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# Influence of Explants Type, Nodal Positions and Two Cytokinins on Cassava *(Manihot esculenta,* Crantz) *In vitro* Organogenesis

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors GHTC and SSH Concepted and designed the study. Authors FGP and JAH collected data. Authors SSH and FGP analyzed and interpreted the data. Authors GHTC and JAH wrote the manuscript. Author JAH made a critical revision of manuscript. Author SSH did the statistical analysis. Author CA funded the work. Authors GHTC and CA supervised the work and author CA gave the final approval. All authors read and approved the final manuscript.*

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## ABSTRACT

Cassava *In vitro* regeneration is essentially influenced by exogen factors as well as explants nature. This work aimed to evaluate explants type and positions influence, phytohormone on cassava plantlets regeneration in modified MS media. The apex and nodes of the position 3, 5, and 7 were initiated on Murashige and Skoog (MS) based media supplemented with Gamborg B5 vitamins and growth regulators such as naphthalene acetic acid (NAA), 6-benzylaminopurine (BAP) and zeatin (ZEAT) in different concentrations. The results indicated a significant difference (p=0.0001) among explants sprouted regarding the nodes position and the cultivar genotype during the time of culture. The highest rate of regenerated plantlets was found with nodes of position 5 (95.33%) followed respectively by nodes of the position 7 (93.33%), nodes of the position 3 (89.33%), and the apex (76%). Regarding the growth regulators, NAA (0.5 mg/l) + BAP (0.5 mg/l) combination was favorable for height growth (26.2cm), and leaves development (7.84 cm) in most of cultivars, whereas NAA (0.5 mg/l) + ZEAT (0.5 mg/l) combination was more favorable to rhizogenesis in overall cultivars. Among the cultivars, the best performance in stem height (26.2 cm), in leaf length (7.84 cm), and roots number (4.64) were observed with INA 1er cultivar. The cultivars RB89509, BEN 86052, and DOKU were less grown on MS supplemented with 0.5 mg/l of BAP or ZEAT. These findings could contribute of using nodes of the positions 5, 7 as potential explants and Zeatin for cassava plantlets rhizogenesis.

*Keywords: Cassava micropropagation; In vitro culture; nature of explant; growth regulators; plantlets regeneration.*

## 1. INTRODUCTION

*Cassava is* the most grown root crop in tropical and sub-tropical Africa [1]. It constitutes the most important staple food and has multiple medicinal, and industrial importance [2]. It is processed into different products such as gari, chips or tapioca etc… [3, 4]. Cassava can be efficiently produced on a small scale, without the need for agricultural mechanization or inputs purchasing even if the soil is poor with unpredictable rainfall [5]. It has been identified to increase food security on a global scale because of cassava can produce in any climate and soil conditions [6]. Thus, cassava tends to become a cash crop such as cotton, coffee, and oil palm by providing substantial income to farmers in developing countries. It is considered as a versatile culture that provides a response to developing countries priorities [7]. In Benin, cassava cultivation is facing many challenges including pests and diseases, shortage of planting materials, etc. [8]. These challenges are very crucial for cassava massive production in the country. Thus, disease free with good quality and homogenous planting materials is requested to enhance cassava production. Few years ago, cassava breeding research in Benin has leaded in the clearance of some local cultivars with important economic value [9, 10]. Since the multiplication of cassava cultivars in field by cutting was limited in the numbers of planting materials, bulky, and chore intensive, research works have been started for *in vitro* culture of cassava [11- 15]. Therefore, the improvement strategies for producing planting material through *in vitro* tissue culture remained an alternative and fast propagation method [16]. Thus, the micropropagation method has been optimized under the effect of growth regulators [12, 13, 17] and explants in several cassava cultivars [15 -18]. Its micropropagation and sanitation method was established and commonly used for massive production of healthy planting materials. The buds have been used to achieve a high rate of microshoots regeneration success, but the axillary buds in position 2 and 3 from the apex have revealed the explants of choice [19]. Furthermore, the explants origin and its age constitute the influencing factors for microshoots regeneration [20]. The explants in the positions out of 3 were not established yet. Thus, the position of buds in other species like pineapple [21] have been shown very influencing plantlets regeneration. It is also reported a high correlation between the type of explants, its positions, and the type of growth regulators used for plantlets regeneration [22]. From the past studies on cassava *in vitro* propagation, Naphthalene acetic acid (NAA), 6-Benzylaminopurine (BAP), Kinetin (Kin) and gibberellic acid (GA3) were commonly used in different concentration for cassava plantlets regeneration [23]. What's more, Zeatin have shown impressionable results in plantlets rhizogenesis that may greatly increase cassava nodes regeneration. The combined effect of BAP

and Zeatin, and their combination with NAA effects are not clearly established on cassava buds' organogenesis especially in the local cultivars of Benin. In some cultivars, Sessou et al [15] have established to regeneration methods based on TDZ, kinetin, and BAP for microshoots induction and on NAA, and IBA concentrations for microshoots rooting which did not well respond for overall cultivars. Understanding the influence of types and nodes explants in positions 4, 5, 6, and 7 on the plantlet's regeneration with antagonism effects of Zeatin, BAP, and NAA in the local cassava cultivars will accelerate cassava planting materials production and generate new knowledge for future research.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Explants Preparation

Three (03) cassava varieties including RB 89509, BEN 86056, INA 1er were collected from National Institute of Agricultural Research Center of Benin (INRAB/CRA-Sud Niaouli), and three (03) cassava cultivars including, DOKU, ASSAM-BANKYE, and WAVE-CI-RB were collected from University of Abomey-Calavi in Central and West African Virus Epidemiology Program (WAVE) cassava germplasm of 2020. Cuttings of 25 – cm - long were submerged in a hot water bath (varying between 45–50 °C) for 30 min and potted. The pots were watered once per day and twice per week until sprouting. Plants were grown under greenhouse at 28 °C, with relative humidity > 60%, with an approximate light/dark cycle of 12/12 h for four weeks. Four weeks after potting, the plants were indexed to be free for common virus (ACMV, and EACMV) by PCR as described by Houngue et al. [24]

Microcuttings from four weeks old stem were collected from each cassava cultivars in baker containing tap water and mouse to Central Laboratory of Biotechnology and Plant Breeding for tissue culture manipulation. Once in the laboratory, microcuttings were first washed with soap water and rinsed correctly with tap water. Under laminar flow hood, the explants were categorized in four groups for different cultivars. The first group is constituted of apex explants, the second group of node explants in the position 3, the third group of node explants in the position 5, and the fourth group of the node explants in the position 7. Explants of each group collected from different cassava cultivars were then sterilized by using ethanol 70° for 3 min followed by immersion in 0.1% mercuric chloride ($HgCl_2$) to which a few drops of Tween 20 were added for 5 min. Thereafter, explants were rinsed twice with sterile distilled water for 5 min [21].

### 2.2 Culture Condition and Plant Growth Regulators Treatment

Apex and node explants were cultured in solid Murashige and Skoog (MS) basic medium [25], supplemented with Gamborg B5 vitamins, 2 µmol CuSO4 [26] 0.01 mg/l of NAA, and 0.05 mg/l of BAP for explants initiation [27]. For organogenesis, initiation medium was modified by adding NAA (0.5 mg/l), BAP (0.5 mg/l), and ZEAT (0.5 mg/l). In total, four (4) different media varying in their concentration of growth regulators were tested. Media M1 and M2 were MS supplemented respectively with BAP (0.5 mg/l) and ZEAT (0.5 mg/l) whereas media M3 and M4 were MS supplemented with NAA (0.5 mg/l) + BAP (0.5 mg/l) and NAA (0.5 mg/l) + ZEAT (0.5 mg /l) respectively.

### 2.3 Experimental Design and Data Analysis

In order to test each category of explants with regard to genotype, a total of 24 treatments were used in overall cultivars for initiation. Regarding the organogenesis, a total of 360 explants were initiated at a rate of 15 explants per treatment. A completely randomized design was adopted with three replications of experiment. Data were collected during five weeks and assessed for regeneration kinetics and plantlets regeneration rate of different category of explants in overall cultivars. Data were analyzed using XLSTAT software version 2014. $\chi^2$ test of independence was used to show the difference in plantlets regeneration rate of different category of explants. Also, a generalized linear model was applied to determine the influence of growth regulators on the leaves number, roots number, leaves length, and stem height for each cultivar during the five weeks of culture. Analysis of variance (ANOVA) was performed to determine significant difference among category of explants regeneration using Newman and Keuls test at the 5% threshold [21].

## 3. RESULTS

### 3.1 Effect of Nodal Positions on Regeneration Kinetics

Plantlets regeneration were significantly ($p < 0.0001$) influenced by the node positions, as well as cassava genotypes, and culture time variation. However, the interaction between the

culture duration and nodal position did not significantly ($p > 0.05$) influence plantlets regeneration. Also, the genotypes, nodal position, and culture duration interaction was not significant [Table 1].

The plantlets regeneration according to nodes positions showed that the nodes, whatever their position, started regeneration from the first week at different rates. Thus, nodes in positions 5 and 7 were earlier with 90% of sprouted explants against 76.67% for the nodes in position 3 at the end of the first week. Thirty percent (30%) of apices were regenerated into plantlets in the first week and reached 100% by the end of the fourth week. Similar result was seen with the nodes of position 5 [Fig. 1].

Whatever the position of nodes, explants were sprouted in overall cultivars within the week 1 at different rates. BEN 86052 variety was earlier with 85% of sprouted explants in the first week and 100% of established plantlets in the second week of culture. On the other hand, the cultivars DOKU, WAVE-CI-RB, and RB 89509 presented respectively 50%, 60%, and 80% of sprouted plantlets in week 1 and 100% of established plantlets in week 4 [Fig. 2].

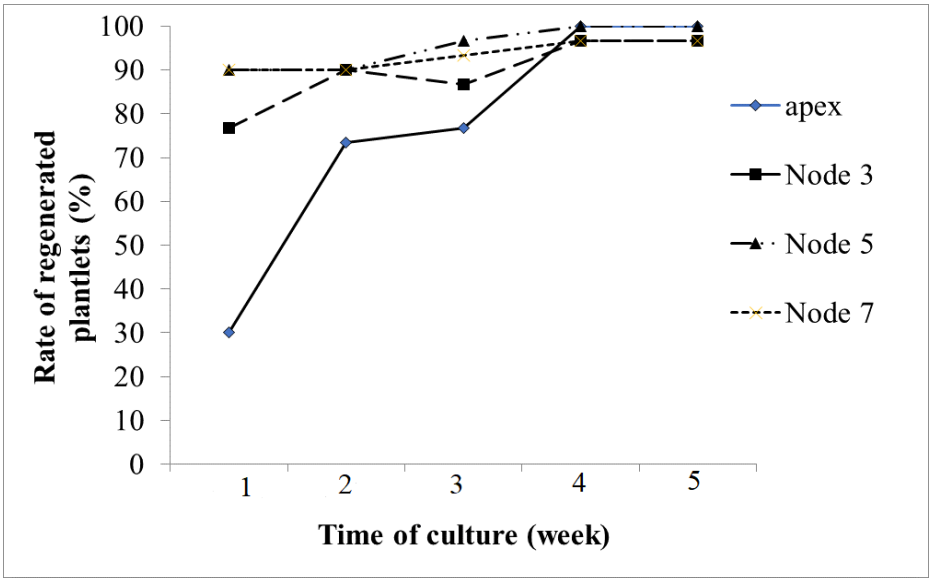


**Fig. 1. Plantlets regeneration rate according to the nodal position during 5 weeks of culture**

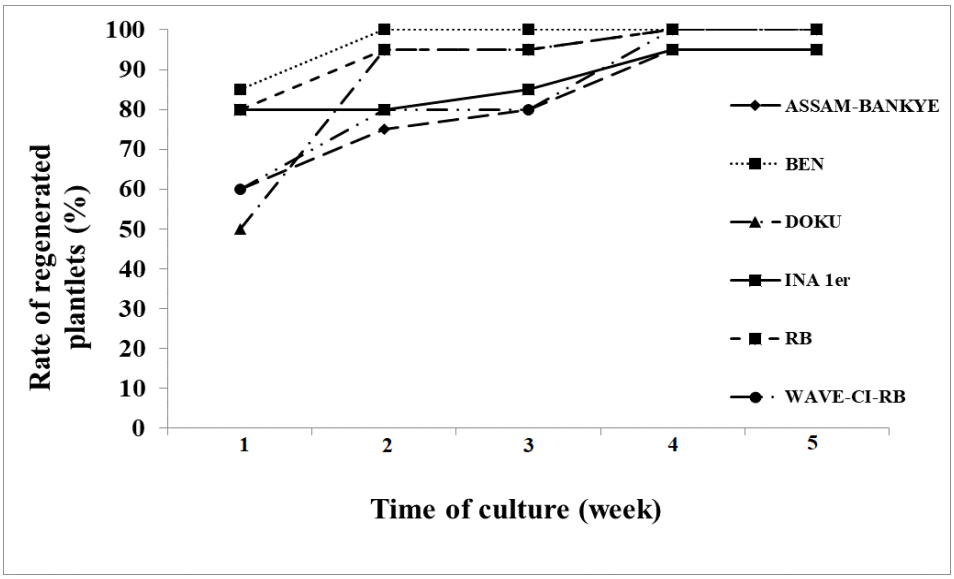


**Fig. 2. Plantlets regeneration rate according to the cultivars during five weeks of culture**

**Table 1. Effect of culture duration, genotypes, and nodal position on plantlets regeneration**

| Source | DF | Khi² (Wald) | Pr > Wald | Khi² | Pr |
|---|---|---|---|---|---|
| Culture duration | 4 | 3455.358 | < 0.000 | 12816.77 | < 0.000 |
| Genotypes | 5 | 20.695 | 0.000 | 20.028 | < 0.000 |
| Nodal Position | 3 | 40.193 | < 0.000 | 34.815 | < 0.000 |
| Genotypes x Culture duration | 20 | 3989.216 | < 0.000 | 14081.38 | < 0.000 |
| Culture duration x Nodal Position | 12 | 0 | 0.999 | 0 | 0.999 |
| Genotypes x Duration x Nodal position | 60 | 0 | 0.999 | 0 | 0.999 |

*DL: Degree of Freedom Pr: Probability*

### 3.2 Effect of Genotypes and Nodal Positions on Plantlets Regeneration Rate

There is a significant difference (P < 0.0001) in the regeneration rate throughout the genotype of cultivars and nodes position. The interaction between the genotypes and nodal position had a significant effect on the plantlets regeneration [Table 2].

In general, nodes of position 5 presented the highest rate of regenerated plantlets (95.33%) followed respectively by nodes of position 7 (93.33%); nodes of position 3 (89.33%), and the apex (76%). The regenerated plantlets from apex varied from 56% to 92% respectively in WAVE-CI-RB and INA1$^{er}$ cultivars. The rate of plantlets regeneration with the nodes of position 3, varied from 64% to 100% respectively for INA 1$^{er}$ and BEN 86052 cultivars. Nodes of positions 5 and 7 gave regenerated plantlets varying from 84% to 100% in the cultivars ASSAM-BANKYE, INA 1$^{er}$, RB 89509, and BEN 86052 varieties. With DOKU cultivar, the regenerated plantlets rate is identical with nodes of position 3 and the apex (83%) [Fig. 3].

**Table 2. Effect of genotypes and nodal position on *Cassava* bud burst**

| Source | DF | Somme of squares | Mean of square | F | Pr > F |
|---|---|---|---|---|---|
| Genotypes | 5 | 1.8150 | 0.3630 | 5.3778 | < 0.0001 |
| Nodal position | 3 | 3.4050 | 1.1350 | 16.8148 | < 0.0001 |
| Genotypes x Nodal position | 15 | 4.5650 | 0.3043 | 4.5086 | < 0.0001 |

*DL: Degree of Freedom ; Pr: Probability*

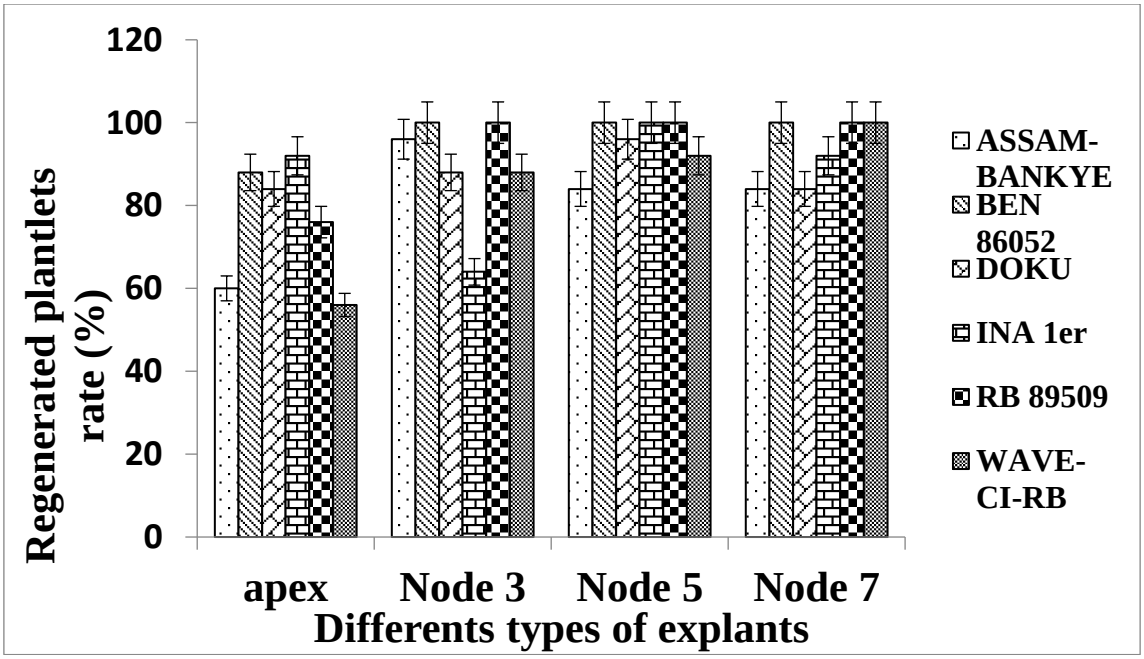


**Fig. 3. Plantlets regeneration according to the cassava genotypes and nodes position**

### 3.3 Combined Effects of Zeatin and BAP Concentrations on Plantlets Regeneration

The growth regulators such as zeatin, NAA, and BAP alone or in combination significantly (p = 0.0001) influenced the height of regenerated plantlets, the leaves length, the number of leaves, and the roots number throughout the cassava cultivars [Table 3]. The highest height of plantlets were found under the hormonal combination of NAA (0.5 mg/l) + BAP (0.5 mg/l) for the cultivars ASSAM-BANKYE (13.44 cm), DOKU (15.8 cm), RB 89509 (9.92 cm); WAVE-CI-RB (11.36 cm), and INA 1er (26.2 cm) while the lowest were found MS supplemented with ZEAT (0.5 mg/l) for the cultivars ASSAM-BANKYE (8 cm), DOKU (9 cm), RB 89509 (4.4 cm); WAVE-CI-RB (6.32 cm), and INA 1er (7.16 cm). Plantlets of BEN 86052 cultivar with minimal height (4.4 cm) were obtained on MS supplemented with BAP (0.5 mg/l) whereas plantlets with maximal height (13 cm) were obtained on MS supplemented with NAA (0.5 mg/ l) + ZEAT (0.5 mg/l) (Fig. 4).

Leaves length was significantly (P < 0.0001) influenced by the cassava genotypes and the growth regulators. The interaction between the genotypes and the medium had a significant (p = 0.0001) effect on the leaf's length of the regenerated plantlets [Table 3]. The longest leaf lengths were obtained on the modified MS supplemented with NAA (0.5 mg/l) and BAP (0.5 mg/l) for the cultivars ASSAM-BANKYE (2.24 cm), DOKU (7.6 cm), INA 1er (7.84 cm), RB 89509 (2 cm), and WAVE- CI-RB (2.12 cm) whereas the shortest lengths were obtained on the modified MS supplemented with ZEAT (0.5 mg/l) for overall cultivars [Table 3]. BEN 86052 cultivar presented on modified MS supplemented with BAP (0.5 mg/l) plantlets without leaf (0.0 cm) and while the longest leaf (13 cm) was observed on the modified MS supplemented with NAA (0.5 mg/ l) + ZEAT (0.5 mg/l).

**Table 3. Effect of culture media on the leaves, roots, and stem height of regenerated plantlets**

| Cultivars | MC | MSH (cm) | MLL(cm) | MNL | MRN |
|---|---|---|---|---|---|
| ASSAM-BANKYE | 0.5 mg/l BAP | 9.68 | 1.84 | 1.04 | 0.52 |
| | 0.5 mg/l ZEAT | 8 | 0.36 | 0.08 | 1.24 |
| | 0.5 mg/l NAA.ZEAT | 9.4 | 1.2 | 0.44 | 3.68 |
| | 0.5 mg/l NAA.BAP | 13.44 | 2.24 | 1.08 | 0.88 |
| BEN 86052 | 05 mg/l BAP | 4.4 | 0 | 0 | 1.32 |
| | 0.5 mg/l ZEAT | 6.88 | 2.24 | 0.68 | 1.44 |
| | 0.5 mg/l NAA.ZEAT | 13 | 4.76 | 1.04 | 2.88 |
| | 0.5 mg/l NAA.BAP | 9.8 | 2.33 | 0.96 | 0.56 |
| DOKU | 0.5 mg/l BAP | 16 | 4.68 | 2 | 0.04 |
| | 0.5 mg/l ZEAT | 9 | 1.8 | 0.64 | 2.2 |
| | 0.5 mg/l NAA.ZEAT | 9.12 | 2.72 | 0.44 | 4.12 |
| | 0.5 mg/l NAA.BAP | 15.8 | 7.6 | 1.64 | 1.8 |
| INA 1er | 0.5 mg/l BAP | 9.64 | 3.76 | 1.8 | 1.76 |
| | 0.5 mg/l ZEAT | 7.16 | 2.08 | 0.6 | 3.76 |
| | 0.5 mg/l NAA.ZEAT | 19.76 | 4.4 | 1.12 | 4.64 |
| | 0.5 mg/l NAA.BAP | 26.2 | 7.84 | 1.88 | 3.08 |
| RB 89509 | 0.5 mg/l BAP | 6.96 | 3.19 | 1.84 | 1.2 |
| | 0.5 mg/l ZEAT | 4.4 | 0 | 0 | 0.84 |
| | 0.5 mg/l NAA.ZEAT | 6.82 | 3.35 | 1 | 4 |
| | 0.5 mg/l NAA.BAP | 9.92 | 5.4 | 2 | 1.28 |
| WAVE-CI-RB | 0.5 mg/l BAP | 7.24 | 1.24 | 0.52 | 0.32 |
| | 0.5 mg/l ZEAT | 6.32 | 0.68 | 0.2 | 2.16 |
| | 0.5 mg/l NAA.ZEAT | 6.4 | 0.52 | 0.2 | 2.44 |
| | 0.5 mg/l NAA.BAP | 11.36 | 2.12 | 0.88 | 0.56 |
| **P-value** | $R^2$ (%) | 41.9 | 34.44 | 31.04 | 44.88 |
| - | Cultivars | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| | Culture media | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| | Cultivars x Media | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

*MS: Murashige and Skoog; NAA: Naphalene-acetic acid; BAP: 6- benzyl-aminopurine; ZEA: Zeatin; MSH: Mean of stem height; MLL: Mean of leaf length; MNL: Mean of leaves number; MRN: Mean of roots number*

Regarding the number of leaves, there is a significant (p = 0.0001) influence of growth regulators on the number of leaves, as well as the interaction between the medium and the cassava genotypes [Table 3]. The combination of NAA (0.5 mg/l) and BAP (0.5 mg/l) on the modified MS medium gave the highest leaves number for the cultivars ASSAM-BANKYE (1.08), INA 1er (1.88), DOKU (1.64), and RB 89509 (2) while the lowest leaves number were obtained on modified MS supplemented with ZEAT (0.5 mg/l) in overall cultivars. Modified MS supplemented with BAP (0.5 mg/l) produced a high number of leaves in DOKU (2) while there were no leaves in BEN 86052 cultivar [Table 3].

Number of roots was significantly (p = 0.0001) influenced by the hormonal growth regulators throughout the cassava genotypes. MS Medium supplemented with NAA (0.5 mg/l) and ZEAT (0.5 mg/l) gave the highest number of roots in overall cassava cultivars with a high value in ASSAM-BANKYE (4.64) cultivar. The lowest number of roots was obtained on MS supplemented with BAP (0.5 mg/l) in *Doku* (0.04) cultivar. BEN 86052 and RB 89509 cultivars gave the lowest roots number on MS supplemented with ZEAT (0.5 mg/l) in respective values of 0.44, and 0.36 [Table 3] (Fig. 5).

## 4. DISCUSSION

The current study was conducted to determine the influence of the types and positions of explants associated with the growth regulators on *in vitro* propagation of cassava cultivars. The findings indicated a significant difference in the plantlet's regeneration regarding the nodes position, genotypes, and the time of culture. The node position constitutes an important factor to be considered for cassava *in vitro* regeneration according to the physiological maturity of the explant. Also, the cells' activities are differed in each node position of the plant. Cells in the youngest nodes are more active in multiplication than the oldest but in contrast the oldest nodes sprouted better than the youngest. The findings showed that the nodes of positions 5 and 7 had early regenerated plants about 90% in the week 1 as compared to the nodes of position 3 and apex which sprouted with low rate (30%) in week 1. The sprouted rate of the node of position 7 was stable within four and six weeks. The lowest rate observed with the apex may be attributed to the disinfection protocol applied which did not better promote the apex regeneration because of their fragility [20].

According to Gitonga et al. [ 19] on *Macadamia spp*, nodal explants from the 1st, 2nd and 3rd nodal position were more suitable for *in vitro* culture than those obtained from the 4, 5, and 6 because of the rapid multiplication of the cells in the youngest explants. Gubis et al, [ 20] explained the differential response between the apical and axillary nodes by their position and their age. The cultivar BEN 86052 was earlier as compared to DOKU, WAVE-CI-RB, and RB 89509 cultivars. Considering overall cultivars, nodes of the position 5 favored had better rate (95.33%) of plantlets regeneration by comparing to other positions, especially the apex (76%). Research work carried out on taro had shown the influence of genotype on the rate of plantlets regeneration [28]. Apex and the node of position 3 contain sufficient endogenous auxins than those of the position 5 and 7. By adding exogenous auxin (NAA) in culture medium may increase the concentration of auxins in the tissue that will influence the early sprouting of the explants [19]. Thus, the nodes of positions 5 and 7 were more suitable for *in vitro* culture than those taken from apex and node position 3. The difference observed can be related to the concentration of endogenous growth regulators between apical and axillary nodes which could influence their organogenesis response. A significant influence of variety, culture medium as well as their interaction on stem height was revealed. The best height growth was found on MS supplemented with NAA (0.5 mg/l) and BAP (0.5 mg/l) for the cultivars ASSAM-BANKYE, RB 89509; WAVE-CI-RB, and INA 1er. Conversely, these better growths were achieved on MS supplemented with NAA (0.5 mg/l) and ZEAT (0.5 mg/l) for the cultivar BEN 86052. ZEAT or BAP, in combination with NAA, achieved better height growth of plants than the alone application. However, low height was observed on MS supplemented with NAA (0.5 mg/l) and BAP (0.5 mg/l) for the cultivar *DOKU*. For the remaining cultivars, there is a synergetic action between NAA and BAP or ZEAT. A high significant influence of the factors and their interaction was noted on the number and length of leaves. MS supplemented with NAA (0.5 mg/l) + BAP (0.5 mg/l) combination is more favorable to leaf formation than MS supplemented with NAA (0.5 mg/l) and ZEAT (0.5mg/l). The combination BAP (0.5 mg/l) and NAA (0.5 mg/l) promotes better leaves formation in ASSAM-BANKYE, INA 1er, and RB 89509, whereas BAP (0.5 mg/l) only is sufficient for better leaf formation in *DOKU* cultivar with negative effect on BEN86052 cultivar. Similarly, ZEAT (0.5 mg/l)

in combination with NAA (0.5 mg/l) had improved leaf formation. Regarding the number of leaves, a high number of leaves was observed on MS supplemented with BAP (0.5 mg/l) or NAA (0.5 mg/l) + BAP (0.5 mg/l) compared to MS supplemented with ZEAT (0.5 mg/l) or NAA (0.5 mg/l) + ZEAT (0.5 mg/l). A synergistic effect of NAA with one of two cytokinins was observed on leaf number and lengths in overall cultivar. Regarding plantlets rhizogenesis, a highly significant effect was observed on MS supplemented with NAA (0.5 mg/l) + ZEAT (0.5 mg/l) and NAA (0.5 mg/l) + BAP (0.5 mg/l) that highlighted the role of NAA in roots formation [15]. These results therefore indicated that each variety may require a particular combination

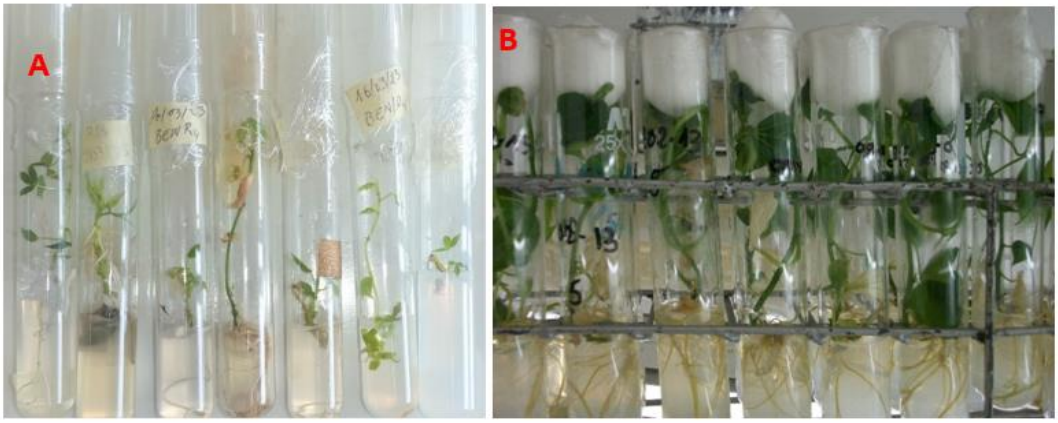


**Fig. 4. Plantlets of BEN 86052 cultivar regenerated on: (A) MS supplemented with 0.5 mg/l of BAP, and (B) MS supplemented with NAA (0.5 mg/ l) + ZEAT (0.5 mg/l)**

**Fig. 5. Roots development in the plantlets regenerated on MS supplemented with NAA and ZEAT**

of growth regulators. Thus, in the same variety, this combination differs according to the expected response, whether for example; stem multiplication, rhizogenesis, and phylogenesis. In addition, MS supplemented with BAP and NAA was more favorable to stem height growth and phylogenesis while ZEAT associated with NAA was more appropriate for rhizogenesis. The type of cytokinins and its combination with NAA at the concentration of 0.5 mg/L influenced cassava *in vitro* organogenesis. Based on the findings of Kbiach, [29] on *Quercus Suber* L., BAP appears to be the best suited for shoots development.

The suitable combination of growth regulators that induces positive response at a given concentrations may repress the same response when used at higher concentrations [30]. These results therefore indicated that the growth regulators could affect or control the development in plants and the used depend to the tissue and the kind of manipulations

## 5. CONCLUSION

The implementation of cassava micropropagation techniques revealed that the nodes of positions 5 and 7 on the stem are the earliest for plantlets regeneration with better rates at week 5. BAP (0.5 mg/l) combined with NAA (0.5 mg/l) is recommended for the height growth and leaf formation for overall, while ZEAT (0.5 mg/l) especially combined with NAA (0.5 mg/l) is more favorable to *cassava plantlets* rhizogenesis. In the process of producing cassava plantlets, BAP would be recommended for micropropagation, and ZEAT for rooting.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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