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Effect of Thermotherapy in the Elimination of Viruses on Four (4) Mosaic Diseased Cassava Cultivars

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Abstract
In Ghana, cassava is the most favoured among all the root crops and indeed all food crops by consumers. Its per capita consumption (PCC) index is as high as 148kg/year followed by that of plantain (83kg/year: Annor-Frempong, 1991). Cassava mosaic disease (CMD) is considered the most important biotic constraint as it greatly reduces yields (Calvert and Thresh, 2002) significantly. Application of meristem culture combined with thermotherapy is reported to increase the survival rate of *in vitro* explants (Manganaris *et al*., 2003). It is against this background that this study was undertaken to ascertain the effectiveness of thermotherapy to generate large numbers of virus-free plants. The study assessed the effect of thermotherapy in the regeneration rate and eradication of cassava mosaic virus disease in four (4) local cassava cultivars. The cuttings of the four cassava accessions showed varying degrees of foliar symptom severity typical of the cassava mosaic disease. Shoots that were subjected to pre-culture thermotherapy at 35-37°C appeared to be disease-free of the cassava mosaic symptoms after three to four weeks of treatment. Thermotherapy treated meristem showed better survival rate in larger explant size than smaller ones in the cassava accessions studied indicating that virus-free planting materials could be produced using thermotherapy.
Keywords: Thermotherapy, cassava mosaic, meristem tip, tissue culture.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial semi-woody shrub with edible starchy roots that is widely grown in the tropical regions of the world. Cassava roots give a carbohydrate production per hectare of 40% higher than that of rice and 25% more than maize (Tonukari, 2004), making it the cheapest source of calories for both human nutrition and animal feeding. The fresh leaves provide a valuable source of protein, vitamins and minerals in most cassava producing areas (Latham, 1979).

In Ghana, cassava is the most favoured among all the root crops and indeed all food crops by consumers. This is reflected by the per capita consumption (PCC) index. The PCC for cassava is high (148kg/year) followed by that of plantain (83kg/year: Annor-Frempong, 1991). It is widely consumed in various forms in many parts of Ghana, therefore playing a role as the leading food security base.

Cassava mosaic disease (CMD) is considered the most important biotic constraint and it greatly reduces yields (Calvert and Thresh, 2002). The disease is transmitted by the whitefly, *Bemisia tabaci* Genn., which is further disseminated through the stem cuttings used routinely for propagation (Pita *et al.,* 2001). The most visible symptom of the disease is the expression of the characteristic leaf chlorotic blotches, distortion and a reduction of the leaf area, thereby adversely affecting photosynthetic efficiency and the overall root yield and quality of leaves as vegetables (Almazan and Theberge, 1989).

Resistance of cassava genotypes to ACMV has not yet been reported (Jennings, 1994). However, in genotypes that show resistance, the virus seems to occur mainly towards the base of shoots so that uninfected cuttings for use as planting material could be obtained from the shoot tips (Cours-Darnes, 1968; Jennings, 1994). According to Thresh *et al.* (1994), spread of the disease within and between plants of resistant varieties is relatively slow.

Unlike bacterial and fungal diseases, viral diseases have no effective chemical control on infected plants, thus, causing heavy yield losses in most vegetative propagated plants. The supply of virus-free planting materials is therefore pivotal to sustainable crop production. Efficient methods developed for production of healthy vegetative propagated crops has been established (Roca, 1984). Three methods currently in use are thermotherapy, tissue culture and chemotherapy (Kassianof, 1992).

It was found that CMV particles could invade meristems (Walkey and Web, 1968) and that a gradient of increasing virus concentration from the dome to the successive leaf primordia exists. Therefore, to improve virus elimination using meristem culture, the use of thermotherapy as antiviral treatment of donor explants is necessary. According to Dodds *et al.,* 1989; Griffiths *et al.,* 1990 heat treatment complement well in those plants in which viruses cannot be eradicated just by meristem tip culture alone.

Propagative materials by this method can serve as guideline for the safe movement of cassava planting materials as outlined by Frison (1994), since the production of virus-free plants is prerequisite for the international exchange of clonal material to avoid risks of introducing diseases to uninfected areas.

It is against this background that this study investigated the effect of thermotherapy on the elimination and regeneration of clean plants of four (4) local disease cassava cultivars. Specifically, the study assessed the effect of heat therapy on the survival and growth of the four cultivars.

MATERIALS AND METHODS

Source and establishment of plant materials

Four cassava accessions namely "Amakuma", "Esi-Abaya", "Afisiafi" and "UCC-Bankye" showing various degrees of severe CMD symptoms in cassava fields were collected from the CSIR-Crops Research Institute (CRI) at Fumesua, near Kumasi. Ten (10) dormant woody stem cuttings (5-7 nodes long) taken from diseased mother plants, were randomly selected in two batches of five cuttings from each of the four accessions. Batch-1 cuttings were planted in buckets and subjected to thermotherapy, while batch-2 cuttings were also planted in buckets and placed outside the insectary of the Crop Science Department of the KNUST, to serve as untreated plants. Buckets were filled with sterile topsoil. The buckets of soil containing the cuttings were watered regularly to maintain adequate water regime for the spouting and growth of plants.

Establishment of heat-treated (Thermotherapy) plants.

A batch of five (5) cuttings each selected from the four diseased cassava accessions were subjected to a heat treatment at 37±2°C for four to six weeks. The five cuttings (representing 5 replications) per cultivar were planted in buckets filled with sterile topsoil and placed in a heat chamber to sprout and establish shoots (Figure 2). The heat chamber was a 0.9m^3 wooden box fitted with side glasses (Figure 3), and 100watts incandescent bulbs to generate the heat. Two thermometers were placed in the box to monitor the heat generated. Each bucket was placed 15cm between rows apart in the heat chamber. The nursed cuttings in the buckets were periodically watered to prevent desiccation of the growing plants. The temperature was kept within the range (35-39°C) until the sprouted shoots were grown into seemingly hard wood. Different node cuttings were excised and cultured in an insectary to grow into matured plants.

Figure 1. Five cassava cuttings each of the four cultivars nursed in buckets placed in a heat chamber to sprout.

Figure 2. Sprouted cuttings of four cultivars of cassava growing in the heat chamber.

Culture of Cassava Ministem cuttings

Three (3) node cuttings were nursed in buckets in the insectary at different sizes (D1+1 measuring 0.2-1.0cm with one pair of leaf primordium, D2+2 measuring 1.0-2.0cm with 2 leaf primordia and D3+3 measuring 2.0-3.0cm with 3 leaf primordia) were excised from the shoot-tips with a scalpel blades. They were then transferred into sterilised soil medium contained in the buckets. Each explant was embedded with a node in the soil medium in the insect-free chamber and left until new shoots were grown into matured stems.

Virus detection by visual inspection

Pre and Post-cultured shoots derived from cuttings of the four diseased cultivars with differential disease symptoms of African cassava mosaic virus (ACMV) were evaluated for the presence and severity of symptoms by physical observation of plant leaves.

A month after planting the cuttings, initial disease severity symptoms of the four accessions were scored by visual inspection of sprouted shoots of the heat treated and untreated nursed cuttings. After plantlets were established from the insectary explants, disease score was again assessed to determine the status of derived plantlets. Scored data was subjected the square root transformation and analysed. The severity of disease symptoms was scored using a range of 1-5 where

1= no symptoms
2= a mild chlorotic pattern over the entire leaf while the latter appears green and healthy
3= a moderate mosaic pattern throughout the leaf narrowing and distortion in the lower one-third of the leaflets
4= severe mosaic distortion in two-third of the leaflets and general reduction in leaf size.
5= severe mosaic and distortion in the entire leaf.

Index of severity of symptoms based on all plants (ISS_{AP}), and index of severity of symptoms based only on diseased plants (ISS_{DP}) were used. The data was subjected to angular transformation (Hahn et al., 1989; Njock, 1994) and analysed:

$$\text{Incidence (\%)} = (X/Y)\ 100$$
$$ISS_{AP} = (a+2b+3c+4d+5e)/ (a+b+c+d+e)$$
$$ISS_{DP} = (2b+3c+4d+5e)/ (b+c+d+e)$$

where X is the number of diseased plants and Y the total number of plants scored; a, b, c, d, and e are the number of plants scored under the respective severity classes 1, 2, 3, 4 and 5.

Shoots derived from cuttings of the four diseased cultivars with differential disease symptoms of African cassava mosaic virus (ACMV) were assessed for index of severity of symptoms (ISS) to determine their status by scoring young resultant plants in a potted experiment. Data was analysed after square root transformation and are in parenthesis (Table 5).

Table 2. List of Positive controls used

Well no.	Description
1	"UCC-Bankye" leaf DNA extracts taken after heat treatment only
2	"Esi-Abaya" leaf DNA extracts taken after heat treatment only
3	"Afisiafi" leaf DNA extracts taken after heat treatment only
4	"UCC-Bankye" leaf DNA extracts taken from the field
5	"Esi-Abaya" leaf DNA extracts taken from the field
6	"Amakuma" leaf DNA extracts from meristem-tip culture only.
7	"UCC-Bankye" leaf DNA extracts from meristem-tip culture only
8	"Esi-Abaya" leaf DNA extracts from meristem-tip culture only
9	"Afisiafi" leaf DNA extracts from meristem-tip culture only
10	"Afisiafi" leaf DNA extracts submitted to thermotherapy and meristem-tip culture

RESULTS

The disease scores on the shoots of the four accessions with differential disease symptoms of African cassava mosaic virus (ACMV) are shown in Table 3. There were significant differences ($p \leq 0.05$) between cassava cultivars prior to thermotherapy, with "Amakuma" and "Esi-Abaya" showing 100% disease incidence in the pre-cultured shoots, while "Afisiafi" and "UCC-Bankye" showed 85 and 80% incidence, respectively.

Table 3. Scores of African Cassava Mosaic Virus disease status on shoots before thermotherapy on the four (4) cassava accessions

Cultivar	ISS_{AP}	ISS_{DP}	Incidence (%)
"Afisiafi" (AFC)	2.15 (1.62)	2.30 (1.67)	85 (9.11)
"Amakuma" (AMC)	2.80 (1.81)	2.80 (1.81)	100 (10.02)
"UCC-Bankye" (UCCC)	2.00 (1.58)	2.08 (1.60)	80 (8.94)
"Esi-Abaya" (EAC)	3.30 (1.95)	3.30 (1.95)	100 (10.02)
Grand mean	(1.74)	(1.76)	(81.1)
Lsd	(0.18)	(0.16)	(21.68)
Cv (%)	(6.5)	(5.8)	(9.5)

ISS_{AP} = Index of severity of symptoms on all plants
ISS_{DP} = Index of severity of symptoms on diseased plants only
Parenthesis = Transformed data used for analysis

Effects of thermotherapy and meristem size on plant regeneration of cassava accessions

The three (3) different explant sizes excised from the four cassava accessions showed sharp differences when shoots from which they derived were heat-treated (Table 5). The large (2.0-3.0cm) explant size recorded the highest (58.3%) plant regeneration, obtaining 7 plants out of 12 explants cultured. The least (7.7%) plant regeneration was obtained by the small (0.2-1.0mm) explant sizes cultured, recording 1 plant out of 13 explants cultured. Three (3) of the accessions ("Afisiafi", "Amakuma" and "UCC-Bankye") produced 66.7% plant regeneration with the large explant size, but 0% regeneration with the small explants cultured. "Esi-Abaya" produced 33.3% plant regeneration from the large explants cultured. "Esi-Abaya" was the only cassava accession that regenerated from the small explant size cultured. These grew into whole plants with well-differentiated shoot and root systems.

Table 4. Effect of thermotherapy and size of meristem on plant regeneration from four (4) cassava accessions

Variety	D1+1(0.2-1.0mm)				D2+2(1.0-2.0mm)				D3+3(2.0-3.0mm)			
	Treated		Control		Treated		Control		Treated		Control	
	Inoc.	Surv	Inoc.	Surv	Inoc.	Surv	Inoc.	Surv.	Inoc.	Surv	Inoc.	Surv
"Afisiafi"	4	0	4	1	3	2	4	0	3	2	3	0
"Amakuma"	3	0	4	1	3	1	4	1	3	2	4	2
"UCC-Bankye"	3	0	4	2	3	1	3	0	3	2	3	1
"Esi-Abaya"	3	1	4	1	3	0	3	0	3	1	3	1
Total	13	1	16	5	12	4	14	1	12	7	13	4

****Inoc*** = Number of explants cultured
****Surv*** = Number of plants regenerated

The plant regeneration was high (31.3%) obtaining 5 plants out of 16 explants cultured with the small (0.2-1.0cm) explant size cultured when their donor shoots were not heat-treated (control). The large (2.0-3.0cm) explant size was slightly close with 30.8% (thus, 4 plants out of 13 explants cultured) plant regeneration. The medium (1.0 -2.0cm) explant size recorded the least (7.1%) plant regeneration. "Afisiafi" recorded 25% regeneration, obtaining 1 plant out of 4 explants from the small explant-size cultured, but obtained 0% both from the medium and the large explant sizes cultured. "Amakuma", "UCC-Bankye" and "Esi-Abaya" recorded 25, 50 and 25% plant regeneration from the small explant sizes and 50, 33.3% with the large explant sizes respectively.

Incidence of African cassava mosaic geminivirus in shoots regenerated from *in vitro* thermotherapy

Scores of the presence and severity of symptoms on regenerated plantlets derived from the three explant sizes (0.2-1.0mm, 1.0-2.0mm and 2.0-3.0mm) recorded 0% incidence of the disease after mother plants were heat-treated and cultured (Table 6). The derived plantlets from the large and medium explant size scored 1 on the scale of Index of severity of symptoms (ISS) on both diseased and all plants assessed for the four cassava accession regenerated. The small explant size for "Afisiafi", "Amakuma" and "UCC-Bankye" scored 0 for both ISS_{AP} and ISS_{DP} because there was no plant regenerated to assess for disease symptoms. "Esi-Abaya" scored 1 for index of disease symptoms on the regenerated plants from small and the large explant sizes.

Similarly, derived plantlets from untreated shoots scored 1 on the scale of both ISS_{AP} and ISS_{DP} for regenerated plants of the small (0.2-1.0mm) explant sizes cultured from "Afisiafi", "Amakuma", "UCC-Bankye" and "Esi-Abaya". However, the ISS_{AP} and ISS_{DP} score of 2 for "Amakuma", and "UCC-Bankye" was observed on the regenerated plants of the large explant size cultured, with 33.3% incidence on the two accessions (Table 6), respectively.

Table 5. Scores of ACMV disease symptoms on derived plantlets from different sizes of heat-treated and meristem culture explants.

Explant size	Parameter	"Afisiafi"		"Amakuma"		"UCC-Bankye"		"Esi-Abaya"	
		H	**C**	**H**	**C**	**H**	**C**	**H**	**C**
D1+1 (Small)	ISS_{AP}	0	1	0	1	0	1	1	1
	ISS_{DP}	0	1	0	1	0	1	1	1
	Incidence	0	0	0	0	0	0	0	0
D2+2 (medium)	ISS_{AP}	1	0	1	1	1	0	0	0
	ISS_{DP}	1	0	1	1	1	0	0	0
	Incidence	0	0	0	0	0	0	0	0
D3+3 (large)	ISS_{AP}	1	0	1	2	1	2	1	1
	ISS_{DP}	1	0	1	2	1	2	1	1
	Incidence	0	0	0	33.3	0	33.3	0	0

****H***= Thermotherapy
****C***= Control treatment

DISCUSSIONS

The cuttings of the four cassava accessions showed varying degrees of foliar symptom severity typical of the cassava mosaic disease. The disease was characterized by leaf chlorotic blotches, distortion and a reduction of the leaf area, depending on the degree of severity. "Esi-Abaya" (ISS_{DP}= 3.30) and "Amakuma" (ISS_{DP}= 2.80) were the most severely affected by the disease among the four cultivars studied recording a 100% incidence, respectively. Unlike "Esi-Abaya" and "Amakuma", "UCC-Bankye" and "Afisiafi" exhibited mosaic symptoms with reduced chlorotic pattern over the entire leaf while the leaf appeared green as these two accessions recorded the ISS_{DP} of 2.0 and 2.30, with disease incidence of 80 and 85% respectively. This result agrees with what was observed by Hahn *et. al.*, (1980); Jennings, (1994); Thresh *et. al.*, (1994) which stated that differences in disease severity symptoms expressed depended on the level of resistance of the cultivar.

Shoots that were subjected to pre-culture thermotherapy alone at 35-37°C appeared to be disease-free of the cassava mosaic symptoms (Plates 4 and 5) when the leaves were assessed for incidence and severity of symptoms, after three to four weeks of treatment. Similar effect of thermotherapy on disease suppression has been reported to produce clean cassava planting materials (Garcia *et al*., 1993; Zok, 1993; Delgado and Rojas, 1993), suggesting that thermotherapy alone might be sufficient to produce virus-free plants. This is because of the ability of heat to inhibit the multiplication of the virus during its application in many plants, including fruit trees (Manganaris *et al.,* 2003).

Response of explant size to thermotherapy and *in vitro* culture

Among the 12 explants each of the small, medium and large sizes cultured from thermotherapy shoots, only the large explant (D3+3) size recorded 7 survival, representing 58.3%. This was followed closely by the medium sized (D2+2) explants cultured recording 4 survival representing 33.3%. The lowest survival of explants was the smallest (D1+1) size excised and cultured, obtaining 1 plantlet out of 13 explants cultured, representing 7.7%. This result shows that survival rate was directly proportional to the size of meristem. This observation is consistent with findings of Walkey and Web, (1968) who proposed that the smaller the size of the meristem explant, the lower the viability as well as the regeneration potential. Adejare and Coutts (1981) also observed that the survival rate of meristem tips was highly correlated with size of cultured tip when cassava explants were obtained from shoots that were subjected to thermotherapy, thus the larger the tip the higher the survival rate. However, among the explant sizes excised and cultured from untreated shoots, the small sized (D1+1) explants recorded the highest (5) number of regenerated plantlets, representing 31.3% survival, close to the number of regenerated plantlets (4) from the large (D3+3) size of explants cultured (30.8%), regenerating into virus-free plantlets. The results presented in this study show that the survival rate of the explants is high even though they were not heat-treated, especially in the large explant sizes that were more likely to transmit disease pathogens and the smallest meristem that may be having reduced chances of regeneration into plantlets (Appiano and Pennazio, 1972). However, this observation is contrary to the findings reported by Hu and Wang (1983) and Kartha (1986). With this result, smaller explants survived well without heat treatment to mother plant.

Among the four cultivars studied under the three categories of explant sizes, "Afisiafi" recorded 2 plantlets each for the medium (D2+2) and the large (D3+3) explant sizes cultured after shoots were submitted to thermotherapy, representing 66.7% survival respectively. Similarly, "Amakuma" and "UCC-Bankye" recorded 2 (66.7%) regenerated plantlets each from the large (D3+3) explant size, but recorded 1 plantlet (33.3%) each from the medium (D2+2) explant size cultured and 0 from the small (D1+1) size. "Esi-Abaya" was the poor performing variety in terms of number of survival, though it recorded one survival each in both the smallest and largest explant sizes. "Afisiafi" which recorded highest number of survival of treated explants performed badly when the shoots were not treated, trailing behind "Amakuma", "UCC-Bankye" and "Esi-Abaya", which yielded higher numbers of explant survival.

CONCLUSION

Thermotherapy treated meristem showed a better regeneration potential in larger explant size than smaller ones in the cassava accessions studied. There were varietal differences in the response to both thermotherapy and meristem tip culture. Donor explants subjected to thermotherapy showed no amplification of the virus in the cassava genome while non-treated explants showed amplification band. Thus, the results presented in this study clearly showed that virus-free planting materials could be produced using thermotherapy-meristem culture for farmers to increase their yield.

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References:

Adejare, G. O. and Coutts, R. H. A. 1981. Eradication of cassava mosaic disease from Nigerian cassava clones by meristem-tip culture. *Plant Cell, Tissue and Organ Culture.* 1:25-32.

Almazan, A. M. and Theberge, R. L. 1989. Influence of cassava mosaic virus on cassava leaf-vegetable quality. *Tropical Agriculture.* 66(4): 305-308.

Annor-Frempong, C. 1991. A survey of cassava cultivation practices in Ghana. In: *Proceedings of the 9th symposium of the Inter. Society for Tropical Root Crops*. (ISTRC) Held at Accra, Ghana. 20-26 October 1991. Ed. F. Ofori and S. K. Hahn.

Appiano, A. and Pennazio, S. 1972. Electron microscopy of potato meristem tips infected with potato virus X. *Journal of General Virology*. 14: 273–276.

Calvert, L. A. and Thresh, J. M. 2002. The viruses and virus diseases of cassava. In: Hillocks, R. J., Thresh, J. M. and Bellotti, A. C., (eds). Cassava: Biology, Production and Utilization. Wallingford, UK: CAB International, 237–60.

Cours-Darne, G. 1968. Improving cassava in Africa. *The Abidjan Conference*. *Agric. Research Priorities for Economic Development in Africa* 2: 330-339.

Delgado, G. E. and Rojas, C. 1992. Cassava seed production program by meristem culture in UNPRG-LAMBAYEQUE (Peru). *Proceedings of the First International Science Meetings*, CBN. Cartagena de Indias, Colombia. 25-28 August. Pp 146-148.

Dodds, J. H., Lizarraga, R., Griffiths, H. and Slack, S. A. 1989. Methods of virus eradication. In: *planning conference on control of virus and virus-like diseases of potato and sweet potato*, 3. Lima. Report**.** Lima: International Potato Centre. 228p.

Frison, E. A. 1994. Sanitation techniques for cassava. *Tropical Science.* 34:146–53.

Garcia, G., Vega, V. M. and Rodriguez, M.S. 1993. Effect of meristem culture on vigour and yield of the cassava clone Senorita. *In: Proceedings of the First International Scientific Meeting, Cassava Biotechnology Network,* Cartagena, Colombia, 25-28 August 1992. Thro, A.M. and Roca, W. (Eds.), pp. 149-153. CIAT Working Document. No. 123.

Griffiths, H. M., Slack, S. A. and Dodds, J. H. 1990. Effect of chemical and heat therapy on virus concentrations in *In-vitro* potato plantlets. *Canadian Journal of Botanic.* 68:1515-1521.

Hahn SK, John C, Isoba G, Ikotun T (1989). Resistance breeding in root crops at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Crop Prot. 8: 147-168.

Hahn, S. K., Terry, E. R and Leuschner, K. 1980. Breeding cassava for resistance to cassava mosaic disease. *Euphytica.* 29: 673-683.

Hu, C. Y. and Wang, P. J. 1983. Meristem, shoot tip and bud cultures. *In*: *Handbook of Plant Cell Culture,* Evans, D. A, Sharp, W. R., Ammirato, P. V. and Yamada, Y. (Eds). 1: 177- 227. MacMillan Publishing Company, New York.

Jennings, D. L. 1994. Breeding for resistance to African cassava mosaic geminivirus in East Africa. *Tropical Science. 34:110-122.*

Kartha, K. K. 1986. Production and Indexing of Disease Free Plant. Plant Biotechnology Institute, Saskatchewan, Canada. *Cambridge University Press.*

Kassianof, E. P. 1992. Biomass partitioning characteristics, tissue culture and transformation for the genetic improvement of cassava (*Manihot esculenta* Crantz). Department of Crop Science, Faculty of Agriculture, University of Zimbabwe. 312 pp.

Latham, M. C. 1979. Human Nutrition in Tropical Africa. Rome, Italy. *FAO Report*.

Manganaris, G. A., Economou, A. S., Boubourakas, I. N. and Katis, N. I. 2003. Elimination of PPV and PNRSV through thermotherapy and meristem-tip culture in nectarine. *Plant Cell Report*. 22: 195–200.

Njock TE (1994). Epidemiology and disease recovery phenomenon of African cassava mosaic virus in resistant and susceptible cassava clones. Ph.D. thesis. University of Ibadan, Ibadan, Nigeria, p. 213.

Pita, J. S., Fondong, V. N., Sangare, A., Otim-Nape, G. W., Ogwal, S. and Fauquet, C. M. 2001. Recombination, pseudo-recombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *Journal of General Virology 82*, 655-665.

Roca, W. M. 1984. Root and Tuber Crops, cassava In: Hand book of Plant Cell Culture, (Eds). Sharp, W., Evans, D., Ammirator, P. and Yamada, Y. MacMillan Publishing Co., New York. 2: 269-301

Thresh, J. M., Fishpool, L. D. C., Otim-Nape, G. W. and Fargette, D. 1994. African cassava mosaic virus disease: An underestimated and unsolved problem. *Tropical Science 34* (1): 3-14.

Tonukari, N. J., 2004. Cassava and the future of starch. *Electronic Journal of Biotechnology.* 7(1)

Walkey, D. G. A. and Webb, M. J. W. 1968. Virus in plant apical meristems. *Journal of General Virology.* 3: 311-313.

Zok, S., Nyochembeng, L. M., Tambong, J. and Wutoh J. G. 1992. Rapid seed stock multiplication of improved clones of cassava (*M. esculenta* Crantz) through shoot tip culture in Cameroon. *Proceedings of the first International Scientific Meeting of Cassava Biotechnology Network,* Cartagena de Indias, Colombia. 25-28 August. Pp 96-99.