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# EFFECT OF PLANT GROWTH REGULATORS ON *IN VITRO* SHOOT MULTIPLICATION FROM CALLUS OF *MANIHOT ESCULENTA* CRANTZ CV. TMS 92/0326

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**ABSTRACT**

The aim of this study was to test the effect of plants regulators on shoots multiplication in cassava cv TMS 92/0326 grown in liquid media with addition of 6-bezylaminopurine (BAP), and α-naphthaleneacetic acid (NAA). Nodal segments (1.5 cm height) were used as explants and kept *in vitro* MS media containing also mio-inositol (100 mg $l^{-1}$) and sucrose (30 g $l^{-1}$). The pH of the media was adjusted to 5.8 before autoclaving. The nodal cuttings produced callus. These callus were yellowish and friable. The highest percentage of callogenesis 69.8% was obtained with 0.07 mg $l^{-1}$ NAA. The highest fresh weights of 1521.2 mg were obtained with 0.07 mg $l^{-1}$ of NAA. When cultured in the presence of 0.01 to 0.1 mg $l^{-1}$ BAP, the callus induced shoots. The highest percentages of 82.3% were obtained with 0.07 mg $l^{-1}$. Also the highest numbers of shoots per callus 27.2 were obtained with 0.07 mg $l^{-1}$.



## INTRODUCTION

Cassava [*Manihot esculenta* (Crantz)] is grown for its starchy tuberous roots which provide food for over 500 million people, mostly in a small-scale plantation by the farmers in the developing countries (Roca *et al*., 1992). Because of its hardiness and tolerance to the adverse environmental conditions it is a reliable crop, giving adequate yields even it is grown on marginal soils that unable to support other crop plants. Despite its high importance to food security in third world countries, cassava has long been neglected in plant breeding programmes; in addition, the potential for crop improvement by traditional breeding of cassava is constrained by the high heterozygosity, highly outcrossing nature and low natural fertility of the plants. This therefore, necessitates the adoption of breeding programmes to introduce new varieties of higher nutritional quality.

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Tissue culture is one of the most successful, commercially exploited components of biotechnology and has been used for rapid clonal multiplication (micropropagation) of selected genotypes of diverse groups of plant species (Rani and Raina, 2000). The first study on tissue culture of cassava was by Kartha *et al*. (1974) who reported regeneration of shoots from meristems of five cassava varieties cultured on MS medium supplemented with 0.1 mg $l^{-1}$ Benzylaminopurine (BAP), 0.04 mg $l^{-1}$ Gibberellic acid ($GA_3$) and 0.2 mg $l^{-1}$ Naphylacetic acid (NAA). Bhagwat *et al*. (1996) reported regeneration of multiple shoots from nodal explants of cassava using 0.11 to 0.22 µM/l thidiazuron (TDZ), 2.2 µM/l BAP and 1.6 µM/l $GA_3$. Groll *et al*. (2001) regenerate of cassava through secondary somatic embryos in a media supplemented with picloram. A frequent *in vitro* culture manipulation for cassava involves standard media such as the Murashige and Skoog (1962) (MS medium), but with altered macro-and/or micronutrient concentrations. This manipulation was initially

restricted to embryo culture and nodal micro propagation, but was later extended to somatic embryogenesis (Taylor *et al*., 1996). The objective of this study was to test the effect of plants regulators on shoots multiplication in cassava cv TMS 92/0326.

## MATERIALS AND METHODS

### Plant material and disinfection

TMR 92/0326 cassava variety was obtained from the International Institute of Tropical Agriculture (IITA) PMB 2008, Messa Yaounde, Cameroon. Nodal cuttings (explants) for about 1.5 cm long were isolated from stems of this variety and then, cleaned under running tap water for 2 h. These explants were disinfected in 70% ethanol for 5 min followed by 3.5% sodium hypochlorite for 10 min and then rinsed four times (10 min each) in sterilized distilled water. All stages of disinfection were done in the laminar flow hood.

### Induction of callus from nodal cuttings

For the induction of callus, the nodal cuttings with budded buds were sub cultured in the closed test tubes (150 x 80 mm) containing 10 ml of BM supplemented with 0.01 to 0.1 mg $l^{-1}$ NAA. The pH of all media was adjusted to 5.8 before autoclaving. All sub cultures were incubated under the same conditions as during budding of axillaries buds. 50 explants were sub cultured per medium and the experiment was repeated thrice. The percentage of explants inducing callus and the fresh weight of callus were evaluated per medium after 28 days. For all experiments, the control was the BM without phytohormones.

### Induction and proliferation of multiple shoots from callus

The effect of BAP on the induction and proliferation of multiple shoots was used indirectly by using callus as explants. With indirect method, callus induced in the presence of NAA were firstly sub cultured in basal medium without phytohormones for 7 days. This sub culture is followed by a second sub culture in BM supplemented with 0.01 to 0.1 mg $l^{-1}$ BAP. Also, the pH of all media was adjusted to 5.8 before autoclaving. All second sub cultures were incubated under the same conditions as during induction of callus. 50 callus were sub cultured per medium and the experiment was repeated thrice. The percentage of callus inducing multiple shoots and the average number of shoots per callus were evaluated per medium after 45 days. For all experiments, the control was the BM without phytohormones.

### Data analysis

All experiments were set up in a completely randomized design. Differences between means were scored with Duncan's multiplication range test. The analysis of samples from each treatment was statically evaluated by analysis of variance (ANOVA, $p < 0.05$) and the interactive effect of two phytohormones was assessed by two-way ANOVA. The program used was SPSS (version 12 for windows).

## RESULTS

### Effect of NAA on the production and growth of callus from budded nodal cuttings

When cultured in the basal medium supplemented with 0.01 to 0.1 mg $l^{-1}$ NAA, the budded nodal cuttings produce callus after 28 days. These callus were yellowish and friable (Figure 1b). The highest percentage of callogenesis (69.8%) was obtained with 0.07 mg $l^{-1}$ NAA (Table 1). The fresh weight of callus varied with the concentration of NAA. The highest fresh weights of 1324.7 mg and 1521.2 mg were obtained with 0.07 and 0.09 mg $l^{-1}$ of NAA respectively (Table 1). With the other concentrations, the value of fresh weight was comprised between 545.5 mg and 1124.6 mg (Table 1).

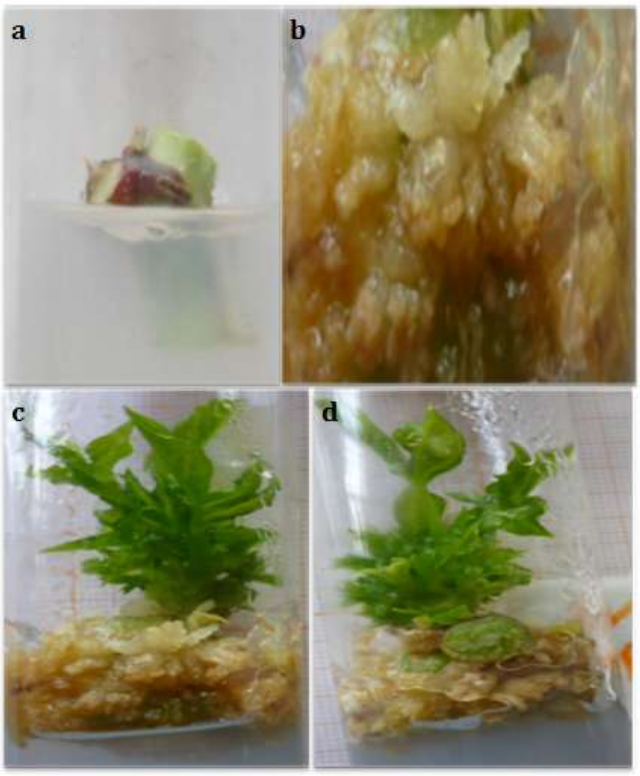


**Fig. 1. Proliferation of multiples shoots from callus of *Manihot esculenta.* (a) Nodal cutting cultured on MS supplemented with 0.05 mg $l^{-1}$ BAP; (b) Callus produced on nodal during 28 days on MS supplemented 0.08 g $l^{-1}$ NAA; (c and d) Multiple shoot proliferated in callus culture during 45 days on MS supplemented with 0.5 mg/$l^{-1}$ BAP.**

**Table 1. Effect of NAA on the callogenesis and fresh weight of callus from nodal cuttings with budded axillary buds of *Manihot esculenta* after 28 days of culture**

| NAA (mg $l^{-1}$) | % of callogenesis | Average fresh weight of callus (mg) |
|---|---|---|
| 0 | 0 | 0 |
| 0.01 | 0 | 0 |
| 0.03 | $2.1^{d}$ | $545.5^{b}$ |
| 0.05 | $6.4^{c}$ | $1124.6^{a}$ |
| 0.07 | $69.8^{a}$ | $1521.2^{a}$ |
| 0.09 | $41.4^{b}$ | $1324.7^{d}$ |
| 0.1 | $27.1^{e}$ | $927.6^{e}$ |

Duncan's multiple range test was used to evaluate the difference in the percentage of callogenesis and fresh weight of callus. Data sharing the same letter in the same column were not significantly different at 5% level.

**Effect of BAP on the induction of shoots from callus**

When cultured in the presence of 0.01 to 0.1 mg $l^{-1}$ BAP, the callus induce shoots after 45 days (Figure 1c and d). The percentages of explants inducing shoots and the average number of shoots per explants varied according to the concentration of BAP. The highest percentage of 82.3% was obtained with callus with 0.07 mg $l^{-1}$ BAP and the lowest percentages of 20.4 for callus were obtained with 0.03 mg $l^{-1}$ BAP (Table 2). Also the highest numbers of shoots per callus (22.4 to 27.2) were obtained with 0.09 mg $l^{-1}$ and 0.07 mg $l^{-1}$ (Table 2).

**Table 2. Effect of BAP on the inducing of shoot from callus of *Manihot esculenta* after 45 days of culture**

| BAP (mg $l^{-1}$) | % of callus inducing shoots | Average number of shoots per callus |
|---|---|---|
| 0 | 0 | 0 |
| 0.01 | 0 | 0 |
| 0.03 | 20.2[e] | 3.6[e] |
| 0.05 | 32.7[d] | 15.7[d] |
| 0.07 | 82.3[a] | 27.2[a] |
| 0.09 | 55.6[b] | 22.4[b] |
| 0.1 | 38.4[c] | 17.6[c] |

Duncan's multiple range test was used to evaluate the difference in the percentage of explants inducing shoots and average number of shoot per explants. Data sharing the same letter in the same column were not significantly different at 5% level.

## DISCUSSION

*M. esculenta* as a many tropical crops can be successfully propagated *in vitro*. Different kinds of explants such as nodal cutting (Konan *et al*., 1994; Smith *et al*., 1986; Fotso *et al*., 2014; Elian *et al*., 2014), meristems (Roca, 1992), axillary buds (Konan *et al*., 1994), shoot-tip (Berbee *et al*., 1974; Kartha *et al*., 1974a; Nair *et al*., 1979) can be used for this propagation. The shoot multiplicaion of *Manihot esculenta* can be indirect to induct by inducing shoot from callus as has been showed on the same the species by Acedo and Labana (2008); Ma and Xu (2002) or other species such as *Saussurea obvvaiiata* (Dhar and Joshi, 2005), *Dioscorea zinguiberensis* (Yongqin *et al*., 2003; Yuan Shu *et al*., 2005), *Dioscorea alata* (Fotso *et al*., 2013) and *Colocassia esculenta* (Yam *et al*., 1990; 1991). In this work, the callus of *Manihot esculenta* was induced from budded nodal cutting in the presence of NAA. Many works have been carried out on the establishment of callus culture from cassava in different laboratories (Eugene *et al*., 2011; Fietosa *et al*., 2007; Atehnkeng *et al*., 2005, Schopke *et al*., 1996). But this is the first time that callus is established from budded nodal cutting of this species. The highest percentage of callogenesis (69.8%) was obtained with 0.07 mg $l^{-1}$ NAA. Similar results were obtained by Peter *et al*. (2011) on two cultivars of the same species (cv Afisiati and cv Afebankye) but with higher concentration of NAA (8 to 10 mg $l^{-1}$). The shoot of cassava was induced indirectly from callus in the presence of BAP. This confirm the highest potential of BAP to induce shoots in different species at as been showed in several works (Anh *et al*., 2007; Konan *et al*., 1997; Fotso *et al*., 2013 and 2014; Davies, 2004). The percentages of explants inducing shoot were ranged from 20.2% (0.03 mg $l^{-1}$ BAP) to 82.3% (0.07 mg $l^{-1}$ BAP). Also with the same concentrations of BAP, the average number of shoots per explants was ranged from 3.6 to 27.2 for callus. These results are comparable to does obtained by Deden and Herni (2011) and Fotso *et al*. (2014) on same cassava varieties but BAP was used in combination with 0.1 to 1 mg $l^{-1}$ thidiazuron and 80 mg $l^{-1}$ adenine sulfate.

**Conclusion**

Indirect shoot multiplication of *M. esculenta is* achieved in this study. Callus was successfully produced from nodal cutting explants on Murashige and Skoog (MS) medium supplemented with 0.08mg $l^{-1}$ Naphthalene acetic acid (NAA). Multiple shoots were initiated from callus on MS medium supplemented with 0.08 mg $l^{-1}$ 6-benzylaminopurine (BAP).

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