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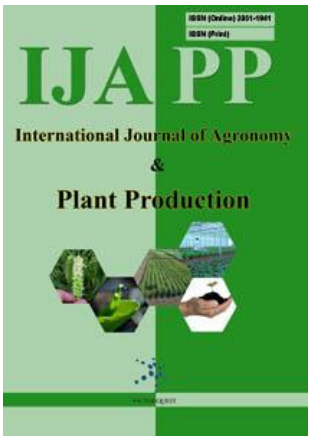

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Eradication of Cassava (*Manihot esculenta*) Mosaic Symptoms through Thermotherapy and Meristems Cultured *in vitro*.

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Abstract

Three cassava accessions (Agric-Sé, Hombètè, Ouémènou) were examined in three different media with different combinations of growth regulators, NAA, 2,4-D (auxins), BAP, Kinetin (cytokinins), and gibberellic acid. Parameters evaluated in thermotherapy conditions were the presence of viral symptom and nodes formation. The response of explants was evaluated by aptitude to regeneration, and stems formation. The ANOVA analysis showed no significant ($P > 0.05$) accessions' reactions to thermotherapy, but significant ($P < 0.05$) accessions' response in deferent media. Thermotherapy revealed a total absence of viral symptom on the cuttings. Highest rate survival was recorded for Ouémènou accession (26 in 30) with combinations MS+NAA+KIN+GA3 and MS+2,4-D+GA3. The combination MS+NAA+BAP+GA3 made regenerate stems for all the accessions with a maximum of 14 in compared to the 30 regenerated by the Ouémènou accession.

Keywords: Growth regulators, *In vitro* culture, *Manihot esculenta*, Meristems, Thermotherapy.
Abbreviations: *BAP:* 6-benzylaminopurine; NAA: Naphthaleneacetic Acid; GA_3: Gibberellic Acid; 2,4-D: 2,4 dichlorophenoxyacetic

Introduction

During the past decade cassava presents a growing importance in tropical countries due to increasingly high food demand for humans and animals, as well as industrial and biofuel (Jamson et *al.*, 2009; Sriroth et *al.,* 2010). Its energy role for the African people is paramount. This is one of the most practiced food crops in the Republic of Benin. This is a potential source of calories especially in countries where malnutrition is a major, and ranks fourth as an energy source after rice, sugarcane and maize (Ceballos et *al.,* 2004).

Cassava is a plant with vegetative propagation major difficulties in its production (Santana et al., 2009). In addition, it is parasitized by several categories of viruses belonging to various groups such as virus cassava brown streak (CBSV), the African mosaic virus and the common mosaic. Associated with bacterial and fungi infections, these viruses cause yield losses of 20-80% in all seasons (Givord et *al.,* 1994). Indeed, the average yield in the tropics is barely 20% of the yield obtained under optimal conditions (Sharkawy, 2004). The lack of healthy cuttings is by far the most important problem of cassava production, followed by low yields of fresh roots. In addition, the rate of adoption and diffusion of new varieties are proving slow because cassava plant can generate only about 10 stem segments (IITA, 1998). The exchanges of materials are certainly needed to improve crop production, but the agriculture of the concerned countries should not incur dangers of food dependence.

Although the work of the various programs on cassava were used to select varieties of cassava highly efficient due to their high productivity and their aptitude to tolerate or resist disease (Hahn et *al.,* 1980), the viral problem remains. Mabanza and Mahouka (2001), noted a decline in the production of clones (MM79, MM92 and NKAAKA) obtained from cuttings unrenovated. The major struggle is to examine the plants to

visually select those which are healthy. Despite the good results obtained in Western and Eastern Africa (Bock, 1983; Fargette et *al.,* 1985), the reinfection rate is still high.

Thermotherapy and meristem culture can be used to remove viruses and regenerate free-virus plants (Zapata et *al.,* 1995). According Mabanza and Mahouka (2001) the combination of the two techniques give a higher yield of cassava sanitized clones (13.7 t / ha) compared to non-sanitized clones (9.9 t / ha) after 18 months of culture. Work in viral sanitation using meristem has been carried out on yam (Saleil et *al.,* 1990) and banana (Wirakarnain et *al.,* 2008; Kabir et *al.,* 2008). The synthesis of researches work on cassava cultivars shows that growth regulators determine organogenesis (Ahanhanzo et *al.,* 2008.), regeneration of meristems (Ng et *al.,* 2010; Wasswa et *al.,* 2010). In the present work, the effects of thermotherapy are evaluated and those of NAA, 2,4-D, BAP and kinetin were studied on the aptitude of *in vitro* regeneration of apical meristems of three accessions of cassava (*Manihot esculenta.* Crantz) from Benin.

Materials and Methods

Collection and preparation of cutting

Three cassava accessions (Agric-Sè, Hombètè, Ouémènou) were obtained from farmers' fields in the departments of Mono and Couffo in Benin. These accessions are identified by their vulnerability to cassava mosaic virus.

Cuttings showing symptoms of mosaic in their environment were disinfected with thiophanate-methyl 70% for ten minutes and established in two diferrent conditions (thermotherapy and greenhouse) at the Laboratory of Genetic and Biotechnology (LGB) at University of Abomey-Calavi in Benin. For thermotherapy, pots containing cuttings are placed in a sterilizer and cultured for four weeks at a temperature of 36 ° C for 8 hours of darkness and 40 ° C for 16 hours light (Wasswa et *al.,* 2010). The apex of stems produced by cuttings in thermotherapy condition is taken for *in vitro* culture.

Disinfection of explants

The explants are washed with running water tap for five minutes to remove soil debris and transferred into laminar flow room . Then they are sterilized in 70% alcohol for three minutes and rinsed with sterile distillate water. This is further disinfected with 10% sodium hypochlorite for 10 minutes and rinsed with sterile distillated water successively three times for 5 minutes each rinse. For each apex, the meristem is excised using forceps, scalpel and a microscope, and placed on culture media.

Phytohormones treatments and culture conditions

The meristms are cultured on solid Murashige and Skoog (MS) basic medium (Murashige and Skoog, 1962), supplemented with NAA or 2,4-D; BAP or Kinetin, and GA_3. Differents culture media were prepared depending on their combination on growth regulators. The medium M1 consisting of MS supplemented with 0.2 mg/l (NAA) + 0,15mg/l (BAP) + 0,02mg/l (GA3) is the reference medium used to IITA by Ng (1990) for cassava culture meristems; the medium M2 consisting of MS supplemented with 0,2mg/l (NAA)+0,15mg/l(KIN)+0,02mg/l(GA3); the medium M3 consisting of MS supplemented with 0,2mg/l (2,4-D)+0,15mg/l (BAP)+0,02mg/l (GA3) with pH adjusted to 5.7 ± 0.1 are solidified by agar (7 g / l) and then autoclaved at 121°C for 15 minutes. The cultures are sealed with parafilm, labeled and kept under incubation in the growth room with temperature maintained 26±1°C (Mapayi et *al.,* 2013), a photoperiod of 12 hours and relative humidity at 80%. After four weeks of growing data were collected on number of nodes and the absence or presence of cassava mosaic symptoms. Four weeks after the meristem culture, data relatives to the frequency of stems were recorded.

Three treatments (media) were used in a completely randomized design. Ten explants were cultured per treatment with three repetitions. Data were analyzed using Minitab 14 and Version 7.1. Analysis of variance was carried out to determine significant difference among accessions and averages were separated using Student Newman and Keuls (SNK) threshold 5%.

Results and Discussion

Effects of thermotherapy on mosaic symptoms and nodes formation

There was no interaction ($P > 0.05$) between the accessions and the conditions of culture (greenhouse and thermotherapy) in the appearance or not of viral symptom. Cutting cultivated in greenhouse registered visual symptoms of virus disease (Figure 1.) against a total absence in thermotherapy condition (Figure 2.).

Valentine et *al..* (2001) working on the metabolic activity of meristematic cells had noted that active division of cell meristem was not favorable to the replication of the virus. Our results were also concordant with those obtained by Wasswa et *al.* (2010) which approved the effect of the temperature on the deceleration of the replication and the movement of the viruses. Likewise, no interaction ($P > 0.05$) was

noted between the accessions and the conditions of culture in the formation of nodes. In thermotherapy condition, the accessions presented high averages of nodes against low averages obtained in greenhouse (Table 1). Thermotherapy increased the production of materials (explants) for the *in vitro* culture compared to the direct culture of the cuttings in greenhouse. This confirmed the stimulative effect of thermotherapy on the growth of the stems of cassava plants (Ferréol, 1978).

Aptitude for the stems regenerated from the meristems of cassava accessions

The frequency of stems regenerated on media M1, M2, and M3 are presented in Figure 3. Data analysed showed that Hombètè accession presented 8 stems in 30 meristems initiated on medium M1 when no stem was obtained on the media M2 and M3. The analysis of variance showed significant ($P < 0.05$) medium M1 compared to others media (M2 and M3) on the regeneration of stems. Agric-Sè accession had 6 stems in 30 meristems initiated on medium M1 and 4 stems in 30 on medium M2. No stem was obtained on the medium M3. Analysis of variance revealed significant ($P <0.05$) media effect for the regeneration of stems. Ouémènou accession generated more stems (14 of 30) in the medium M1 against 6 stems in 30 on medium M2 and 4 stems in 30 on the medium M3. The analysis of variance showed significant ($P <0.05$) media on the regeneration of stems. The aptitude to caulogenesis varied according to accessions and was strongly influenced by the type of hormonal combinations used. The MS basal supplemented with 0.2 mg / l (ANA) + 0.15 mg / l (BAP) + 0.02 mg / l (GA3) allowed the formation of stem for the three accessions with high frequency on Hombètè (8 in 30) and Ouémènou (14 in 30). Ng (1990) also obtained cassava plantlets by growing meristematic cells on the same medium. However, an absence of stem is noted for Hombètè accession on MS media supplemented with 0.2 mg / l (NAA) + 0.15 mg / l (KIN) + 0.02 mg / l (GA3) and MS media supplemented with 0.2 mg / l (2,4-D) + 0.15 mg / l (BAP) + 0.02 mg / l (GA3) on the one hand and the accession Agric Sè on MS medium supplemented with 0.2 mg / l (2, 4-D) + 0.15 mg / l (BAP) + 0.02 mg / l (GA3) on the other. There was no significant defference ($P>0,05$) between media M1, M2 and M3 on the meristems regeneration of Agric-Sè and Ouémènou accessions. It come out from these analysises that cytokinins (BAP and Kinetin) combined to NAA express different effects according to accession. Cacaï et *al.,* (2012) also noted that medium MS supplemented with 0,1mg/l (NAA) + 0,2mg/l (BAP) supported a better regeneration on several accessions of cassava such as Gbèzé, sazoué Agric, Okoyao, Sèkandji, meanwhile medium MS supplemented with 0,1mg/l (NAA) + 0,2mg/l (KIN) supported a better regeneration at other varieties such as Ahouandjan, 92/0057. Thus, substituting the medium used by Ng (1990) NAA by 2,4-D or BAP by Kinetin there was inhibition of organogenesis process on Hombètè accession. However, Ahanhanzo et *al.* (2008), by initiating microcuttings RB 89509, BEN 86052, TMS 30572 cassava varieties on MS medium supplemented with 0.5 mg / l (ANA) + 0.5 mg / l (KIN) obtained stems. Meristems used as explants in this work could be a factor influencing the formation of stems. Mapayi et *al*. (2013) using combinations NAA and BAP obtained high rates of survival at genotype 95/0289: 98% and 100% respectively on media MS+0,1mg/l (NAA) + 0,2mg/l (BAP) and MS/2+ 0.02 mg/l (NAA)+ 0.10 mg/l (BAP) certainly because of the quantity in BAP which is relatively higher than that of the ANA.

Figure 1. Cuttings in greenhouse condition

Figure 2. Cuttings in thermotherapy condition.

Table 1. Effect of culture conditions (thermotherapy and greenhouse) on the formation of nodes.

Accessions	Culture conditions	Averages	Maximum	Ecart-type
Hombètè	Thermotherapy	14,73	21	3,86
	Greenhouse	8,40	12	1,88
Agric-sè	Thermotherapy	12,80	25	4,61
	Greenhouse	8,60	12	1,99
Ouéménou	Thermotherapy	16,93	26	4,58
	Greenhouse	7,13	9	1,24

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