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Microtuberization of *Manihot esculenta* Crantz cv. TMS 95/0211

Elian Hubert Dieu Béni*[1,3], Fotso[1,2], Tchouga Alvine Ornella[1], Djeuani Astride carole[1,4], Zinga Innocent[3], Silla Semballa[3] and Omokolo Ndoumou Denis[1]

[1]Laboratory of Plant Physiology, Higher Teacher's Training College, University of Yaoundé 1. P.O. Box 47, Yaoundé, Cameroon;
[2]Department of Biology, Higher Teachers Training College Bambili, University of Bamenda, P.O. Box 39 Bamenda, Cameroon;
[3]Department of Biology, Faculty of Sciences, University of Bangui, P.O. Box 1450 Bangui, Central African Republic;
[4]Department of plant Biology, Faculty of Sciences, University of Yaoundé I, P.O. Box 824 Yaoundé, Cameroon.

ABSTRACT

The objective of this work was to induce microtuberization in cassava variety TMS 95/0211 grown in liquid media with addition of 6-bezylaminopurine (BAP), Kinetin (Kin) and α-naphthaleneacetic acid (NAA) in different concentration of sucrose. 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 growth and development of shoots to complete plantlets of *M. esculenta* can also be under the control of BAP/NAA complementary and synergistic action. In fact, when subcultured in the same media, shoot from nodal cutting regenerate plantlets at 96 % in presence of 0.5/0.05 mg l^{-1} BAP/NAA. The highest percentages of plantlets forming microtubers (44.6 to 77.4%) were obtained with 30 to 40 g l^{-1} in the presence of these growth regulators. The highest number of microtubers per plantlet (4 to 5), the greatest microtuber diameter (53.3 to 123.8 mm) and higher fresh weight of microtubers (209.4 to 432.0 mg) were obtained when 30 to 40 g l^{-1} sucrose were used.

KEY WORD: Microtuberization, liquid medium, phytohormones, sucrose, *Manihot esculenta*.

Abbreviations: MS: Murashige and Skoog; BAP-6-benzylaminopurine; Kin: Kinetin; NAA: α-naphthalene acetic acid, TMS: Tropical *Manihot* Species, IITA: International Institute of Tropical Agriculture.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial shrub from the American tropics with a tuberous edible root. It is cultivated throughout the tropical world for its starchy roots, from which cassava breads, flour, tapioca and beverages are derived. In the tropics cassava is the third most important source of calories with more than 600 million people in Africa, Asia and Latin America depending on it [1, 2]. Cassava is multiplied mainly by stem cuttings which is a slow process when compared with grain crops. Micropropagation allows rapid multiplication of clones in a short duration under disease free, controlled environment on yearly basis. Cassava can be easily micropropagated [3, 4, 5, 6, 7]. Micropropagated plants, when cultured under suitable conditions, produce *in vitro* microtubers [8]. The cassava is crop restricted to tropical and subtropical regions, which may account for the scanty available literature regarding environmental and endogenous factors involved in the control of the tuberization process. Most of the information available about tuberization was from potato (*Solanum tuberosum* L.) and *Dioscorea sp*. It is well known that the tuberization is a complex process, which is coordinated, by the environment conditions, hormonal control, nutrition and genetics [9]. Potato and yam tuberization is characterized by anatomical modifications, hormone and physiological changes. The use of *in vitro* growth of plants for production of microtuber has the advantage of higher control of the different factors that might affect the tuber formation, compared to plants grown in soil [10]. Furthermore, by using microtubers it is possible to maintain genebank accessions in a much smaller space, and to remove virus- infection in asexually propagated species [11, 12]. Previous potato research focused on the influence of growth regulators and photoperiod on the induction of tuberization [13, 14, 15, 16, 17]. Short day and sugars influence yam tuberization [18, 19]. The addition of carbohydrate on the growing media shifts the source/sink ratio affecting the development of shoots and tuber [20]. This study was aimed to induce microtuberization in cassava variety TMS 95/0211 grown in liquid media with the addition of 6-bezylaminopurine (BAP), Kinetin (Kin) and α-naphthaleneacetic acid (NAA) in different concentration of sucrose. Many factors such as the presence or the absence of growth regulators, the concentration of sugars, the mineral composition of the medium and the

Corresponding Author: Elian Hubert Dieu Béni, Laboratory of Plant Physiology, Higher Teacher's Training College, University of Yaoundé 1. P.O. Box 47, Yaoundé, Cameroon; eliandieubeni@gmail.com
Tel.: (+237) 51911591 (Cameroon) or (+236) 75012301 (Central African Republic)

photoperiod were known to influence the organogenesis *in vitro* [13, 21, 22, 23]. Therefore, the effect of growth regulators (BAP and NAA) and sucrose, together or in combination with the micropropagation of *M. esculenta* were studied.

MATERIAL AND METHODS

Plant material and disinfection

Cassava variety, TMS 95/0211 was obtained from the Internal Institute of Tropical Agriculture (IITA) PMB 2008, Messa Yaoundé, 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.

Regeneration of plantlets

For regeneration of plantlets, disinfected nodal cuttings were cultured in closed test tubes containing each 10 ml of [24] supplemented with [25] vitamins and containing 30 g l^{-1} of sucrose, 0.5 mg l^{-1} of 6-benzylaminopurine (BAP), 0.05 mg l^{-1} of α-naphthalene acetic acid (NAA), 3 g l^{-1} of activated charcoal and 7 g l^{-1} of Difco agar. The pH of the medium was adjusted to 5.8 with NaOH solution (1 N) or HCl solution (0.1 N) before autoclaving at 115 ° C for 30 min under a pressure of 1.6 ± 0.1 kg cm^{-2}. All cultures were incubated under 80 µmol m^2 s^{-1} light provided by cool white fluorescent tube lamps (Mazda) at a photoperiod of 16 h at 26 ± 1° C. 150 nodal cuttings were cultured and the experiment was repeated twice. After 21 days, shoots proliferated at the level of the node and when maintained in the same medium for 15 days, each shoot differentiated into roots and became plantlets. After being isolated from the node, those plantlets constituted the explants for the following experiment.

Induction and production of microtubers

Regenerated plantlets from nodal cuttings were sub cultured in 150 x 80 mm glass culture tubes containing semi liquid (3.5 g l^{-1} Difco agar) of half strength MS/2 supplemented with Morel and Wetmore vitamins and then containing 30 g l^{-1} of sucrose, 5 µM of jasmonic acid, 0.01 to 0.1 mg l^{-1} NAA, 0.1 to 0.6 mg l-1Kin and combination NAA plus Kin. However the effects of these three growth regulators were evaluated on the induction and production of microtubers. The pH of all the media was also adjusted to 5.8 before autoclaving. All cultures were also incubated under the same conditions as during plantlets regeneration. 30 plantlets were subcultured for each concentration per growth regulator and all experiments were repeated twice. The percentage of plantlets inducing microtubers was evaluated after 30 days. 60 days later, without any secondary sub culture, the average number of microtubers produced, the average length and the average fresh weight were evaluated for each treatment. For all experiments the control was the MS/2 semi liquid supplemented with vitamins, sucrose and jasmonic acid. The effect of sucrose was also evaluated during the same period on the induction and production of microtubers. However, MS/2 semi liquid medium was supplemented with 20, 30, 40, 50 and 60 g l^{-1} of sucrose together with 0.07 mg l^{-1} BAP, 0.4 mg l^{-1} Kin and 0.07/0.4 mg l^{-1} NAA/Kin. The control was without sucrose.

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 statistically 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

Regeneration of plantlets from nodal cuttings culture

When cultured on MS supplemented with 0.05 mg l^{-1} BAP and 0.5 mg l^{-1} NAA, the lateral buds on the nodal cuttings appear visible after 7 days. When maintained in the same medium, 96 % of shoots growth to plantlets (Figure 1a) with an high 17.2 ± 2.4 cm, an average number of leaves of 13.1 and an average number of roots of 12.5 within 26 days (Table 1).

Effect of NAA, Kin and NAA/Kin ratio on the production and growth of microtubers from plantlets

When sub cultured in the basal medium supplemented with NAA or Kin or NAA/Kin ratio, the plantlets differentiate microtubers at their base in 35 days (Figure 1b). These microtubers growth and after 65 days, they were isolated from plantlets (Figure 1d) and weighed. The percentages of plantlets producing microtubers, the number of microtubers per plantlet, their diameter and fresh weight varied with the type and the concentrations of phytohormones. The highest percentage of plantlets producing microtubers (66.8 %) was obtained with 0.07/0.4 mg l^{-1} NAA/Kin; follow by 0.07 mg l^{-1} NAA (36.2 %) and by 0.4 mg l^{-1} Kin (35.4 %) (Table 2). The

number of microtubers per plantlet varied from 1 to 3 for all treatments (Table 5). The microtubers with the highest diameter (127.3 mm) were obtained with 0.07/0.4 mg l^{-1} NAA/Kin; follow by 0.4 mg l^{-1} Kin (59.2 mm) and by 0.07 mg l^{-1} NAA (56.7 mm) (Table 2). With all other treatments, the diameter of microtubers was below these higher values (Table 2). The highest fresh weights of microtubers (317.8 mg) was obtained with 0.07/0.4 mg l^{-1} NAA/Kin, follow by 0.4 mg l^{-1} Kin (263.5 mg) and by 0.07 mg l^{-1} NAA (263.5 mg) (Table 2). The lowest fresh weights of microtubers (191.6 mg, 198.7 mg and 185.4 mg) were obtained with 0.01 mg l^{-1} NAA; 0.6 mg l^{-1} Kin and 0.1/0.4 mg l^{-1} NAA /Kin respectively (Table 2).

Effect of sucrose on the production and growth of microtubers from plantlets

When plantlets were sub cultured in the presence of 10 to 60 g l^{-1} sucrose supplemented with 0.07 mg l^{-1} NAA or 0.4 mg l^{-1} Kin or 0.07/0.4 mg l^{-1} NAA/Kin, they differentiate microtubers in 35 days. They were then harvested after 65 days and Weighed. The highest percentages of plantlets producing microtubers (53.4 % and 77.4 %) were obtained with 30 g l^{-1} sucrose in the presence of 0.07 mg l^{-1} NAA and 0.07/0.4 mg l^{-1} NAA/Kin respectively, while in the presence of 0.4 mg l^{-1} Kin, a maximum of 49.6 % plantlets produce microtubers with 40 g l^{-1} sucrose (Table 3). With all concentrations of sucrose tested in the presence of NAA and Kin used separely, the number of microtubers per plantlets varied from 1 to 4, while in the presence of NAA/Kin, a maximum of 5 microtubers per plantlet was obtained with 30 g l^{-1} sucrose (Table 3). The largest microtubers (111.2 to 123.8 mm of diameter) were obtained with 40 and 30 mg l^{-1} sucrose respectively in the presence of NAA/Kin compared to other treatments (Table 2). Also with 40 and 30 mg l^{-1}sucrose and in the presence of 0.07/0.4 mg l^{-1} NAA/Kin, microtubers presented the highest fresh weights of 412.7 mg and 432.0 mg respectively compared to all other treatments (Table 3).

DISCUSSION

The fact that auxin and cytokinin must be combined to induce the proliferation of shoot in *M. esculenta* confirmed the complementary and synergistic action of these growth regulators as has been shown in several accessions of cassava such as Gbèzé, sazoué Agric, Okoyao, Sèkandji, Ahouandjan and 92/0057 [26]. The growth and development of shoots to complete plantlets of *M. esculenta* can also be under the control of BAP/NAA complementary and synergistic action. In fact, when subcultured in the same media, shoot from nodal cutting regenerate plantlets at 96%. This percentage is comparable to that obtained by [27] in *M. esculenta.* The highest growth parameters (high of plantlets, number of leaves and number of roots per plantlet) were obtained with BAP/NAA ratio. In fact, in this work, BAP, NAA and Kin were supplemented in the same medium at a single concentration while in other species they were used separately and sometimes at various concentrations [28]. In this study, the microtubers were produced when the basal medium was supplemented with 2 µM jasmonic acid different concentration of NAA, Kin, NAA/Kin ratio and sucrose. Till date none or may be very few has been reported on microtuberization in cassava species while several has been reported on other crop tuber species such as *Solanum tuberosum* [29]. *Xanthosoma sagitifolium* [30], *Dioscorea sp* [17, 23]. These different works show that, the percentage of plantlet producing microtubers, the number of microtubers per plantlet and their size varies not only according to species but also according to the culture conditions and this is confirmed in this study. In fact, jasmonic acid has been to be shown effective on microtuberization [22], but this effectiveness also depend on other culture condition meanly de type and the concentration of phytohormones used and the concentration of sucrose in the culture media [31, 32, 33,34]. The result of this study shows that, microtubers in *M. esculenta* can be produced in the presence of 2 µM jasmonic acid and 0.01 to 0.1 mg l^{-1}NAA, 0.1 to 0.6 mg l^{-1} Kin used separately or in ratio 0.01/0.4 to 0.1/0.4 mg l^{-1} NAA/Kin. The highest percentage of plantlets producing microtubers were 36.2 % (0.07 mg l^{-1} NAA), 35.4 % (0.4 mg l^{-1}Kin) and 66.8 % (0.07/0.4 mg l^{-1}NAA/Kin). With these concentrations the highest numbers of 3 and 4 microtubers were obtained with the respective diameter of 56.7 mm, 59.2 mm and 127.3 mm and the respective fresh weight of 247.1 mg, 263.5mg and 317.8 mg for NAA, Kin and NAA/Kin. Then, NAA and Kin were more effective on the microtuberization in *Manihot esculenta* when they were used in ratio or combination. These results were comparable to those obtained by [30] in *Xanthosoma sagitifolium* during which the effect of photoperiod and and thermoperiod were also studied on the microtuberization, but they contrasts which those obtained by [29] which showed that NAA or Kin used separately were more effective on the microtuberization in *Solanum tuberosum*. When the basal medium is supplemented with 10 to 60 g l^{-1} sucrose and 0.07 mg l^{-1} NAA or 0.4 mg l^{-1} Kin or 0.07/0.4 NAA/Kin. The results obtained show that under these conditions, the microtuberization is easily obtained with plantlets produced *in vitro* from nodal cuttings. Similar results were reported by [13] on the same species and *D. bulbifera* but under the control of different factors. The percentage of plantlets producing microtubers, the number of microtubers per plantlet, their diameter and their fresh weight varied according to the concentration of NAA, Kin and NAA/Kin used or according to the concentration of sucrose in the medium. The highest percentage of plantlets producing microtubers (77.4 %) the highest number of microtubers per plantlet (5) having bigger sizes (123.8 mm of diameter and 432.0 mg of fresh weight) were obtained with NAA/Kin ratio and 30 g l^{-}

^{1} sucrose. This result contrasts those obtained by [34] in *Pterostylis sanguinea* which showed that in the presence of 1.5 and 2 mg jasmonic acid and NAA or Kin. The microtuberization is induced at least with 60 g l^{-1} sucrose, and those of [35] which show that in *Dioscorea composita*, microtuberization is induced with 80 to 100 mg l-1 sucrose.

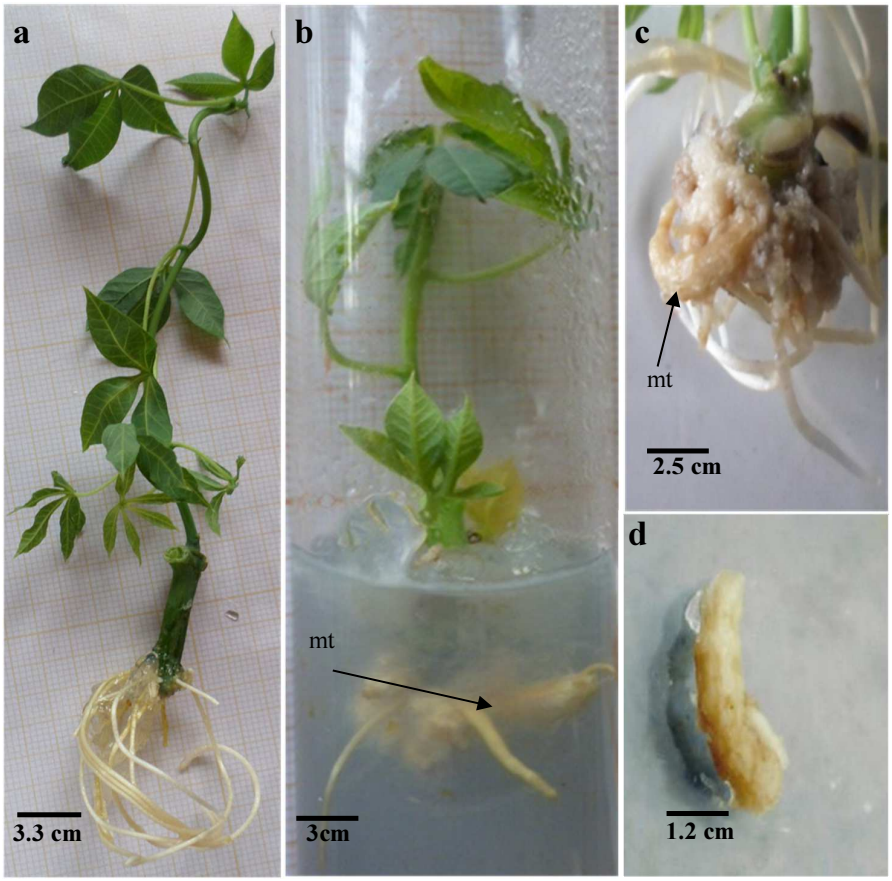

Fig 1. *In vitro* tuberization of cassava. Plantlet regenerated from nodal cutting (a). Plantlet with microtubers (mt) induced on MS/2 medium supplemented with 0.07/0.4 mg l^{-1} NAA/Kin and 30 g l^{-1} sucrose after 65 days of culture (b and c). Isolated microtuber from plantlet cultured in the presence of 0.07 mg l^{-1} NAA or 0.4 mg l^{-1}kin or 0.07/0.4 mg l^{-1} NAA/Kin and 30 to 40 g l^{-1} sucrose.

Table 1. Effect of BAP/NAA ratio on the regeneration and growth of plantlets from isolated shoots of *Manihot esculenta* after 26 days of culture.

BAP/NAA (mg l-1)	% of shoot growth to plantlets	Height of plantlets (cm)	Average number of leaves per plantlets	Average number of roots per plantlets
0	56^{f}	12.7 ± 1.3^{e}	7.1^{e}	8.1^{d}
0.1/0.05	62^{d}	13.6 ± 3.1^{d}	6.9^{e}	6.7^{d}
0.3/0.05	71^{c}	15.2 ± 1.5^{b}	11.0^{c}	11.2^{b}
0.5/0.05	96^{a}	17.2 ± 2.4^{a}	13.1^{a}	12.5^{a}
1/0.05	94^{b}	14.2 ± 1.7^{c}	12.4^{b}	12.1^{a}
3/0.05	58^{e}	12.8 ± 3.2^{e}	10.8^{c}	9.4^{c}
5/0.05	29^{g}	9.8 ± 2.4^{f}	8.2^{d}	8.9^{c}

χ^{2} and Duncan's multiple range test was used to evaluate the high of plantlets, the average number of leaves and average number of roots per plantlets. Data sharing the same letter in the same column were not significantly different at 5 % level.

Table 2. Effect of NAA, Kin and NAA/Kin ratio on the production regeneration and growth of microtubers from plantlets of *Manihot esculenta* after 65 days of microtuber

Phytohormones (mg l^{-1})	Numbers of plantlets cultured	% of plantlets producing microtuber	Growth of microtubers		
			Number per Plantlet	Average diameter (mm)	Average fresh Weight (mg)
NAA					
0	30	0	0	0	0
0.01	30	26.2^{b}	1^{c}	32.1^{e}	191.6^{f}
0.02	30	25.6^{c}	2^{b}	38.8^{d}	211.3^{c}
0.05	30	24.2^{d}	2^{b}	41.4^{b}	219.7^{b}
0.07	30	36.2^{a}	3^{a}	56.7^{a}	247.1^{a}
0.09	30	24.4^{d}	1^{c}	38.6^{d}	197.2^{d}
0.1	30	7.4^{e}	1^{c}	40.4^{c}	196.4^{e}
Kin					
0	30	0	0	0	0
0.1	30	0	0	0	0
0.2	30	15.2^{d}	1^{c}	36.3^{e}	201.3^{d}
0.3	30	20.3^{b}	2^{b}	53.8^{b}	236.5^{b}
0.4	30	35.4^{a}	3^{a}	59.2^{a}	263.5^{a}
0.5	30	17.6^{c}	2^{b}	40.9^{c}	211.7^{c}
0.6	30	14.6^{e}	2^{b}	37.1^{d}	198.7^{e}
NAA/Kin					
0	30	0	0	0	0
0.01/0.4	30	8.6^{e}	2^{b}	40.4^{f}	177.2^{f}
0.02/0.4	30	28.7^{d}	2^{c}	68.5^{d}	205.7^{d}
0.05/0.4	30	30.9^{c}	3^{b}	120.8^{b}	311.6^{b}
0.07/0.4	30	66.8^{a}	4^{a}	127.3^{a}	317.8^{a}
0.09/0.4	30	47.3^{b}	2^{c}	69.8^{c}	265.1^{c}
0.1/0.4	30	5.1^{f}	2^{c}	51.9^{e}	185.4^{e}

Duncan's multiple range test was used to evaluate the difference between the percentage of plantlet production of microtubers, number of microtubers per plantlet, average diameter of microtuber and average fresh weight of microtuber per plantlet. Data sharing the same letter in the same column were not significantly different at 5 % level.

Table 3. Effect of sucrose on the production and growth of microtubers from plantlets of *M. esculenta* after 65 days of culture.

Phytohormones	Sucrose (g l^{-1})	% of plantlets producing microtubers	Average number of microtubers per plantlets	Average diameter of microtubers (mm)	Average of fresh weight of microtuber (mg)
	0	0	0	0	0
	10	6.3^{e}	1^{d}	32.5^{e}	191.3^{e}
NAA	20	22.2^{c}	2^{c}	44.7^{b}	196.6^{d}
(0.07 mg l^{-1})	30	53.4^{a}	4^{a}	48.7^{b}	199.6^{c}
	40	32.1^{b}	3^{b}	56.3^{a}	209.4^{a}
	50	9.7^{d}	1^{d}	41.0^{c}	200.2^{b}
	60	5.1^{f}	1^{d}	38.1^{d}	187.9^{f}
	0	0	0	0	0
	10	5.4^{f}	1^{e}	42.1^{d}	209.2^{d}
Kin	20	38.4^{c}	3^{b}	56.8^{c}	236.7^{a}
(0.4 mg l^{-1})	30	41.3^{b}	4^{a}	63.4^{a}	259.4^{b}
	40	49.6^{a}	4^{a}	61.1^{b}	269.7^{c}
	50	38.5^{c}	2^{d}	32.4^{e}	216.2^{f}
	60	10.1^{d}	1^{e}	20.7^{d}	199.4^{e}
	0	0	0	0	0
	10	14.5^{f}	2^{d}	100.3^{d}	302.8^{d}
NAA/Kin	20	52.8^{b}	4^{b}	104.3^{c}	407.6^{c}
(0.07/0.4 mgl^{-1})	30	77.4^{a}	5^{a}	123.8^{a}	432.0^{a}
	40	50.7^{c}	4^{b}	111.2^{b}	412.7^{b}
	50	38.8^{d}	3^{c}	99.7^{e}	286.4^{e}
	60	17.3^{e}	2^{d}	84.2^{f}	263.2^{f}

Duncan's multiple range test was used to evaluate the difference between the percentage of plantlets production microtubers, average number of microtubers per plantlet, average diameter of microtuber and average fresh weight of microtuber per plantlets. Data sharing the same letter in the same column were not significantly different at 5 % level.

Conclusion

The proliferation of shoot on nodal cutting and their transformation in plantlets were obtained with BAP/NAA ratio. In these conditions, BAP/NAA at 0.5/0.05 mg l^{-1} gave the highest percentage of shoots developed into plantlets (96 %). The induction of microtubers is due to the use of NAA or Kin or NAA/Kin and

sucrose at different concentrations. The treatment containing 0.07 mg l^{-1}NAA + 0.4 mg l^{-1} Kin + 30 g l^{-1} sucrose was the most effective to induce tuberous roots in cassava, which was well differentiated after 65 days of growth in the inducing media.

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REFERENCES

[1] Cock, J.H., 1982. Cassava: a basic energy source in the tropics. Science, 218(19): 755-762.

[2] Ospina, B. and H. Ceballos, 2002. La Yuca en el Tercer Milenio: Sistemas Modernos de Producción, Procesamiento, Utilización y Comercialización. Centro Internacional de Agricultura Tropical (CIAT). Colombia. 1: p. 576.

[3] Bhagwat, Basdeo, Luiz, G.F. Vieiral Larry, and R. Erickson, 1996. Stimulation of in vitro shoot proliferation from nodal explants of cassava by thidiazuron, benzyladenine and gibberellic acid. Plant Cell, Tissue and Organ Culture. Kluwer Academic,The Netherlands. 46:1-7.

[4] Garcia, M.G., V.M. Vega and and S.R. Morales, 1993. Effect of meristem culture micropropagation on the vigor and yield of the cassava clone "Senorita". In W. M. Roca, & A. M. Thro (Eds.), *Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network*, Cartagena, Colombia, 25-28 August 1992. Cali, Colombia: Centro International de Agricultura Tropical.

[5] Jorge, V., M. Fregene, M. Duque, M. Bonierbale, J. Tohme and V. Verdier, 2000. Genetic mapping of resistance to bacterial blight disease in cassava (*Manihot esculenta* Crantz). *Theor. Appl. Gen., 101*,865-872.

[6] Onuoch, C.I. and N.J.C. Onwubiku, 2007. Micropropagation of cassava (*Manihot esculenta* Crantz) using different consentration of Benzyaminiopurin (BAP). *J. Engineering and Applied Sci., 2*(7), 1229-1231.

[7] Staden, J.V., E. Zazimalova and E.F. George, 2008. Plant Growth Regulators II: Cytokinins, their Analogues and Antagonists. In E. F. George, M. A. Hall, & G. De Klerk (Eds.), *Plant Propagation by Tissue Culture* (3 edn) (pp. 205-226). Springer.

[8] Wang, P. and C. Hu, 1982. *In Vitro* mass tuberization and virus- free seed potato production in Taiwan. Am. Potato J. 59*:*33-37.

[9] Villafranca, M.J., J. Veramendi, V. Sota and A.M. Mingo-Castel, 1998. Effect of physiological age of mother tuber and number of subcultures on in vitro tuberization of potato (Solanum tuberosum L.). Plant Cell Rep 17:787-790.

[10] Veramendi, J.L., Willmitzer and R.N. Trethewey, 1999. *In vitro* grown potato microtubers are a suitable system for the study of primary carbohydrate metabolism. Plant Phys Biochem 37:693-697

[11] Ranalli, P., F. Bassi, G. Ruaro, P. Del Re, M. Di Candilo and G. Mandolino, 1994. Microtuber and minituber production and field performance compared with normal tubers. Potato Res 37:383-391

[12] Zobayed, S.M., J. Armstrong, and W. Armstrong, 2001. Micropropagation of potato: evaluation of closed, diffusive and forced ventilation on growth and tuberization. Ann Bot 87:53-59.

[13] Mantell, S.H. and S.A. Hugo, 1989. Effects of photoperiod, mineral medium strength, inorganic ammonium, sucrose and cytokinin on root, shoot and microtuber development in shoot culture of *Dioscorea alata* L. and *D. bulbifera* L. yams. Plant Cell Tiss Organ Cult 16: 23-37

[14] John, J.L., W.H. Courtney, D.R. Decoteau, 1993. The influence of plant growth regulators and light on microtuber induction and formation in *Dioscorea alata* L. cultures. Plant Cell Tiss. Organ Cult. 34:245-252.

[15] Jasik, J. and S.H. Mantell, 2000. Effects of jasmonic acid and its methylester on *in vitro* microtuberisation of three food yam (*Dioscorea*) species. Plant Cell Rep. 19:863-867.

[16] Borges, M., W. Ceiro and Meneses, 2005. Regeneration and multiplication of *Dioscorea alata* germplam maintained *in vitro*. Plant Cell Tiss. Organ. Cult. 76:87-90.

[17] Fotso, M.F.S. Ngo-Ngwe, H.D. Mbouobda, P.F. Djocgoue and N.D. Omokolo, 2013. Micropropagation of *Dioscorea alata* L. from microtubers induced *in vitro.* African Journal of Biotechnology Vol. 12(10), pp. 1057-1067.

[18] Xu, XA., A.M.V. Lammeren, E. Vermeer, D. Vreugdenhil, 1998.The role of gibberellin, abscisic acid, and sucrose in the regulation of potato tuber formation in vitro. Plant Phys 117:575-584.

[19] Omokolo, N.D., T. Boudjeko, J.J. Tsafack-Takadong, 2003. *In vitro* tuberization of Xanthosoma sagittifolium L. Shott: effects of phytohormones, sucrose, nitrogen and photoperiod. Scientia Horticulturae 98:337-345.

[20] Peres, L.E.P., R.F. Carvalho, A. Zsögön, O.D. Bermúdez-Zambrano, W.G.R. Robles and S. Tavares, 2005. Grafting of tomato mutants onto potato rootstocks: an approach to study leaf-derived signaling on tuberization. Plant Sci 169:680-688.

[21] Koda, Y. and Y. Kikuta, 1991. Possible involvement of jasmonic acid in tuberization of yam plants. Plant Physiol 32: 629-633.

[22] Santos, I. and R. Salema, 2000. Promotion by jasmonic acid of buld formation in shoot culture of *Narcissus triandus* L. Plant Growth Regul 30: 133-138.

[23] Ovono Ondo, P., C. Kevers and J., Dommes, 2007. Axillary proliferation and tuberisation of *Dioscorea cayenensis –D. rotundata* complex. Plant Cell Tiss Organ Cult 91: 107-114

[24] Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-479

[25] Morel, G. and R.H. Wetmore, 1951. Fern callus tissue culture. Ann J Bot 38: 141-143

[26] Cacaï, G.H.T., C. Ahanhanzo, J.S. Dangou, S.S. Houédjissin, C. Agbangla, 2012. Effets de différentes combinaisons hormonales sur l'organogenèse *in vitro* de quelques cultivars locaux et variétés améliorées de *Manihot esculenta* Crantz (manioc-Euphorbiaceae) cultivées au Bénin. Int. J. Biol. Chem. 6(4): 1593-1607.

[27] Le, B.V., Anh, B.L., Soytong, K., N.D. Danh and L.T. Anh Hong, 2007. Plant regeneration of cassava (*Manihot esculenta* CRANTZ) plant. Journal of A gricultural Technology 3(1):121-127.

[28] Malaurie, B., O. Pungu, M.F. Trouslot, 1995b. Effect of growth regulators concentrations on morphological development of meristem-tips in *D. cayenensis- D. rotundata* complex and *D. praehensilis*. Plant Cell Tiss. Organ Cult. 41:229-235.

[29] Gami, R.A., S.K. Parmar, P.T. Patel, C.J. Tank, R.M. Chauhan, H.S. Bhadauria, and S.D. Solanki, 2013. Microtuberization, minitubers formation and *in vitro*shoot regeneration from bud sprout of potato *(Solanum tuberosum* L.) cultivar *K. badshah.* African Journal of Biotechnology.12(38), pp. 5641-5647.

[30] Tsafack, T.J.J., G.P. Charles, A. Hourmant, N.D. Omokolo, M. Branchard, 2009. Effect of photoperiod and thermoperiod on microtuberization and carbohydrate levels in cocoyam (*Xanthosoma sagittifolium* (L.) Schott). Plant Cell Tiss. Organ Cult. 96:151-159.

[31] Hoque, M. E. 2010. *In vitro* tuberization. Plant omics Journal. 3(1):7-11.

[32] Yu, W.C., P.J. Joyce, D.C. Cameron, B.H. McCown, 2000. Sucrose utilization during potato microtuber growth in bioreactors. Plant Cell Rep. 19: 407– 413.

[33] Bazabakana, R., M. Baucher, B. Diallo, M. Jaziri, 2003. Effect of jasmonic acid on developmental morphology during *in vitro* tuberisation of *Dioscorea alata.* Plant Growth Regul. 40:229-237.

[34] Debeljak, N., M. Regvar, K.W. Dixon, K. Sivasithamparam, 2002. Induction of tuberization *in vitro* with jasmonic acid and sucrose in an Australian terrestrial orchid, *Pterostylis sanguinea*. Plant growth Regul. 36:253-260.

[35] Alizadeh, S., S.H. Mantell and AM. Viana, 1998. *In vitro* shoot culture and microtuber induction in the steroid yam *Dioscorea composita* Hemsl. Plant Cell Tiss. Organ Cult. 53:107-112.