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Full Length Research Paper

Hormonal influence on the *in vitro* bud burst of some cassava varieties and accessions from Benin

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This work tested the effect of different growth regulators on *Manihot esculenta* explants cultured. Nodal segments were disinfected and cultivated on Murashige and Skoog's basal media. The effects of the different combinations on bud burst were observed after five weeks of culture. The results show an interaction between the growth regulators and the genotypes of the varieties and accessions. Most of the varieties and accessions (Gbèzé, Kpètévikoutou, Ibadan and Sèkandji) obtained the maximal bud burst in the media MS supplemented with 0.2 mg/l BAP and MS supplemented with 0.2 mg/l KIN. However, with segments cultivated in medium, containing 0.2 mg/l KIN, 1 in 15 showed bud burst for the accession Agric Comé. Naphthalene acetic acid effect varied according to the genotype and the cytokinin used, whereas on 0.1 mg/l NAA combined with 0.2 mg/l with the variety 92/0057, 14 in 15 budded. However, no bud burst was observed with 0.1 mg/l NAA combined with 0.2 mg/l BAP.

Key words: *In vitro* culture, *Manihot esculenta Crantz*, bud burst, NAA, BAP, Kinetin.

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is a subsistence crop for the small farmers in the tropics. It is often grown where other crops fail (Thro et al., 1998). Cassava has two major roles in the tropical agriculture: it is used as food safety culture and as a basic production for poor region. It is the fifth of the nutritious vegetable productions in the world, after maize, rice, wheat, and potato (FAO, 2008). Cassava is grown in Benin, particularly in the Central and the Southern regions. Cassava is becoming an export culture after cotton, coffee, palm tree which provides substantial incomes to producers. Cassava holds a privileged position in Beninese agricultural production because of its various uses (food, pharmaceutical and textile industries). It is consumed in various forms: gari and tapioca used as beverages, pounded cassava and in many other dough forms. Cassava's leaves are consumed as leaves for sauces in the southern and central regions of Benin.

Despite its importance, cassava remains one of the cultures whose farming techniques are still rudimentary. Thus, cassava production is facing many challenges such as bacterial and viral diseases, devastators' attacks, edaphic and socio-economic factors which lead to insufficiency of propagation materials. The fact that farmers obtain cuttings, both exotic and indigenous cassava cultivars, from their previous crop or from their neighbours, suggests that without a deliberate program for restricting movement of infected material would perpetuate the Cassava Brown Streak Disease (CBSD), spread (Wasswa, 2010). Research of new seeds through *in vitro* production for a normal regeneration of cassava at the production sites seems to be the best way. Due to the level of spread of diseases and devastators since 1970, many research programs were focused on the collect of local

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Abbreviations: NAA, Naphthalene acetic acid; **BAP,** 6-benzylaminopurine; **MS,** Murashige and Skoog medium; **PGR,** plant growth regulator.

Table 1. Material samples of investigated cassava.

Locality	Ethnic/ meaning	Collected samples of cassava	
		Variety	Accession
Adjohoun		-	Ahouandjan,
Sèmè Poji		-	Sèkandji
Grand Popo		-	Agric Sazoué
Comé		-	Agric Comé
Djakotomey (South of Benin)	Children's cassava	-	Kpètévikoutou
Djakotomey (South of Benin)	The natural choice	-	Gbèzé
Savè		BEN 86052	Okoyao
Savè		92/0057	-
IITA in Ibadan (Nigeria)			Ibadan

cultivars of *M. esculenta Crantz*. However, in *ex-situ* collection of cassava pose some maintenance problems, because of uncontrolled environment conditions and financial difficulties. Taking into account these problems, "*in vitro"* culture techniques have been proposed to be tested. *In vitro* culture should be a useful tool for mass propagation of superior stock plants as well as genetic improvement. Moreover, multiplication can continue throughout the whole season. In addition, micropropagation may be used, in basic research, in production of virus-free planting material, cryopreservation of endangered and elite woody species, applications in tree breeding and reforestation (Mohan and Häggman, 2007; Filiz et al., 2009).

This technique has been proved to be very effective for rapid propagation of rare plant species including medicinal plants (Hassan and Roy, 2005; Sudha and Seeni, 2006; Biswas et al., 2007; Hussain et al., 2008; Roy, 2008; Bhadra et al., 2009). Tissue culture systems allow propagating the plant material with high rates of multiplication, in an aseptic environment. According to some research works, the quick multiplication techniques of *in vitro* culture of plant tissues depends on many factors that include the genotype (Ahanhanzo et al., 2010), the effect of growth regulators (Filiz et al., 2009) and the type of explants.

The presence of different types of organic nitrogen additives viz casein hydrolysate, peptone and tryptone-peptone additives into the basal medium slightly enhanced the number of multiple shoots formed on types of explants (Ng, 2010). For a quick *in vitro* multiplication of *M. esculenta Crantz*, Mabanza and Jonard (1981) used composed medium including MS + 0.5 mg/l of NAA (naphthalene acetic acid), at the IITA (International Institute of Tropical Agriculture) a medium MS supplemented with 0.01 mg/l NAA+ 0.05 mg/l BAP (6-benzylaminopurine) was used. Although, many research works were carried out on *in vitro* culture of *M. esculenta Crantz*, few were focused on bud burst.

Furthermore, it was reported that growth regulators differently influence the *in vitro* morphogenesis of *Dioscorea cayenensis,* and *Dioscorea rotundata complex* (Malaurie et al., 1995). These investigations were of very limited nature where only a few combinations of plant growth regulators (PGRs) were used on a few varieties and accessions. The present study was therefore undertaken with a view to develop an efficient and repeatable protocol for rapid and mass propagation of this plant with detailed evaluation of PGR combinations including auxin and cytokinins group in the background of culture media. Thus in this study, the effects of NAA, BAP and Kinetin on the *in vitro* bud burst of ten varieties and accessions of *M. esculenta Crantz* grown in Benin were evaluated.

MATERIALS AND METHODS

Plant materials and explants preparation

Stems of cassava cultivars plants were collected from six municipalities in Bénin. The districts represented agro-ecologies where cassava is popularly grown. The plant materials include eight (8) cassava accessions and two cassava varieties. The names of the accessions are linked to the names of the localities from where they have been collected or to some ethnic/ meanings (Table 1).

20 stem cuttings collected per cultivar were disinfected with 0.5% Topsin'M for 5 min and established in a screen house at the Laboratory of Genetic and Biotechnology (LGB) at University of Abomey-Calavi in Benin. After one month of plant culture, nodal segments of the healthy plants were collected and used as explants for *in vitro* experiments. The explants were washed under tap water for 10 min. Ten node cuttings from young stems of each of the ten popularly grown cassava cultivars were singly sterilized, first with ethanol 70% for 5 min and then with sodium hypochlorite 10% for 20 min. Both were finally rinsed four times with distilled water.

Culture conditions and phytohormone treatments

The nodes were cultured on solid Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962), supplemented with Kinetin, BAP and NAA. Five different culture media were prepared depending on their growth regulators components. Thus, the culture media MI, MII and MIII, respectively, contained 0.1 mg/l of NAA (naphthalene acetic acid); 0.2 mg/l of BAP (6-benzyl-aminopurine) and 0.2 mg/l of kinétin. The media MIV and MV, respectively, consisted of the combinations between NAA and BAP, and between

Table 2. Effect of plant growth regulators on *Manihot esculenta* (Agric Comé, Agric Sazoué, Kpètévikoutou and Gbèzé) bud burst after five weeks of culture.

Variety accession	Media	Bud burst average value
Agric Comé	MII : MS+BAP	13.0±1[a]
	MIII: MS+KIN	1.0±1[b]
	MIV: MS+NAA+BAP	12.50±0.5[a]
	MV: MS+NAA+KIN	12.50±0.5[a]
Agric Sazoué	MI: MS+NAA	6.5±0.5[b]
	MIII:: MS+KIN	15.0±0[a]
	MIV: MS+NAA+BAP	14.5±0[a]
Kpètévikoutou	MI: MS+NAA	5.0±0[b]
	MII : MS+BAP	15.0±0[a]
	MIII: MS+KIN	11.5±1.5[a]
Gbèzé	MI: MS+NAA	14.0±1[a]
	MII : MS+BAP	15.0±0[a]
	MIII : MS+KIN	13.5±0.5[a]
	MIV: MS+NAA+BAP	12.0±0[ab]
	MV: MS+NAA+KIN	10.0±1[b]

Average in the same row followed by the same letters do not differ significantly ($p < 0.05$).

NAA and kinetin. All growth regulators were added before autoclaving; pH was adjusted to 5.6 ± 0.2 and autoclaving was done at 121°C for 15 min. The cultures were incubated in a growth room at 26 ± 2°C under white fluorescent light with a 16 h photoperiod.

Experimental design and data analysis

In order to test each growth regulators and each auxin-cytokinin combination, a total of five treatments were used. The experiment consisted of completely randomized design and was replicated three times for each treatment for each cultivars/ accessions. Five explants were cultured per treatment. In total (5 x 5 x 3) = 75 explants was obtained per cultivar/accession. Each cassava sample was scored as a binary variable (bud burst / no bud burst). Mean bud burst values were established by subjecting the data to ANOVA. Where the ANOVA indicated significant ($P<0.05$) difference, the means were separated using SNK.

RESULTS

Data related to effect of single NAA, single cytokinin and combination NAA and cytokinin on the accessions or varieties from different localities are presented in Tables 2 and 3. Different plant regulators used singly or in combination had a significant effect on the regeneration of plantlets (Tables 1 and 2). Agric Comé accession exhibited a high average (13.0±1) of bud burst on MS medium supplemented with 0.2 mg/l BAP. Similar results were obtained with the media containing: 0.1 mg/l NAA + 0.2 mg/l BAP and 0.1 mg/l NAA + 0.2 mg/l KIN; average value obtained on media MS supplemented with 0.2 mg/l KIN was too weak (1.0±1). These results show that kinetin (0.2 mg/l) used in single do not enhance bud burst of Agric Sazoué; its combination with 0.1 mg/l NAA improved the bud burst. Concerning Agric Sazoué accession, the maximal bud burst was obtained on MS media supplemented with 0.2 mg/l KIN or 0.1 mg/l NAA+ 0.2 mg/l BAP. The low value (6.5) of medium MS supplemented with 0.1 mg/l NAA showed a significant difference ($P<0.05$) (Figure 1). All explants of Kpètévikoutou accession cultured on MS medium supplemented with 0.2 mg/l BAP had budded. But a bud burst average (11.5±1.5) was obtained on medium MS supplemented with 0.2 mg/l KIN.

However, there was no significant difference concerning the effect of two cytokinins. NAA at 0.1 mg/l shows a low performance. All explants of Gbèzé accession also had budded in the medium MS supplemented with 0.2 mg/l BAP. Media MS supplemented with 0.1 mg/l NAA or 0.2 mg/l KIN enhanced a good bud burst with respective average value of 14.0±0 and 13.5±0.5 with no significant difference. But each combination of NAA with kinetin reduced significantly bud burst value. With Ahouandjan accession, the highest bud burst average (14.0±0) was obtained in the combination 0.1 mg/l NAA+ 0.2 mg/l KIN but the lowest average (9.0±1) was recorded in the combination 0.1 mg/l NAA+ 0.2 mg/l BAP. The same bud burst averages in media MS supplemented with 0.2 mg/l (BAP) and 0.2 mg/l KIN were (13.5±1.5). There were significant difference ($P<0.05$) between the combination NAA+BAP and other media.

According to Sèkandji, the maximal bud burst was obtained on MS media supplemented with 0.2 mg/l (BAP).

Table 3. Effect of plant growth regulators on *Manihot esculenta* (Ahouandjan, Sèkandji,Okoyao, 92/0057, BEN 86052 and Ibadan) bud burst after five weeks of culture.

Variety accession	Media	Bud burst average value
Ahouandjan	MII: MS+BAP	13.5±0.5[a]
	MIII: MS+KIN	13.5±1.5[a]
	MIV: MS+NAA+BAP	9.0±1[b]
	MV: MS+NAA+KIN	14.0±0[a]
Sèkandji	MI: MS+NAA	13.0±0[b]
	MII: MS+BAP	15.0±0[a]
	MIV: MS+NAA+BAP	12.5±0.5[b]
Okoyao	MII: MS+BAP	13.0±1[a]
	MV: MS+NAA+KIN	10.5±0.5[a]
92/0057	MII: MS+BAP	14.0±0[a]
	MIII: MS+KIN	12.5±0.5[b]
	MIV: MS+NAA+BAP	0[c]
	MV: MS+NAA+KIN	14.0±0[a]
BEN 86052	MI: MS+NAA	12.0±2[a]
	MIII: MS+KIN	15.0±0[a]
	MV: MS+NAA+KIN	15.0±0[a]
Ibadan	MI: MS+NAA	11.5±1.5[a]
	MII: MS+BAP	15.0±0[a]

Average in the same row followed by the same letters do not differ significantly ($p < 0.05$).

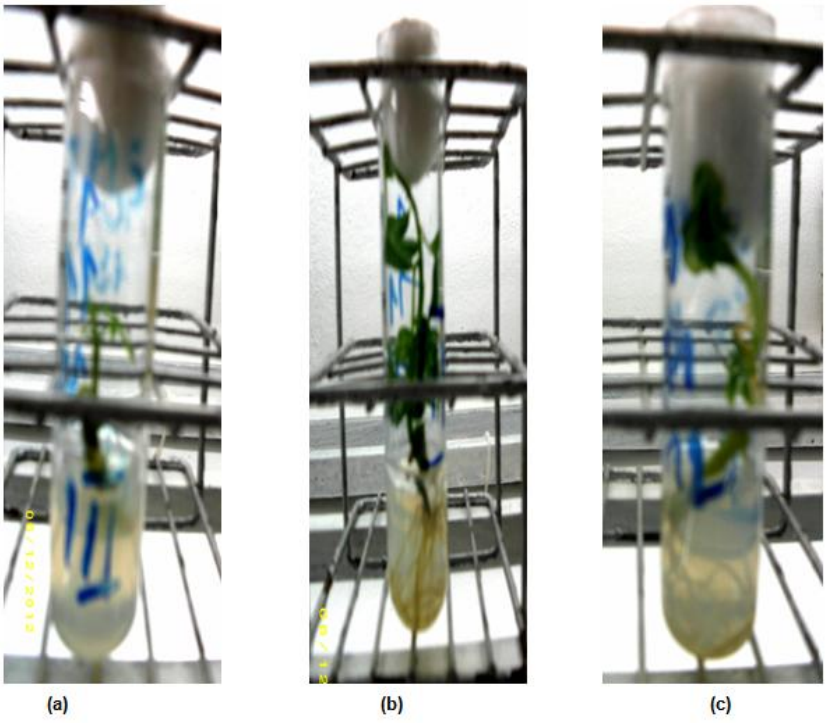
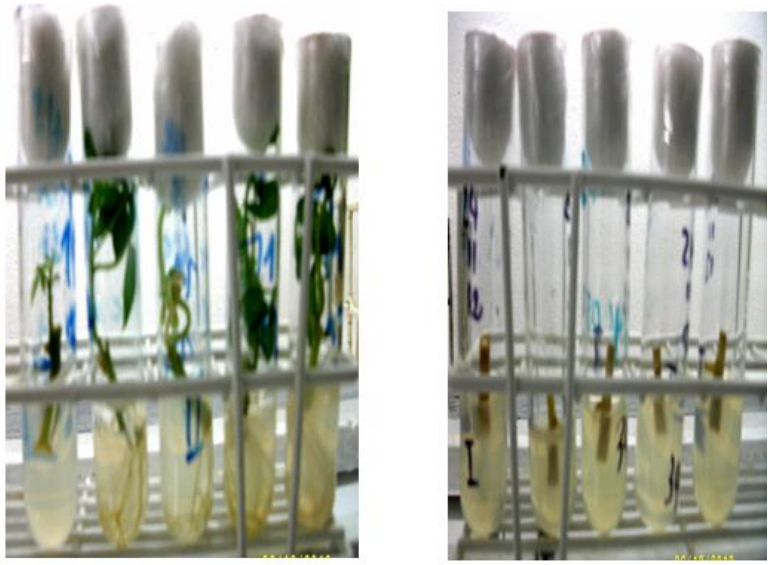


Figure 1. Bud burst of Agric Sazoué on (a) MS medium supplemented with 0.1 mg/l NAA; (b) MS medium supplemented with 0.2 mg/l KIN; (C) MS medium supplemented with 0.1 mg/l NAA+ 0.2 mg/l BAP.



(a) (b)

Figure 2. Bud burst of 92/0057 on (a) MS medium supplemented with 0.2 mg/l BAP; (b) MS medium supplemented with 0.1 mg/l NAA+ 0.2 mg/l BAP.

Media MS supplemented with 0.1 mg/l NAA or with 0.1 mg/l NAA+ 0.2 mg/l (BAP) exhibited similar average value of bud burst (13.0±0 and 12.5±0.5). Okoyao accession showed the best bud burst average in the medium MS supplemented 0.2 mg/l (BAP) with an average of 13.0±1. With Medium MS supplemented with 0.1 mg/l NAA+ 0.2 mg/l KIN a bud burst average (10.5±0.5) was registered. 92/0057 variety revealed a mean value (14.0±0) of bud burst close to the maximum (15) with the medium MS supplemented 0.2 mg/l (BAP) and the medium MS containing the combination 0.1 mg/l NAA+ 0.2 mg/l (KIN). Meanwhile, there was no bud burst with the medium MS with combination 0.1 mg/l NAA+ 0.2 mg/l (BAP) (Figure 2). A significant difference ($P< 0.05$) was revealed by the effect of BAP; kinetin and combination NAA+BAP for this variety. With BEN 86052 variety, it was remarked that all the explants budded on media MS supplemented with 0.2 mg/l KIN and medium MS supplemented with 0.1 mg/L NAA+ 0.2 mg/L KIN (Figure 3). A bud burst average of 12.0±2 was obtained in the medium MS supplemented with 0.1 mg/l NAA. There was no significant difference between NAA and kinetin used singly or in combination. All explants of accession Ibadan budded in the medium MS+BAP but a bud burst average (11.5±1) was obtained in the medium MS supplemented with 0.1 mg/l NAA. Nevertheless, no significant difference in the effect of NAA and BAP was noted.

DISCUSSION

The regulators NAA and BAP used singly or in combination are favorable for bud burst for Agric Comé accession. It was seen that there was no significant difference ($P>0.05$) among the media. However, a high significant difference at 5% was observed between the medium MS+ KIN and the others regulators. Romano et al. (1992) reported that cytokinins (Kinetin and Zeatin) reduce buds development. However, Ndoumou et al. (2003) showed that the presence of Kinetin in the culture medium had a positive effect on the *in vitro* bud burst of the *Irvingia gabonensis* explants. With Kpètévikoutou accession, there was no significant difference between the effects of the different combinations of growth regulators except NAA used in single, which had a significant difference ($P<0.01$) with other combinations. Used alone, NAA did not enhance the bud burst of that accession in the medium (MS).

Miller and Skoog (1957) reported that auxins had an inhibitory effect on buds development. With Ibadan accession, the combinations of NAA with each of the two cytokinins showed similar bud burst average (13.5±0.5). This value is higher than those of the other media, except for the medium containing BAP. Contrary to Agric Comé and Kpètévikoutou accession, Sèkandji accession had in the media MS+ KIN and MS+NAA bud burst averages higher to the one presented in the combination NAA+ KIN with respective values of 13.5±0.5 and 13.0±0. In the same way, the combination NAA+BAP reduced the bud burst average of each of the two growth regulators taken individually. Still, BAP was the most favorable regulator to the bud burst among Sèkandji accession. This probably could justify the reactivity of those genotypes against cytokinins. These results are in accordance with those of Ahanhanzo et al (2010); El Kbiach et al (2002) who reported that BAP appears to be the best adapted to bud

Figure 3. Bud burst of BEN 86052 on (a) MS medium supplemented with 0.2 mg/l KIN; (b) MS medium supplemented with 0.1 mg/l NAA+ 0.2 mg/l KIN.

burst of auxiliary yam buds and of auxiliary *Quercus suber* L, respectively. The combination NAA+KIN showed a highly significant difference at 5% with media (three) that had only one type of growth regulator. However, there was no significant difference with the combination NAA+BAP when compared to NAA+KIN. It could be assumed that the combined regulators NAA and Kinetin in the medium MS did not support bud burst of Gbèzé accession but when used separately, they enhanced a better bud burst. There was an interaction between NAA and Kinetin on this variety. Used alone, Kinetin was more favorable than its combination with NAA.

Abdelhadi et al. (2005) obtained similar results by using Zeatin for the young olive explants. In contrast, the regulators NAA and BAP yielded the best bud burst ave-rages when they were used in combination. It could be assumed that used alone in the medium MS, NAA did not promote a good bud burst of Agric Sazoué accession. This confirms the report of Ahanhanzo et al. (2008) that NAA had not allowed a better formation of sprouts among RB 89509, BEN 86052 and TMS 30572 varieties of cassava. But, the combined effect of NAA and BAP did not promote bud burst of Ahouandjan accession. The combined effect of NAA and Kinetin improved bud burst. There was a synergetic action of those two growth regulators. In opposite, the combination NAA+BAP showed a bud burst average lower to that of the two regulators taken individually with Okoyao accession. The bud burst inhibitory effect that NAA had in the presence of BAP on this genotype is thus put in evidence. This effect was stronger among all 92/0057 variety because it made the average drop from 14.0±0 to 0.0±0. In the same way, used alone, Kinetin had yielded a better bud burst than its combination with NAA. It seems that the combination auxin + cytokinin was not favorable to the bud burst of Okoyao accession, probably, either due to the strong dose of NAA used (0.1 m/L) or to the species.

According to Chalupa (1984), the addition of weak concentrations of auxins (IAA or NAA at 0.25 to 0.5 µM) does not significantly affect the bud burst of *Quercus robu*r. Probably, the combinations auxin + cytokinin were not favorable to the bud burst of Okoyao accession.

REFERENCES

Abdelhadi A, Najiba B, Dou el Macane WL (2005). Essais de prolifération et d'enracinement de matériel issu de rajeunissement par bouturage d'oliviers adultes (*Olea europaea* L.*)* et de germination *in vitro :* Effets de cytokinine et d' auxines. Biotechnol. Agrom. Soc. Environ. 9(4): 237-240.

Ahanhanzo C, Gandonou C, Agbidinoukoun A, Dansi A, Agbangla C (2010). Effect of two cytokinins in combination with acetic acide α-naphthalene on yams (*Discorea spp*.) genotypes response to *in vitro* morphogenesis. Afr. J. Biotechnol. 9(51): 8837- 8843.

Ahanhanzo C, Agbangla C, Agassounon DTM, Cacaï G, Dramane K (2008). Etude comparative de l'influence des régulateurs de croissance sur la morphogénèse (*in vitro)* de quelques variétés de *manihot esculenta Crantz* (manioc-euphorbiaceae) du Bénin. Rev. CAMES - Série A, 07: 47-52.

Bhadra SK, Akhter T, Hossain MM (2009). *In vitro* micropropagation of *Plumbago indica* L. through induction of direct and indirect organogenesis. Plant Tissue Cult. Biotechnol. 19(2): 169-175.

Biswas A, Roy M, Miah MAB, Bhadra SK (2007). *In vitro* propagation of

Abrus precatorious L. - a rare medicinal plant of Chittagong Hill Tracts. Plant Tissue Cult. Biotechnol., 17(1): 59-64.
Chalupa V (1984). I*n vitro* propagation of oak (*Quercus robus L*.) and Linden (Tilia cordata Mill). Biol. Plant. 265: 374-377.
El Kbiach ML, Lamart A, Abdali A, Badoc A (2002). Culture *in vitro* des bourgeons axillaires de chêne-liège (Quercus suber). Bull. Soc. Pharm. Bordeau 141: 89-104.
Filiz A, Çi dem I, Süreyya N, Bekir Erol A (2009). Effect of plant growth regulators on *in vitro* shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki. Afr. J. Biotechnol. 8 (22): 6168-6174;
Hassan A, Roy SK (2005). Micropropagation of *Gloriosa superb L*. through high frequency shoot proliferation. Plant Tissue Cult. 15(1): 67-74.
Hussain TM, Chandrasekhar T, Gopal GR (2008). Micropropagation of *Sterculia urens* Roxb. - an endangered tree species from intact seedlings. Afr. J. Biotechnol. 7(2): 95-101.
Mabanza J, Jonard R (1981). La multiplication des clones de manioc (*Manihot esculenta* Crantz) à partir d'apex isolés *in vitro*. CR Acad. Sci. Paris. 292: 839-842.
Malaurie B, Pungu O, Trouslot MF (1995). Effect of growth regulators on Dioscorea cayenensis D. rotundata complex and D. praehensilis. *Plant cell*, Tiss. Org. cult. 41:229-235.
Miller CO, Skoog F (1957). Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp. Soc. Exp. Biol. 11: 118-130.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tissue culture. *Physiologia. Plantarum*, 15 : 473-497.
Ndoumou DO, Fosto, Oumar, Duclaire M (2003). Propagation d'*Irvingia gabonensis* par microbouturage *in vitro* Fruits. 59(1): 31–38.
Ng CY, Norihan MS Faridah QZ (2010). *In vitro* multiplication of the rare and endangered slipper orchid, *Paphiopedilum rothschildianum* (Orchidaceae). Afr. J. Biotechnol. 9(14) : 2062-2068.
Romano A, Noronha C, Martins-Loução MA (1992). Influence of growth regulators on shoot proliferation in *Quercus suber* L. Ann. Bot. London 70(6): 531-536.
Roy PK (2008). Rapid multiplication of *Boerhaavia diffusa* L. through *in vitro* culture of shoot tip and nodal explants. Plant Tissue Cult. Biotechnol. 18(1): 49-56.
Sudha GG, Seeni S (2006). Spontaneous somatic embryogenesis on *in vitro* root segment culture of *Rauvolfia micrantha* Hook. F. – A rare medicinal plant. *In Vitro* Cellular and Development Biology. Plant, 42(2): 119-123.
Thro AM, Roca W, Iglesias C, Henry G, Ng SYC (1998). Contributions of *in-vitro* biology to cassava improvement. Afr. Crop Sci. J. 6(3): 303-315.
Wasswa P, Alicai T, Mukasa SB (2010) . Optimisation of in vitro techniques for Cassava brown streak virus elimination from infected cassava clones. Afr. Crop Sci. J. 18(3): 235 – 241.