contact technique was not explored yet to transmit the mosaic virus. Improving the greenhouse studies of CMD is important to get a credible understanding of the etiology of the disease and to look for the best techniques of transmission in order to screen cassava genotypes to virus resistance. In general, plants used in mechanical transmission studies are free to virus because they are regenerated by *in vitro* meristems culture [**7, 8**]. However, thermotherapy is efficient method to produce clean planting material in short time with little means [**11, 12**]. Indeed, heat causes dehydration of cuttings reducing the survival rate of cuttings but at the same time acts on viruses by blocking their multiplications. Thus, the aim of this study is to (1) to evaluate the effect of heat on cuttings sprouting and virus elimination, (2) to determine the efficiency of mechanical transmission techniques of cassava mosaic disease, (3) to evaluate the influence of transmission technique and plants genotype on disease severity.

2. MATERIALS AND METHODS

2.1. Work site and plant material

These experiments were done at Central Laboratory of Biotechnology and plant breeding of Genetic and Biotechnology Department of University of Abomey-Calavi located at 6°30.150' longitude North and 1°66.104' latitude east at an altitude of 15.6 m. Cuttings from (08) different cultivars largely adopted by famers [**13**] were used.

Table 1: Monthly cycle and clones of different cultivars

N◦	Cultivars	Cycle in months	Clones of cultivar
1	Adjatindaho	10	Rb; Affodjouba; Ahossou faingnin; Atinwi; Awonlinfaignin ; Holegoumin ; Houlamè kloklo ; Okpa doundoun ;
2	Agric-rouge	6-8	Bobirin; Carder rouge; Gohoto; Faingni olowo Okè; Okoyawo doundoun
3	Agric-blanc	6-8	Agric-blanc
4	Atinwewe	12-18	Atinwewe
5	Hombete	12-18	Holly faingnin

6	Soukounon	7	Adja ; Alexifaingnin ; Kpessimon
7	Djadjakor	6-8	Djadjakor
8	Ornania	8-10	Ornania

2.2. Thermotherapy of cassava cuttings

Cuttings were collected from symptomatic cassava plants of eight cultivars preferred by the farmers and treated by heat. A part was treated with 50°C in the drying oven during two hours, the second part was treated with 50°C in the drying for twenty four hours. The cuttings were potted in the greenhouse under the growth conditions. After two weeks, the young leaves were collected to index pants to mosaic virus. Plant genomic DNA were extracted from young leaves using DNeasy^{R} Plant Mini Kit (cat. Nos. 69104 and 69106) followed using manufacture. The sequence of 906 pb length of virus DNA-A were amplified using the primers JSP1, 5'-ATGTCGAAGCGACCAGGAGAT-3' and JSP2 5'-TGTTTATTAATTGCCAATACT-3' to amplify the ACMV and JSP1, 5'-ATGTCGAAGCGACCAGGAGAT-3' and JSP3, 5'-CCTTTATTAATTTGTCACTGC-3' for EACMV [**14**]. These primers amplified 770 pb of the coat protein sequence of each virus. The volume of 12.5 µl of each PCR reaction contains 2 µl of DNA template (150 ng/µl), 0.5 µl of JSP1/JSP2 and JSP1/JSP3 (10 µM), 0.25 µl of dNTPs (10mM), 2.5 µl of PCR buffer , 1.25 µl of MgCl2 (25 mM) and 0.05 µl of Taq polymerase (5unit/µl). PCR was run under the conditions 5 min at 94 °C and 35 cycles of 45 sec at 94 °C, 45 sec at 45 °C and 55 sec at 72 °C followed by the final elongation time of 7 min at 72 °C. The PCR products are separated on 1.8% Agarose gel using ethidium bromite followed by UV revelation.

2.3. Virus Inoculation

The source of inoculum was provided by the same plant to be sure that the same virus was inoculated in the four experiments Sap, Latex, Graft and Contact techniques. The inoculation experiments were conducted in the greenhouse for 16 Weeks.

2.3.1. Sap

Five grams of virus infected leaves were grinded with 20 ml of 0.01 M phosphate buffer (pH 7.0) in a sterilized mortar. The EDTA was added as a stabilizing agent and the suspension was gently injected with syringe in the

shoot of the plants. The mortar and pestles were disinfected and treated with diethyl pyrocarbonate (DEPC) water prior using.

2.3.2. Graft

Side cleft grafting options was made in which a cleft was made tangentially in the main stem near one of the leaf nodes. The grafting was performed according to Wagaba *et al.* [**7**]. Axillary buds between 3 mm and 6 mm were obtained from the source of inoculums. Buds with the petiole and leaf attached were excised under the first five nodes of the apex from virus infected and control plants by making a triangular cut a double-edged razor blade. Excision was done at depth about 2 mm to expose the cambium layer. Axillary buds of similar size were excised from plants free to virus at the 6^{th} floor of the nodes 8 weeks after potting. The buds used as inoculum were inserted and fixed on the healthy plants. The graft portions were tightly taped with parafilm to promote union and prevent desiccation. Two weeks after the grafting, the parafilm was removed, and the success rate was evaluated. Grafted plants were maintained in the greenhouse and monitored for symptoms expression.

2.2.3. Contact

The infected plants and the healthy plants were placed in contact after wounding the both plants. The joining point was tightly taped with parafilm to avoid desiccation. Two weeks later, the parafilm was removed and both plants were maintained in contact. The success rate of contact successful was evaluated after two weeks. The plants were maintained under greenhouse and monitored for symptoms expression. The data were recorded each two weeks for two months.

2.2.4. Latex

The latex from the infected plants was used to infect the free cassava plants. Four to five wounds were made on the shoot of the free plants and the latex was pressed on the wound points of the free plants. Inoculated plants were maintained under greenhouse and the data on the symptoms expression were recorded for two months at regular period of two weeks.

2.4. Data analysis

The general linear regression was performed to determine the influence of the time of exposition of cuttings to high temperature. The test of comparison of two means was also performed to know the significant different of the infected plants rate. Data processing and analysis were performed using R software version 3.1.0

3. RESULTS

3.1. Influence of heat on cuttings sprouting and virus indexing.

The exposure time of cuttings to the heat influenced the cuttings sprouting. Four cultivars (Agric-rouge, Agric-Blanc, Hombete and Atinwewe) showed a sprouting rate greater to 80% (fig.1) at two hours of treatment. when the processing time was 24 hours, three cultivars (Adjatindaho, Ornania and Soukounon) had respective sprouting rates of 66.66 %; 78.57 % and 64.28 % (fig. 1) which were greater than those obtained at two 2 hours.

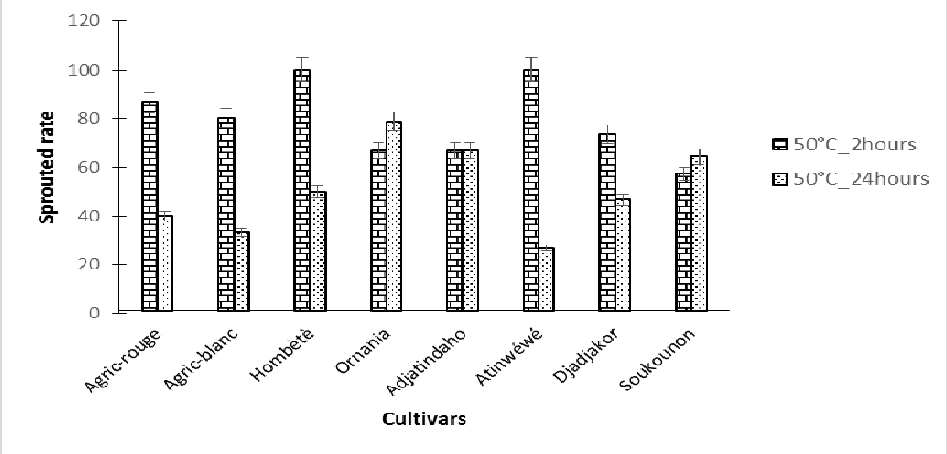

Figure 1: Variation sprouted rate by varieties in function of heat exposure time

The linear regression of the sprouting rate according to exposure time showed a significant difference (df = 14; P = 0.0069) of sprouting rate of different cultivars. The low magnitude observed between the values of 1Q (-4.9502) and 3Q (4.0649) showed that the residues had a normal distribution with a minimum of -13.8035 and a maximum of 15.2791 (fig. 2).

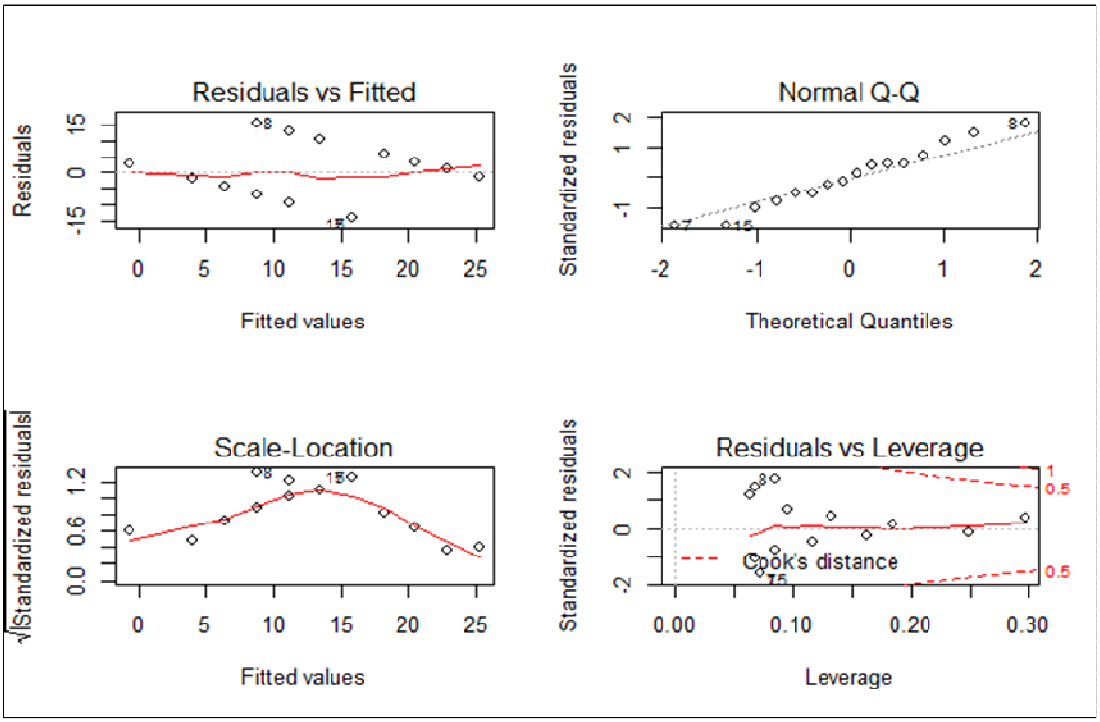

Figure 2: Regression plots of residuals

The adjusted coefficient of determination (R^2 = 0.5741) indicates that 57.41% of the traits were taken into account and determined the quality of the regression model. The plants obtained from heat cuttings treatment did not show any particular visual signs of mosaic regardless the exposure time. Furthermore, any band was not detected by PCR using a specific primers of African Cassava Mosaic Virus (ACMV) and East African Cassava Mosaic Virus (EACMV) (fig. 3).

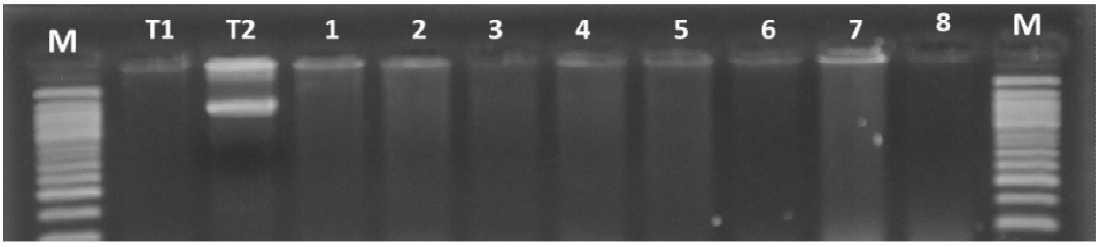

Figure 3: Results concerning PCR amplified products (770 bp ACMV and EACMV coat protein gene fragment) for samples testing plants using CMV-specific primer pair JSP1 and JSP2; JSP1 and JSP3. 1 = Adjatindaho, 2 = Agric-rouge, 3 = Agric-blanc, 4 = Atinwewe, 5 = Hombete, 6 = Soukounon, 7 = Djadjakor, 8 = Ornania.

3.2. Effectiveness of mechanical transmission techniques of cassava mosaic disease

3.2.1. Contact

Two cultivars (Soukounon and Agric-blanc) were used in this experiment. Sixteen healthy plants of each cultivar were placed into contact with infected plants. Two weeks after contact, 75% (12/16) of Soukounon plants had a perfect adhesion against 87.5% (14/16) of Agric-blanc (table 2). A total of 68.75% (11/16) of Soukounon plants exhibited mosaic symptoms with the severity average of 2.7 after 8 weeks versus 75% (12/16) of Agric-blanc with severity average of 2.4 (table 2). There was not a significant difference (P = 0.912) between the infection rate of the plants. Furthermore, there was not dependence (P = 0.823) between cultivars, infestation rate and the mean of severity (table 2).

3.2.2. Graft

Two buds from infected plants at scale 4 were grafted to each cassava plants of the cultivars Djadjakor and Agric-rouge. A total of 32 buds were grafted to the plants shoot of Djadjakor with success rate of 68.75% (22/32) after two weeks versus 75% (24/32) success for the cultivar Agric-rouge (table 2). There was not a significant difference in the grafting success and the successful rate did not depend to the type of cultivar (P = 0.578). Eight weeks after grafting, 100% (16/16) plants of Djadjakor showed mosaic symptoms with severity average of 3.1 versus 00% for Agric-rouge (table 2). There was a dependence (P = 0.042) between the cultivars, the infestation rate and the mean of severity.

3.2.3. Sap

Twelve plants of Atinwewe cultivar and fourteen plants of Adjatindaho cultivar inoculated with 2 ml of inoculum prepared from leaves of infected plants at scale 4 did not present disease symptoms after eight weeks after inoculation (table 2).

3.2.4. Latex

Fourteen plants of Ornania cultivar inoculated with latex showed an infestation rate of 42.85% (6/14) eight weeks after inoculation against ten plants of Hombete cultivar with an infestation rate of 21.42% (3/10). The plants of Ornania cultivar had an average severity of 1.6 against 1.3 of the plants of Hombete cultivar (table 2). The test of proportion and the dependence test showed that there was not a significant difference (P > 0.05) between infestation rates, cultivars and mean of severity.

3.3. Influence of transmission technique and genotypes on disease severity

The severity mean of the disease by the contact technique was 2.7 for the plants of Soukounon versus 2.4 for the plants of Agric-blanc. The highest severity mean (3.1) was obtained by grafting technique than all other inoculation techniques (table 2). Analysis of variance showed that there was a significant difference ($P < 0.05$) between the transmission techniques and disease severity. Inoculation by latex was positive with a severity mean of 1.6 in Ornania cultivar and 1.3 in Hombete cultivar. When the cultivars were considered in pairs by inoculation technique, Soukounon and Agric-blanc, Djadjakor, Ornania and Hombete cultivars showed a susceptibility to the disease, whereas the Agric-rouge cultivar showed a tolerance to the disease. Moreover, the cultivars Atinwewe and Adjatindaho which were inoculated by sap technique did not show the disease symptoms.

Table 2: Transmission of CMD by different mechanical inoculation techniques to cassava plants

Techniques	Cultivars	Successful[++] /Number of performed (%)	Plants presenting CMD-symptoms/total (%)	Inoculated plants	Mean** of severity	Scale** of Inoculum
	Soukounon	12/16 (75)	11/16 (68.75)	16	2.7	4
Contact						
	Agrik blanc	14/16 (87.5)	12/16 (75)	16	2.4	4
	Djadjakor	22/32 (68.75)	16/16 (100)	16	3.1	4
Graft						
	Agric-rouge	24/32 (75)	00	16	1	4
	Atinwewe	---	00	12	1	4
Sap						
	Adjatindaho	---	00	14	1	4
	Ornania	---	6/14 (42.85)	14	1.6	4
Latex						
	Hombete	---	3/10 (21.42)	10	1.3	4

[++] Successful plants were those had parfait union with the infected plants for contact technique and created a jointing point between the infected and clean plants. For the graft technique, the successful were those maintained the green color of the grafted buds.
** Severity scale of IITA, (1990) [**15**] was used to choice the inoculated plants and the severity were calculated by the following formula: $S = \Sigma$ Severity of plants presented the symptoms / Total number of plant presented the symptoms.

The disease infestation rate varied with the time. By the contact technique, more than 31.25% of the plants of Soukounon cultivar presented the mosaic symptoms versus 43.75% plants of Agric-blanc cultivar (fig. 4). Four

weeks after inoculation, the infestation rate in Agric-blanc was increased from 56.25% to 62.5%. After six weeks, the infection rate increased to 68.75% remained stable until the eighth week in Soukounon cultivar against 75% of infestation rate in Agric-blanc until the eighth week.

The grafted plants of Djadjakor showed 100% of mosaic symptoms after four weeks, compared with any of Agric-rouge cultivar during the eight weeks. No plants showed symptoms by the Sap technique for the two cultivars tested. A low level of infestation (21.42%) of the plants of Hombete cultivar was observed versus 42.85% for the cultivar Ornania after the fourth week to the eighth week by latex inoculation (fig.4). The best infestation rate was obtained by grafting (100%) against 75% obtained by the contact technique. A lower rate of transmission of the disease was observed by the latex inoculation technique and 0% by the sap inoculation technique.

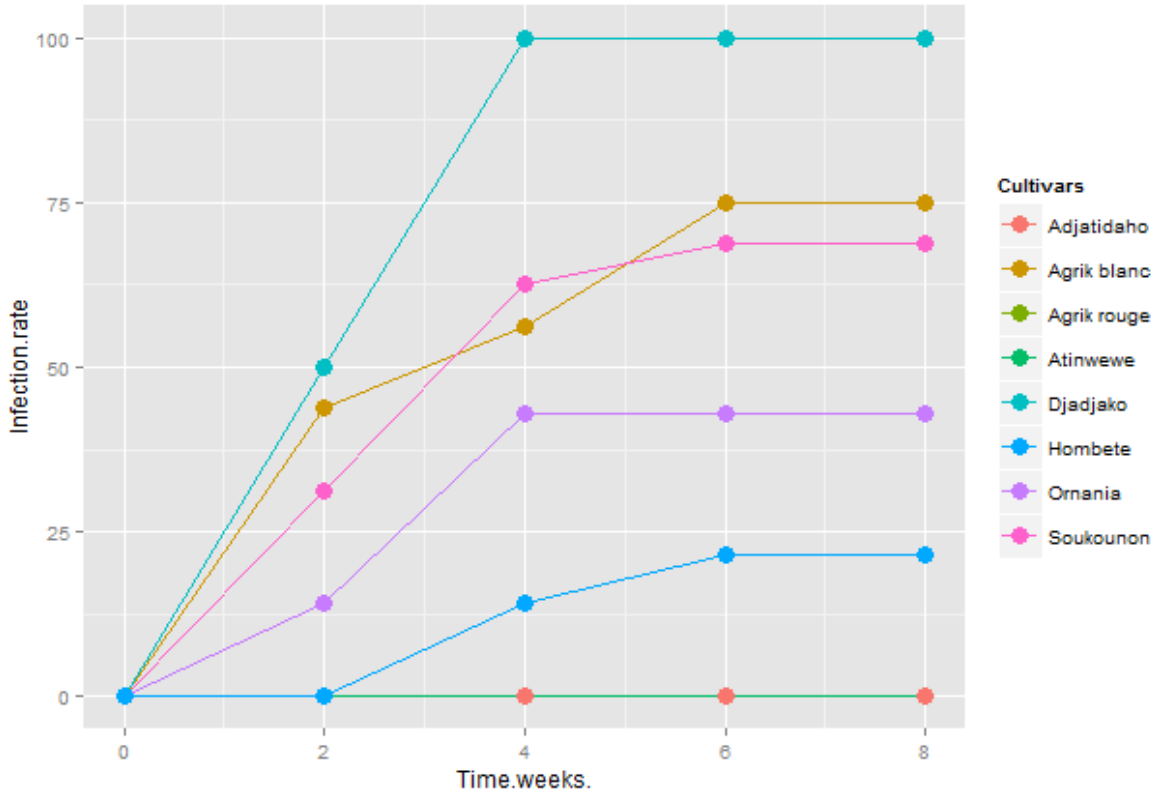

Figure 4: Evolution of infestation rates in cassava plants

4. DISCUSSION

Pre-treatment of cassava cuttings by heating prior to use was very effective means of controlling viral diseases [**16, 17**]. However, the exposure time of cuttings to the heat was a major factor affecting the sprouting of

cuttings. Some cultivars such as Soukounon, Ornania and Adjatindaho gave the best sprouting rates after 24 hours of heat at 50°C. The genotype of plants and the exposure time of cuttings to high temperatures were very influential factors in the sprouted cuttings. The tolerance of cuttings to heating of different genotypes varied from one cultivar to another. Kaiser and Louie [**18**] also showed this variation of tolerance to heating in cassava plants. The heat plants indexed by PCR did not reveal the presence of the mosaic virus, which explained the health status of the plants that were inoculated. It would therefore be advisable to associate thermotherapy with mechanical methods of virus inoculation to ensure the health status of the plants. It was not evident that a cassava plant in its natural environment was certified to be 100% free to virus even if it was derived from the meristem culture, it could be recontaminated after its introduction in real environment. The pre-treatment of the cuttings by heating before using for mechanical transmission of viruses constituted an effective means to maintain the sanitary condition of the plants. These results confirm those of Cacai *et al.*, **[11**] who demonstrated the effectiveness of thermotherapy in the elimination of cassava plant viruses. Moreover, some of explored transmission techniques revealed a systematic morphological efficiency on cassava plants. Thus, transmission of the disease by contact technique was an explored way of transmission that resulted a high transmission of the disease. Up to 75% of the plants tested expressed the disease through the contact technique. It was an effective means of disease transmission. Two cultivars on which this technique was applied, revealed a sensitivity to the mosaic virus. Inoculation by sap did not reveal a particular visual signs of the mosaic on the plants. Molecular analysis were therefore necessary to check the presence of disease and to determine the effectiveness of the sap technique. This technique was ineffective for viral cassava transmission **[7**]. Therefore, the infectivity of sap technique would be linked to the method of preparation of the inoculum. The absence of any particular signs on the plants may also be due to the resistance of the cultivars to mosaic. These cultivars had to be inoculated also by grafting for a better evaluation of the technique. The inoculation by buds axillary grafting to the plants revealed an efficiency in the transmission of the disease. Hundred per cent (100%) of the plants of Djadjakor revealed a sensitivity to mosaic but at the same time none of the plants of Agric-rouge did not express the disease. These results, were in agreement with those obtained by Wagaba *et al.,* **[7]**, which demonstrated the effectiveness of the grafting technique in the transmission of cassava brown streak virus and cassava mosaic virus. The severity observed on the plants explained the effectiveness of the techniques and the susceptibility of the cultivars to the mosaic

viruses. The time of appearance of the first symptoms of mosaic was between the first six weeks after inoculation. Plants of the same cultivar did not show symptoms at the same time. Which means that each plant had a system of self-defense against the disease before being weakened especially in sensitive cultivars. This was also related to environments such as the availability of nutrients in the substrate of each plant. In this work, contact inoculation and latex techniques were also mechanical transmission methods that can be used for greenhouse resistant screening.

5. CONCLUSION

The pre-treatment of cuttings by heating before using in mechanical transmission of virus was request for ensuring the health of the tested plants. It should be noted that contact and latex inoculation techniques were also viral transmission techniques and can be used for the screening of resistant cultivars. However, the grafting technique was always the best. It was also apparent from this work that Djadjakor, Agric-blanc, Soukounon and Ornania were the cultivars sensitive to mosaic. Agric-rouge was the only cultivar that has shown resistance against mosaic. The using of grafting or contact techniques on the cultivars Atinwewe, Adjatindaho and Hombete was requested to draw a reliable conclusion on their sensitivity to the mosaic. Sensitive cultivars will be subject to breeding program against cassava mosaic disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Okogbenin E, Egesi CN, Olasanmi B, Ogundapo O, Kahya S, Hurtado P, Marin J, Akinbo O, Mba C, Gomez H, de Vicente C, Baiyeri S, Uguru M, Ewa F, Fregene M. Molecular Marker Analysis and Validation of Resistance to Cassava Mosaic Disease In Elite Cassava Genotypes in Nigeria. *Crop Sci.* 2012: 52:2576 - 2586
2. Storey H. Virus diseases of east African plants: VI - a progress report on studies of the diseases of cassava. *Afri. Agri. J*. 1936: 2:34-39.
3. Legg JP, Jeremiah SC, Obiero HM, Maruthi MN, Ndyetabula I, Okao-Okuja G, Bouwmeester H, Bigirimana, S, Tata-Hangy W, Gashaka G, Mkamilo G, Alicai T, Kumar PL. Comparing the regional epidemiology of cassava mosaic and cassava brown streak virus pandemics in Africa. *Virus Res.* 2011: 159:161–170
4. Fauquet CM, Stanley J. Geminivirus classification and nomenclature: progress and problems. *Ann Appl Biol*, 2003:142:165-189.
5. Stanley J, Bisaro DM, Briddon RW, Brown JK, Fauquet CM, Harrison BD, Rybicki EP, Stenger DC. Geminiviridae. In Virus Taxonomy, VIIIth Report of the ICTV 8 th edition. Edited by: Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U. and Ball, L.A. London, Elsevier/Academic Press. 2004: 301-326.
6. De Bruyn A, Villemot J, Lefeuvre P, Villar E, Hoareau M, Harimalala M, Abdoul-Karime A L, Abdou-Chakour C, Reynaud B, Harkins GW, Varsani A, Martin DP, Lett JM. East African cassava mosaic-like viruses from Africa to Indian Ocean islands: Molecular diversity, evolutionary history and geographical dissemination of a bipartite begomovirus. *Evol. Biol*. 2012:12(228): 1471-2148
7. Wagaba H, Beyene G, Trembley C, Alicai T, Fauquet CM, Taylor NJ. Efficient transmission of Cassava brown streak disease viral pathogens by chip bud grafting. *BMC Res. Notes* 2013: 6:516
8. Rwegasira GM, Chrissie MER. Efficiency of non-vector methods of cassava brown streak virus transmission to susceptible cassava plants. *Afri. J. food Agri. Nutrn. and Dev.* 2015: 15(4): 10335-10351
9. Prasangika HMS, Salim N, Razak MM. Evaluation of susceptibility of cassava germplasm to cassava mosaic disease *J. Natn. Sci.* 2008:*36 (1): 99-102*

10. Ogwok E, Patil BL, Alicai T, Fauquet CM. Transmission studies with Cassava brown streak Uganda virus (Potyviridae: Ipomovirus) and its interaction with abiotic and biotic factors in Nicotiana benthamiana. *J. Virol. Meth.* 2010: 169: 296 –304.
11. Cacaï GHT, Adoukonou-Sagbadja H, Kumulugui BS, Ondo OP, Houngue J, Ahanhanzo C. Eradication of cassava (*Manihot esculenta*) mosaic symptoms through thermotherapy and meristem cultured *in vitro. Int. J. Agr. Pl. Pro.* 2013: 4(S): 3697-3701.
12. Houngue JA, Cacaï GHT, Zandjanakou-Tachin M, Azalou-Tingbe NC, Ahanhanzo C. Impact of thermotherapy and chlorothalonil on plantlets production of some genotypes of cassava (*Manihot Esculenta* Crantz) produce in Benin. *Int. J. Sci & Tech Res.* 2017: 6(1): 170-174
13. Agre AP, Dansi A, Rabbi IY, Battachargee R, Dansi M, Melaku G, Augusto B, Sanni A, Akouegninou A, Akpagana K. Agromorphological Characterization of Elite Cassava (*Manihot esculenta* Crantz) Cultivars Collected in Benin. *Int. J. Curr. Res. Biosci. Plant Biol.* 2015, 2(2): 1-14
14. Pita JS, Fondong VN, Sangare A, Otim-Nape GW, Ogwal S, Fauquet CM. Recombination, pseudo-recombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *J Gener Virol.* 2001: 82:655-665.
15. IITA. Annual Reports, Ibadan, Nigeria. 2016. Accessed in 25 December 2017. Available on http://www.iita.org/wp-content/uploads/2017/08/2016-annual-reportLOWRES.pdf
16. Wasswa P, Alicai T, Mukasa SB. Optimization of *in vitro* techniques for Cassava brown streak virus elimination from infected cassava clones. *Afr. Crop Sci. J.* 2010: 18: 235 – 241.
17. Zinga I, Chiroleu F, Kamba E, Giraud-Carrier C, Harimalala M, Komba EK, Yandia S, Semballa S, Reynaud B, Dintinger J, Lefeuvre P and Lett JM. Field Evaluation of the Effectiveness of Thermotherapy against Cassava Mosaic Disease in Central African Republic. *Ameri. J. Exp. Agri.* 2014, 4:1232-1241
18. Kaiser WJ, and Loui R. Heat therapy of cassava infected with African cassava mosaic disease. *Plant Dis.* 1982: 66:475-477

DEFINITIONS, ACRONYMS, ABBREVIATIONS

ACMV African cassava mosaic virus, EACMV East Africa cassava mosaic virus, CMD, cassava mosaic disease, DNA Desoxyribose nucleic acid, PCR, Polymerase chain reaction.