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Effect of cytokinins and auxin on bud burst and direct organogenesis *in vitro* of some sweet potato landraces (*Ipomoea batatas* L.) grown in Benin.

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ABSTRACT

Objectives: Ipomoea batatas L. is a tuberous root plant of great nutritional and economic importance in Benin. This study aims to analyze the effects of two cytokinins (Benzylaminopurine and kinetin) and a one auxin (Naphthalene Acetic Acid) on direct organogenesis *in vitro* of six sweet potato landraces in Benin.

Methodology and Results: Ten uninodal stems disinfected of each variety are cultivated on a Murashige and Skoog (MS) medium then transplanted onto other MS media with different combinations of Benzylaminopurine, Kinetin and Naphthalene Acetic Acid. Their vitroplants are also acclimatized. Analysis of variance was used for data analysis. The results showed that " Amitchéwin, Vobodouaho and Koïdokpon " have recorded the highest average bud burst (12.67, 12.33 and 11.67 respectively). The media MS + 1 mg.l^{-1} BAP + 0.1 mg.l^{-1} NAA and MS + 1 mg.l^{-1} Kin + 0.1 mg.l^{-1} NAA were found more effective for the organogenesis of the varieties. Vitroplants of each variety acclimatized well with survival rates ranging from 56.66% to 83.33%.

Conclusion: and application of results: The results showed that the combination of 1 mg.l^{-1} of Benzylaminopurine or kinetin with 0.1 mg.l^{-1} of Naphthalene Acetic Acid was effective for *in vitro* organogenesis of sweet potato landraces used with well-acclimated vitroplants. This study paves the way for the establishment of *in vitro* collection of sweet potato landraces in Benin with a view to their *ex situ* preservation.

Keywords: *Ipomoea batatas*; growth regulators; organogenesis *in vitro*; Acclimation; Benin.

INTRODUCTION

Sweet potato *(Ipomoea batatas)* is part of roots and tubers reproducing mainly by vegetative way whose performance is high and ranked second in the world after potatoes (Deng *et al.*, 2012; Ndagijimana *et al.*, 2014). It is increasingly produced in Benin, especially in the south of the country by small producers as a food during the lean season because of its ability to adapt to difficult conditions and its nutritional importance (Adégbloba, 2003; Sanoussi *et al.*, 2013). Previous work on the species in Benin has shown the existence of a diversity of sweet potato landraces, some of which are increasingly neglected because of the many constraints that hinder production and whose conservation becomes a priority (Doussoh *et al.*, 2016; Paraïso *et al.*, 2013; Sanoussi *et al.*, 2016). *In vitro* culture techniques are

an alternative that is widely used for regeneration and transformation of sweet potato plants (Onwubiko *et al.*, 2015). Thus, in vitro vegetative propagation is an important tool for the recovery, the multiplication and *ex situ* of preservation germplasm of species. It is also useful for the future improvement of breeding programs for the production of new more efficient and tolerant (Kamal *et al.*, 2015;Saucedo-Ruiz *et al.*, 2006;Sivparsad and Gubba, 2012). The success of micropropagation depends on several factors including the genotype and type of explants used (uninodal stems or leaf fragments, meristems), the disinfection protocol used (sodium hypochlorite, mercuric chloride), the growing conditions (temperature, brightness, humidity), the culture medium and its composition in growth regulators (Ahanhanzo *et al.*, 2010; Cacai *et al.*, 2012; Glato *et al.*, 2013). Growth regulators play a decisive role in the orientation of culture an organogenesis or somatic embryogenesis (Delgado-Paredes *et al.*, 2016; Demeke *et al.*, 2014; Onwubiko *et al.*, 2015). Auxins and cytokinins or their synthetic analogs are the most used growth regulators and the ratio of their combination determines the proliferation of roots, leaves and shoot length (Ezeibekwe *et al.*, 2009). Auxins commonly used are naphthalene acetic acid (NAA), indole acetic acid (IAA), while cytokinins most used are kinetin, benzyl amino purine (BAP). Preliminary work on the introduction of sweet potato *in vitro* culture in Benin has been completed. These work used explants and disinfected uninodal stems and mercuric chloride respectively with an effective dose of disinfection of 0.5% on three sweet potato landraces (Doussoh *et al.*, 2017). Similarly, MS (Murashige and Skoog) with or without naphthalene acetic acid and benzyl amino purine have been used to regenerate sweet potato *in vitro* with vegetative organs formation including leaves, roots, shoots. However, no study has been conducted on the organogenesis in vitro for creation of in vitro collection of sweet potato landraces and the use of their apex and meristems in ex situ preservation protocols. The present work aims to improve the production of vitroplants of sweet potato landraces from uninodal stems fragments on MS media with different combinations of naphthalene acetic acid, benzyl amino purine and kinetin. Specific objectives were: (a) to evaluate the rate of bud burst of six sweet potato landraces on an MS initiation medium; (b) to determine the influence of the hormonal combinations of naphthalene acetic acid, benzyl amino purine and kinetin on the formation of shoots, leaves and roots; (c) to acclimate vitroplants rooted on an organic substrate.

MATERIAL AND METHODS

Plant material and production of mother plants : The plant material constituted six (6) sweet potato landraces (Table 1) collected in southern and central Benin during September 2015 to February 2016 (Doussoh *et al.*, 2016). "Dokoui carotte" and "Koïdokpon" were selected because they are increasingly neglected while their colored flesh represents an indicator of richness in vitamins A. Amitchéwin, Dokoui èlèhin akpao, 'Bombo wéwé and Vobodouaho were selected on the basis of the preference criteria of the producers (Precocity, high multiplication rate, high market value and good post-harvest conservation) (Doussoh *et al.*, 2016). These different landraces are morphologically different from each other. Their cuttings were disinfected with a fungicide, the methylthiophanate at 70% and were grown in polyethylene pots filled with treated soil by nematicide (carbofurant). The pots were maintained in the ender greenhouse of Genetic and Biotechnology Department of Central Laboratory of Plant Biotechnology and Breeding Plant (LCBVAP) of the Faculty of Science and Techniques of the University of Abomey-Calavi. After six weeks of culture, young shoots were then obtained as mother plants.

Table 1: Characteristics of landraces used.

Landraces	Characteristics of tubers	Collecting municipality	Production cycle (months)
Amitchewin	Dark purple skin, yellow flesh	Sissèkpa (adjohoun)	4
Bombo wéwé	White skin, yellow flesh	Sokan (Abomey-Calavi)	4
Doki èlèhin akpao	Red skin, white flesh	Takou (Kétou)	3-4
Dokoui carotte	Orange skin and flesh	Glo Fanto (Abomey-Calavi)	3-4
Koïdokpon	Red skin, yellow flesh	Glo Fanto (Abomey-Calavi)	4-5
Vobodouaho	White skin and flesh	Kpoto (Zangnanando)	3

Culture media : Seven (7) culture media were used with MS medium (Murashige and Skoog, 1962) as a base supplemented with naphthalene acetic acid (NAA), benzyl amino purine (BAP) and kinetin at different concentrations (Table 2). The pH of the media is adjusted to 5.7 ± 0.1. The media supplemented with 30 $g.L^{-1}$ sucrose and 8 $g.L^{-1}$ agar were distributed in tubes (25 mm x 150 mm), sterilized at 121 ° C for 15 minutes in an autoclave and transferred to the culture chamber. The seeded media were placed in the culture chamber at 27 ± 1 ° C and were subjected to a photoperiod of 16 hours per day under a light intensity of 5000 lux provided by Philips TLD18W and Sibalec lamps. The relative humidity is maintained at 80%.

Table 2 : Composition of culture media

Culture media	Composition in growth regulators
M_I	MS without growth regulators
M_{II}	MS + 1 mg. l^{-1} BAP
M_{III}	MS + 1 mg. l^{-1} BAP + 0,1 mg.l^{-1} NAA
M_{IV}	MS + 1 mg. l^{-1} Kin
M_V	MS + 1 mg. l^{-1} Kin + 0,1 mg. l^{-1} NAA
M_{VI}	MS + 0,5 mg. l^{-1} BAP + 0,5 mg.l^{-1} Kin
M_{VII}	MS + 0,5 mg. l^{-1} BAP + 0,5 mg.l^{-1} Kin + 0,1 mg.l^{-1}NAA

Disinfection of explants and initiation of shoots. : The disinfection protocol applied was that using 0.5% mercuric chloride (Ahanhanzo *et al.*, 2008; Doussoh *et al.*, 2017). Fragments of uninodal stems of the six landraces were used as explants and are then taken from the mother plants. Fragments were cleared of their leaves and rinsed with tap water and were transferred to the culture chamber under hood. They were then immersed in 70% alcohol for 1 min and then dipped in a 0.5% mercuric chloride solution containing two drops of Tween 80 for 10 min. The explants were rinsed three times with distilled water sterilized for five minutes per rinsing. The disinfected explants were rid of their necrosed parts by a scalpel. Each explant of about 3 cm with a node was placed in a test tube containing MI medium (MS without growth regulators). Each seeded tube is closed with sterile cotton and sealed with parafilm. The tubes were transferred to the culture chamber.

Micropropagation of the shoots formed: To evaluate the effect of the hormonal combinations on the *in vitro* organogenesis of the six landraces, the shoots from the MI medium are cut into uninodal 2 cm fragments with a node and subcultured onto the seven culture media, the MI medium serving as the witness.

Acclimatization: The vigorous vitroplants of each landrace having formed roots were acclimated to a substrate contained in plastic pots perforated at the base and ¾ filled. The substrate used consisted of a mixture of compost, sawdust and soil in the proportions 2: 1 : 1. This substrate was sterilized in an oven at 150 ° C for 2 hours. These pots were placed in acclimation tanks then in the greenhouse and watered with Shives solution.

Evaluation parameters: The number of explants budded and formed shoots of each landraces was determined after observations made every 48 hours for 14 days. As for *in vitro* organogenesis, the number of leaves, nodes, roots and the height of the shoots formed were evaluated after six weeks of culture. Finally, the survival rate of the acclimated vitroplants of each local variety was determined after four weeks.

Data analysis: The experimental device used was a completely random block. For bud burst, fifteen (15) explants per landrace were used with three repetitions. For organogenesis, each culture medium was considered a major factor and varieties as secondary factors. For each parameter evaluated, an average of ten (10) vitroplants was considered as the sample unit with three repetitions. An analysis of variance (ANOVA) preceded

the tests of RYAN-JOINER and LEVENE was realized. The variables number of leaves, number of nodes and number of roots do not follow a normal distribution, the two first have undergone a logarithmic transformation and the number root variable, a square root transformation allowing them to tend towards normality. The Student-Newmann-Keuls test at the 5% threshold was also used to compare averages. In terms of acclimation, ten (10) vitroplants of each local sweet potato variety were used with two repetitions. The different data analysis was carried out with XLSAT software.

RESULTS

Bud burst of the different landraces on the MS medium as a function of time: Bud burst of the different landraces on the MS medium was evaluated after 14 days. The analysis of variance showed a highly significant difference for the variety factor ($p < 0.001$) (Table 3).

Table 3 : ANOVA of burd burst

Source of variation	Degree of freedom	Average squares	Probability
Varieties	5	27.66	<0.001
Error	12	2.5	
Total	17		

Figure 1 showed the average bud burst of different sweet potato landraces. " Amitchéwin " and " Vobodouaho " had the highest average bud burst (12.67 and 12.33 respectively) while; the lowest average bud burst (5.67) was obtained with " Doki èlèhin akpao ". The landraces " Bombo wéwé " and " Koïdokpon " were the first to bud up after 2 days compared to " Doki èlèhin akpa " whose first budding explants were observed from the 6th day (Figure 2).

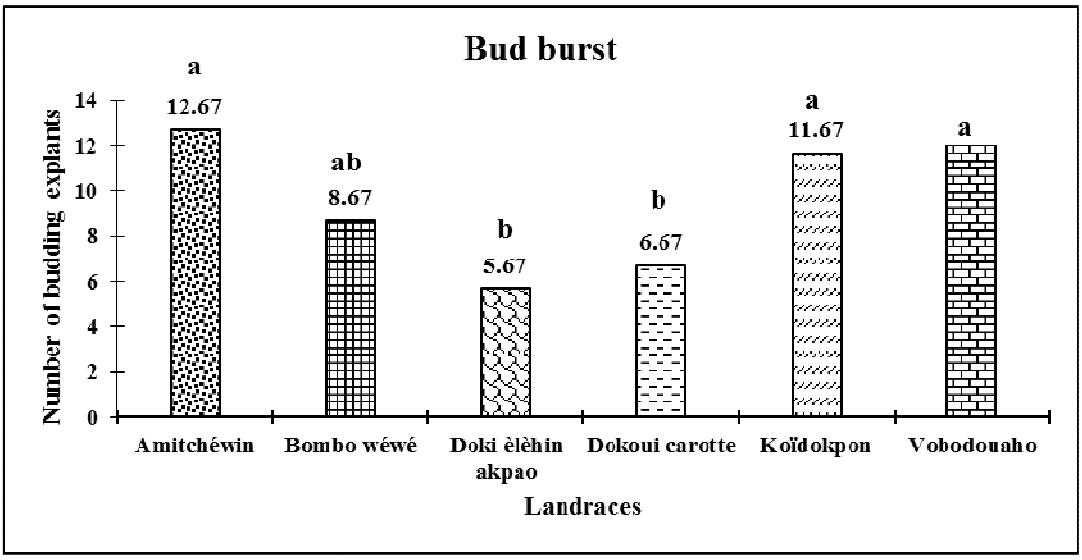


Figure 1 : Averages bud burst of six sweet potato landraces

Histograms with the same letter are not significantly different according to the Student, Newman and Keuls averaging test at the 5% threshold.

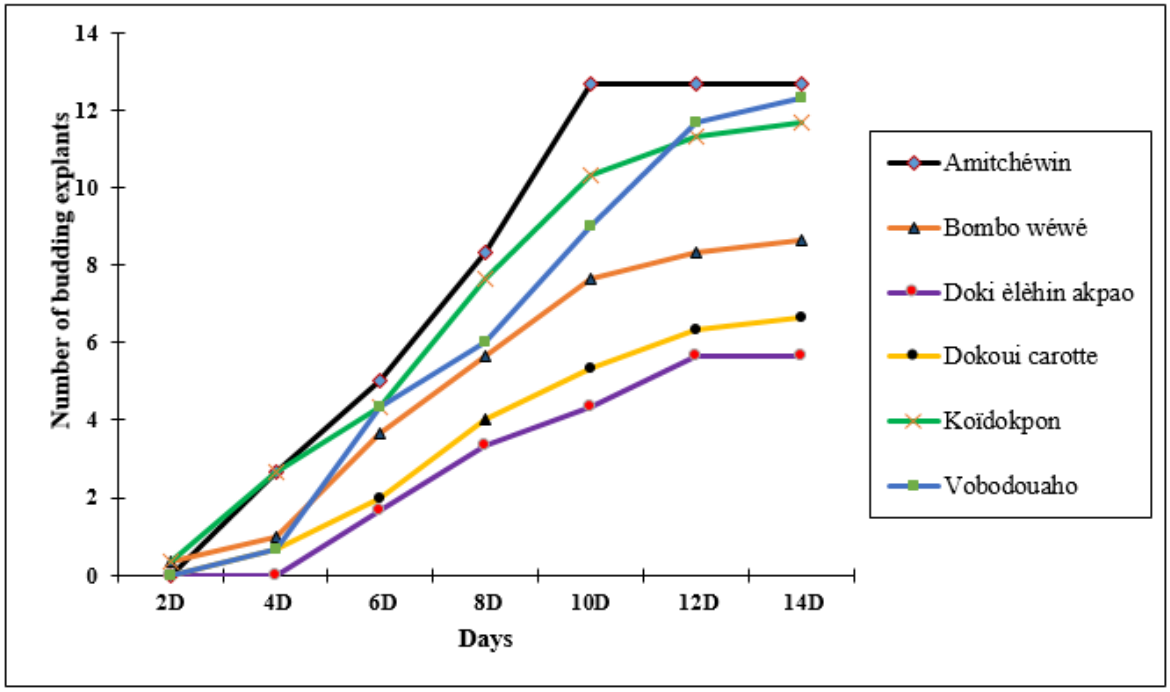


Figure 2 : Kinetics of bud burst of six sweet potato landraces over time.

Effect of the growth regulators on the organogenesis *in vitro* of six sweet potato landraces: *In vitro* organogenesis of sweet potato landraces was characterized by the formation and development of different growth organs (Figure 3). Analysis of variance of regulators on the formation of nodes, leaves, roots and shoot height were presented in Table 4.

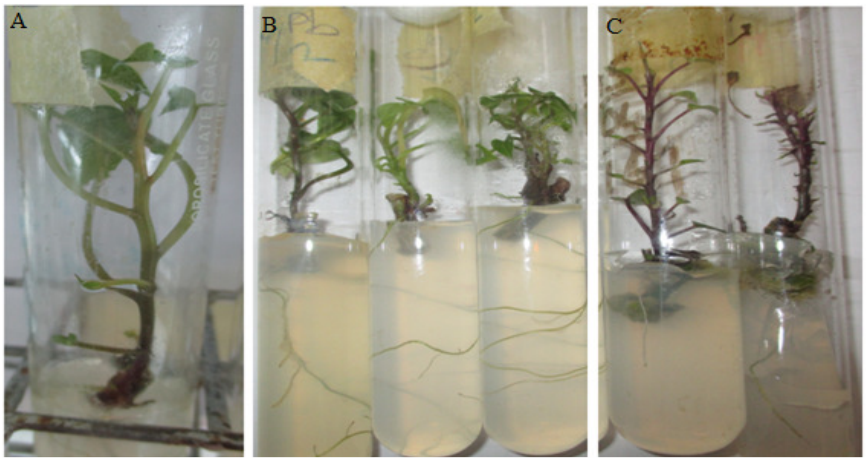


Figure 3 : Effect of grows regulators on organogenesis of three sweet potato landraces

A- Leaf formation of ''Dokoui èlèhin akpao'' on MII medium ; B- Root formation of ''Vobodouaho on MIII medium ; C- Shoot formation of ''Amitchéwin'' on MI medium

Table 4 : ANOVA of the number of nodes, leaves, roots and shoots height.

Source of variation	Degree of freedom	Average squares	Probability (P)
Number of nodes			
Varieties	5	1.42	<0.001
Medium	6	0.12	0.010
Varieties *Medium	30	0.25	<0.001
Error	84	0.04	
Total	125		
Number of leaves			
Varieties	5	1.46	<0.001
Medium	6	0.32	<0.001
Varieties *Medium	30	0.18	<0.001
Error	84	0.04	
Total	125		
Number of roots			
Varieties	5	0.27	0.229
Medium	6	2.79	<0.001
Varieties *Medium	30	0.23	0.118
Error	84	0.16	
Total	125		
Shoots height			
Varieties	5	31.50	<0.001
Medium	6	5.42	<0.001
Varieties *Medium	30	3.93	<0.001
Error	84	0.58	
Total	125		

Effects of different growth regulators on nodes formation: The "Varieties" and "Medium" factors was showed respectively a highly significant difference (P <0.001) and a significant difference (P = 0.010) for the Logarithm of the number of nodes formed (Table 4). The interaction between these two factors is also highly significant (P <0.001). Thus, the formation of the average number of nodes was varied significantly from one medium to another and from one variety to another. Landraces ''Amitchéwin'', ''Bombo wéwé'' and ''Koïdokpon'' on MIII medium (MS + ANA + BAP) recorded the highest average of nodes (12.66 ; 5.67 and 8.33 respectively) while the highest averages of nodes were obtained for ''Dokoui carotte'' (5.67) and ''Doki èlèhin akpao'' (3.5) on MVI medium (MS + BAP + KIN) and Vobodouaho (6.67) on MV medium (MS + KIN + ANA) (Table 5).

Table 5: Average of nodes.

Media / Varieties	M I	M II	M III	M IV	M V	M VI	M VII
Amitchéwin	4.33 ± 1.15c	7.00 ± 3.6 ab	12.66 ± 2.52 a	7.33 ± 0.57ab	5.33 ± 1.15bc	5.67 ± 1.52bc	5.00 ± 1.73bc
Bombo Wéwé	3.33 ± 0.57c	5.33 ± 0.57a	5.67 ± 0.57a	5..67 ± 0.57a	4.67 ± 1.15b	2,67 ± 0.57c	4.33 ± 0.57bc
Dokouin Carotte	4.33 ± 0.33b	5.00 ± 0.00 ab	5.00 ± 0.57ab	4.33 ± 0.33b	4.00 ± 0.57b	5.67 ± 0.67a	5.33 ± 0.67 a
Doki èlèhin akpao	2.73 ± 0.28ab	3.43 ± 0.29a	3.16 ± 0.12ab	2.03 ± 0.31b	2.67 ± 0.33ab	3.50 ± 0.28a	3.07 ± 0.24ab
Koïdokpon	2.33 ± 0.57e	6.33 ± 1.52b	8.33 ± 0.57a	4.67 ± 1.15c	6.00 ± 1.00bc	4.67 ± 0.57c	3.67 ± 0.57d
Vobodouaho	4.33 ± 1.55c	6.33 ± 0.57ab	4.33 ± 0.57c	5.33 ± 1.55bc	6.67 ± 0.57a	5.67 ± 0.57b	6.00 ± 1.00b

M_I = MS without growths regulators ; M_{II} : MS + 1 mg. l-1 BAP ; M_{III} = MS + 1 mg.l^{-1} BAP + 0,1 mg.l^{-1} NAA ; M_{IV} = MS + 1 mg.l^{-1} Kin ; M_V = MS + 1 mg.l^{-1} Kin + 0,1 mg.l^{-1} NAA ; M_{VI} = MS + 0,5 mg.l^{-1} BAP + 0,5 mg.l^{-1} Kin ; M_{VII} = MS + 0,5 mg.l^{-1} BAP + 0,5 mg.l^{-1} Kin + 0,1 mg.l^{-1}NAA.

Effects of different growth regulators on leaves formation: The factors " Varieties " and " Medium " and the interaction between these two factors were showed for each a highly significant difference (P <0.001) for the logarithm of the number of leaves formed (Table 4). Thus, for the leaves, their average number also varied significantly from one variety to another and from one medium to another. For ''Amitchéwin'', ''Bombo wéwé'' and ''Koïdokpon'' landraces, MIII medium (MS + BAP + ANA) was favoured the highest average leaves (respectively 11.86; 6 and 6.67). In contrast, the highest averages leaves were obtained for MVI medium for ''Dokoui carotte'' (5.67), MII medium for ''Doki elehin akpao'' (3.67) and MV medium for ''Vobodouaho'' (5.33). (Table 6).

Table 6 : Average of leaves

Media / Varieties	M I	M II	M III	M IV	M V	M VI	M VII
Amitchéwin	5.66 ± 0.57c	6.66 ± 3.06b	11.86 ± 2.20a	6.33 ± 1.15bc	5.33 ± 0.57c	6.67 ± 0.57b	6.00 ± 1.00bc
Bombo Wéwé	2.67 ± 1.15d	5.33 ± 0.57bc	6.00 ± 1.00a	4.67 ± 0.57c	5.67 ± 1.15a	2.33 ± 0.57d	4.33 ± 1.15c
Dokouin Carotte	4.33 ± 0.57bc	5.00 ± 1.00ab	5.33 ± 0.57a	4.67 ± 0.57b	3.67 ± 0.57c	5.67 ± 0.57a	4.67 ± 1.15b
Doki èlèhin akpao	1.33 ± 0.57c	3.67 ± 0.57a	3.33 ± 0.57ab	2.33 ± 0.57b	2.67 ± 0.57b	3.33 ± 0.57ab	3.33 ± 0.57ab
Koïdokpon	3.00 ± 1.00c	6.33 ± 1.15a	6.67 ± 0.57a	4.33 ± 0.57bc	5.00 ± 1.00b	4.33 ± 1.15bc	4.33 ± 0.57bc
Vobodouaho	2.67 ± 0.57d	5.17 ± 1.28a	4.67 ± 1.15ab	3.33 ± 1.15cd	5.33 ± 1.15a	4.33 ± 0.57b	4.67 ± 1.52ab

M_I = MS without growths regulators ; M_{II} : MS + 1 mg. l-1 BAP ; M_{III} = MS + 1 mg.l^{-1} BAP + 0,1 mg.l^{-1} NAA ; M_{IV} = MS + 1 mg.l^{-1} Kin ; M_V = MS + 1 mg.l^{-1} Kin + 0,1 mg.l^{-1} NAA ; M_{VI} = MS + 0,5 mg.l^{-1} BAP + 0,5 mg.l^{-1} Kin ; M_{VII} = MS + 0,5 mg.l^{-1} BAP + 0,5 mg.l^{-1} Kin + 0,1 mg.l^{-1}NAA.

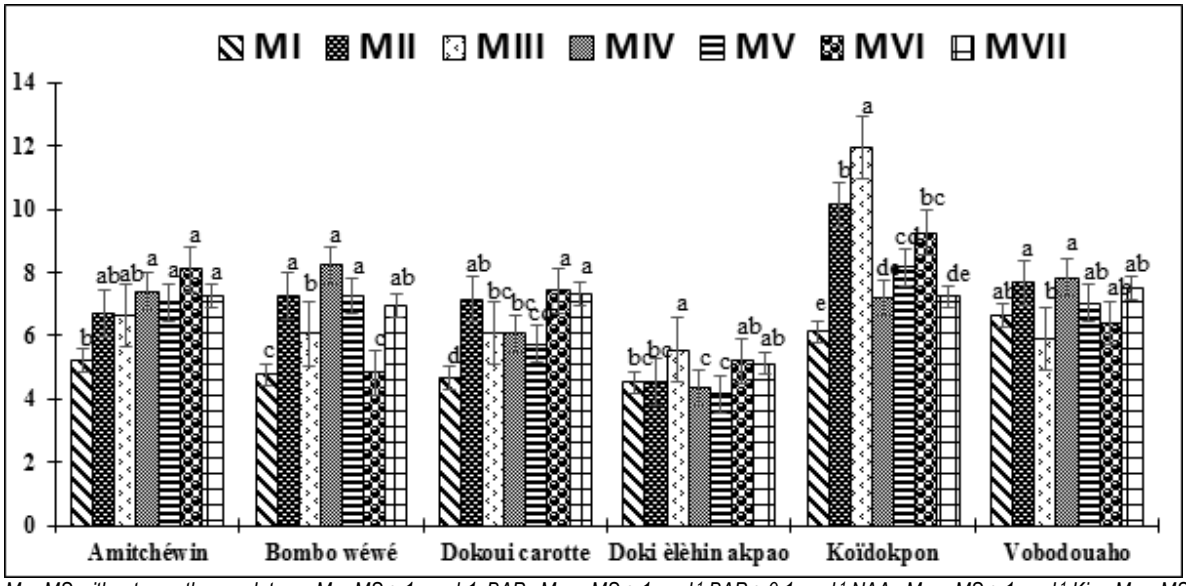
Effect of different growth regulators on roots formation: The factors " Varieties " and the interaction Varieties * Media were showed no significant difference (p = 0.22 and 0.11 respectively) while the " Media " factor was showed a highly significant difference (P <0.001) for the square root formed. Thus, for the roots, their average number varied significantly from one medium to another. The high average numbers of roots were obtained for ''Amitchéwin'' (2.33), ''Dokoui carotte'' (3.00) and ''Koïdokpon'' (2.93) on the medium MVII, ''Bombo wéwé'' (2.67) and ''Doki èlèhin akpao'' (3, 27) on MVI medium and ''Vobodouaho'' (3,00) on MIII (Table 7).

Table 7 : Average of roots

Media / Varieties	M I	M II	M III	M IV	M V	M VI	M VII
Amitchéwin	2.33 ± 0.57a	1.53 ± 0.61b	2.00 ± 1.0ab	0.67 ± 0.57c	1.33 ± 0.57b	0.67 ± 0.57c	2.33 ± 1.15a
Bombo Wéwé	2.33 ± 0.57a	0.67 ± 0.57c	1.67 ± 0.57b	0.33 ± 0.27c	2.67 ± 0.77a	0.33 ± 0.57c	2.33 ± 0.57a
Dokouin Carotte	2.33 ± 0.57bc	0.67 ± 0.15de	2.67 ± 0.57b	0.33 ± 0.57e	1.67 ± 1.15c	0.67 ± 0.57d	3.00 ± 0.10a
Doki èlèhin akpao	1.67 ± 0.57c	1.20 ± 1.00d	3.20 ± 0.72a	0.67 ± 0.15d	3.27 ± 0.76a	1.82 ± 0.20c	2.40 ± 0.36b
Koïdokpon	1.67 ± 0.57c	0.67 ± 0.57d	1.67 ± 0.57c	2.00 ± 1.00b	2.46 ± 0.53ab	0.83 ± 0.64cd	2.93 ± 0.37a
Vobodouaho	2.67 ± 0.57ab	1.93 ± 1.30b	3.00 ± 0.10a	0.67 ± 0.57d	1.667 ± 0.577c	1.33 ± 0.57c	1.67 ± 0.57c

M_I = MS without growths regulators ; M_{II} : MS + 1 mg. l-1 BAP ; M_{III} = MS + 1 mg.l^{-1} BAP + 0,1 mg.l^{-1} NAA ; M_{IV} = MS + 1 mg.l^{-1} Kin ; M_V = MS + 1 mg.l^{-1} Kin + 0,1 mg.l^{-1} NAA ; M_{VI} = MS + 0,5 mg.l^{-1} BAP + 0,5 mg.l^{-1} Kin ; M_{VII} = MS + 0,5 mg.l^{-1} BAP + 0,5 mg.l^{-1} Kin + 0,1 mg.l^{-1}NAA.

Effects of different growth regulators on the height of the shoots formed: The factors " Varieties " and " Media " and their interaction showed a highly significant difference (P <0.001) (Table 4). Thus, for shoot height, their average varied significantly from one variety to another and from one medium to another. The highest average shoot height was obtained for ‘’Amitchéwin’’ (8.10 cm) and ‘’Dokoui carotte’’ (7.43 cm) on medium MVI, for ‘’Bombo wéwé’’ (8.23 cm) and ‘’Vobodouaho’’ (7.83 cm) on the medium MIV and for ‘’Koïdokpon’’ (11.96 cm) and doki èlèhin akpao (5.56 cm) on MIII medium (Figure 4).



M_I = MS without growths regulators ; M_{II} : MS + 1 mg. l-1 BAP ; M_{III} = MS + 1 mg.l^{-1} BAP + 0,1 mg.l^{-1} NAA ; M_{IV} = MS + 1 mg.l^{-1} Kin ; M_V = MS + 1 mg.l^{-1} Kin + 0,1 mg.l^{-1} NAA ; M_{VI} = MS + 0,5 mg.l^{-1} BAP + 0,5 mg.l^{-1} Kin ; M_{VII} = MS + 0,5 mg.l^{-1} BAP + 0,5 mg.l^{-1} Kin + 0,1 mg.l^{-1}NAA.

Figure 4 : Averages of the height of shoots formed.

Acclimatization of sweet potato vitroplants: Figure 5 showed the survival rates of the acclimated vitroplants of the landraces after six weeks of culture. The landraces ‘’Amichéwin’’ and ‘’Dokoui carrot’’ had the highest survival averages (80.33% and 80%, respectively) compared to ‘’Bombo wéwé’’ and ‘’Doki èlèhin akpao’’. The lowest survival averages (60% and 56.66% respectively) were obtained. These acclimated vitroplants presented growth organs whose stem and leaves are well developed (Figure 6) and can be transferred to the field.

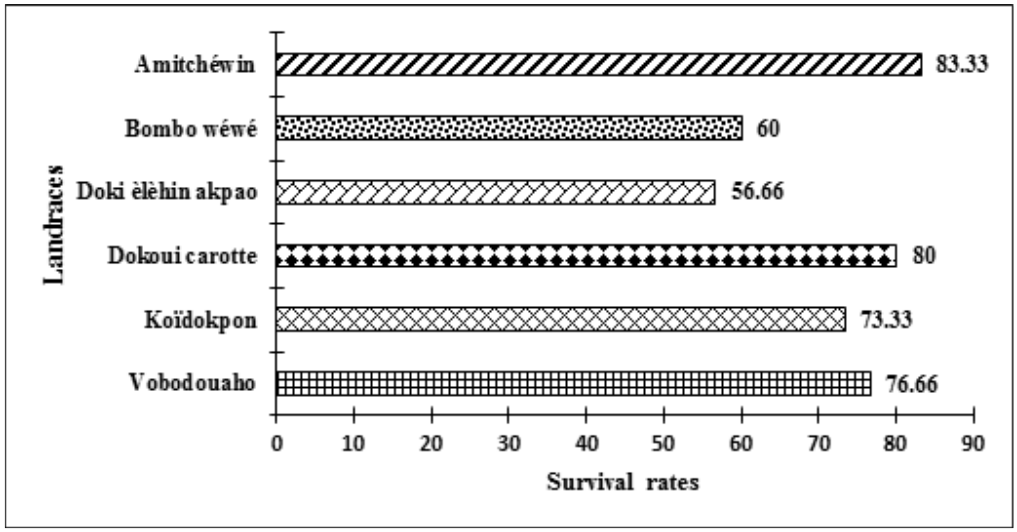


Figure 5 : Averages survival rates of the acclimated vitroplants of sweet potato landraces

Figure 6 : Vitroplants of the landrace ''Amitchéwin'' after four weeks.

DISCUSSION

For bud burst, the response was varied from one landrace to another (12.67 for ''Amitchéwin'' against 5.67 for ''Doki èlèhin akpao''), confirming the analysis of variance which showed a highly significant difference. These results showed that the buds on the same medium would be dependent on the genotype of the different sweet potato landraces. Several works carried out on the sweet potato on the cassava by several authors have shown the influence of genotype on the rate of bud burst on a culture medium (Cacaï *et al.*, 2013; Glato *et al.*, 2014; Kamal *et al.*, 2015). These authors have shown that the MS medium associated with various combinations of growth regulators has an influence on the rate of bud burst, which can reach 100% in some varieties. An assessment of the rate of bud burst of these landraces in different media with combinations of growth regulators should therefore be considered, especially in landraces with a low bud burst rate on the MS medium without growth regulators. For the effect of Auxin (NAA) and cytokinins (BAP and kinetin) on organogenesis, the analysis of variance of the number of nodes, leaves, roots and shoots height were showed significant differences for the factors varieties, media and their interactions. Only the number of roots formed has not presented significant difference for the "varieties" factor and interaction "varieties *media. This shows the existence of an interaction between growth regulators and genotypes of different landraces. Moreover, the type of cytokinins and its combination or not with the naphthalene acetic acid (NAA) has influenced the formation of nodes, leaves, roots and shoots height. Media containing a combination of BAP and NAA or BAP alone favoured more to the formation of a significant number of nodes and leaves most of the varieties. Thus the MIII medium (MS + 1 mg.l^{-1} BAP + 0.1 mg.l^{-1} NAA) allowed to have a maximum number of nodes and leaves in the ''Amitchewin'', ''Bombo wéwé'' and ''Koïdokpon'', as well as the MII medium (MS + 1 mg.l^{-1} BAP) from ''Doki èlèhin akpao''. Only ''Vobodouaho'' landrace has recorded a significant number of nodes and leaves on MV medium containing kinetin and NAA (MS + 1 mg.l^{-1} kin + 0.1 mg.l^{-1} NAA). MVI medium containing a combination of BAP and kinetin (MS + 0.5 mg.l^{-1} BAP + 0.5 mg.l^{-1} Kin) has favoured a high number of nodes and leaves in the ''Dokoui carotte''. These results showed that, on the one hand, the cytokinin-NAA combination was more favourable to the formation of nodes and leaves in most landraces. On the other hand, the BAP has been more effective in the formation of nodes and leaves compared with kinetin for the same concentration. Indeed, even if the media V and VI (MS + 1 mg.l^{-1} Kin + 0.1 mg.l^{-1} NAA and MS + 0.5 mg.l^{-1} BAP + 0.5 mg.l^{-1} Kin) have produced a significant number of nodes and leaves in ''Vobodouaho'' and ''Dokoui carotte'', these media haven't presented significant differences with the MII medium (MS + 1 mg.l-1 BAP). These results revealed that at equal concentration, BAP is more effective for phyllogenesis compared with kinetin. Onwubiko *et al* (2015), Sivparsad and Gubba (2012) showed respectively on cassava and sweet potato that a BAP + NAA association with a high BAP /NAA ratio (10/1) is favourable for phyllogenesis and the responses were dependent on the genotypes used. As for the average of the shoots formed, it was found that the medium MIV (MS + 1 mg.l^{-1} Kin) allowed to have the highest shoot height for ''Amitchéwin'', ''Bombo wéwe'' and ''Vobodouaho'' as well as medium MIII (MS + 1 mg.l^{-1} BAP + 0.1 mg.l^{-1} NAA) for ''Doki èlèhin akpao'' and ''Koïdokpon''. Only ''Dokoui carotte'' produced tall shoots with MVI medium (MS + 0.5 mg.l^{-1} BAP + 0.5 mg.l^{-1} Kin). It follows from this result that kinetin alone or in

combination with BAP was favourable for growth of the stem. However, the formed leaves are not developed compared to the MIII medium. Dolinski and Oleck on sweet potato and George *et al* on cassava have shown that a combination of BAP and kinetin was favourable for growth in height of formed shoots (Doliński and Olek, 2013; George *et al.*, 2008). Similarly, a combination of a high concentration of BAP associated with a low NAA concentration is used for better growth of vegetative organs in sweet potato in vitro culture (Wondimu *et al.*, 2012; Yasmin *et al.*, 2011). Thus, the combination BAP/NAA or kinetin/NAA in a ratio greater than one is essential for the production of vitroplants as seed and the establishment of a collection of vitroplants of several sweet potato landraces for purposes preservation. With the roots, analysis of variance (Table 4) showed that only the medium factor was presented a highly significant difference in the number of roots formed. The results obtained (Table 7) showed that most of the media having in addition to cytokinins, the NAA have promoted the formation of the roots, the number of which is high. MV medium (MS + 1 mg.l^{-1} Kin + 0.1 mg.l^{-1} NAA) improved root formation of the landraces ''Bombo wéwé'', ''Doki èlèhin akpao'' and ''Koïdokpon''. Similarly, MIII medium (MS + 1 mg.l^{-1} BAP + 0.1 mg.l^{-1} NAA) was favourable for the formation of roots for ''Vobodouaho''. These results indicate that NAA is essential for the formation and elongation of the roots. These results differ from those obtained by Cacai *et al* (2012) on cassava and which showed that the MS with 0.2 mg/l kinetin was favourable for root formation. In terms of acclimatization of vitroplants, this study results showed a difference in survival rate of the acclimated vitroplants of each landrace. Outside ''Bombo wéwé'' and ''Doki èlèhin akpao'' landraces that gave survival rates of 60% and 56.66% respectively, other landraces had a survival rate of over 70%. However, these rates are lower than those obtained by several authors who have worked on sweet potato and have approached 100% (Demeke *et al.*, 2014; Doliński and Olek, 2013; Glato *et al.*, 2014). This difference observed in the survival rate of vitroplants could be related to the substrate this study used (mixture of compost, sawdust and soil in proportions 2: 1: 1) which is different from that used by these other authors (sterilized compost or soil mixed with sand).

CONCLUSION

In this study, the effects of different hormonal combinations on the in vitro organogenesis of sweet potato landraces were determined. Of the landraces initiated on the MS medium, four (''Amitchéwin'', ''Vobodouaho'', ''Koïdokpon'' and ''Bombo wéwé'') showed high bud burst averages. Organogenesis further revealed that the medium MIII (MS + 1 mg.l-1 BAP + 0.1 mg.l-1 ANA) was more effective for the formation of vegetative organs (leaves, nodes and roots) and growth in height of the vitroplants. Finally, the vitroplants produced were successfully acclimatized with survival rates ranging from 56.66% to 83.33%. This study will contribute for the establishment of *in vitro* collection of different sweet potato landraces in Benin for their *ex situ* preservation.

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