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Comparison of *in vitro* morphogenetic capacities of different clones of three local cultivars of sweet potato (*Ipomoea batatas*) from Togo

GLATO Kodjo*, ETSE Kodjo Djidjolé, SANVEE Mensah, TOZO Koffi and AIDAM Atsou

Laboratory of Plant Physiology and Biotechnology, University of Lomé, P Box. 1515, Lomé, Togo.

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Sweet potato *(Ipomoea batatas*) is an hexaploide plant with complex incompatibility that reduces chances of self-fertilization. *In vitro* culture of sweet potato by means of biotechnological approaches is an advantage for its study and variety improvement. Three sweet potato cultivars from Togo: *Damadoami, Tombolo, Nagohé* were grown on Murashige and Skoog (MS) medium with or without indole-3-butyric acid (IBA), and the morphogenetic properties of the plantlets obtained were compared. Uninodal stem explants were used. The reactivity of explants grown on MS differed according to the cultivar and the clone. Addition of IBA induced embryogenesis and root neoformations. The growth depended on the genotype, the clone, and the cultivar. The cultivar *Damadoami* was the most reactive on MS medium. Plantlets were acclimated easily and can be transferred to the field

Key words: Tuberous root, *in vitro*, morphogenesis, neoformations, indole-3-butyric acid (IBA), Togo.

INTRODUCTION

Sweet potato (*Ipomoea batatas*) is a food crop of tropical and temperate zones. According to the FAOSTAT statistics, the annual sweet potato production in the world was about 106 million tons (Faostat, 2010). Essentially, it is grown for its tuberous root and its leaves. These two types of organs are used in human and animal alimentation (Sihachakr and Ducreux, 1993). The roots are eaten raw, boiled or fried and the leaves serve as vegetables or fodders. For many years, the storage roots of the sweet potato have been used for industrial alcohol production. *I. batatas* roots, of different shapes and colours, are energy rich and made of starch (20%) and simple carbohydrate (5%) (Hattorie et al.,1985). The leaves are rich in proteins (4 to 27%) whereas the roots contain only 1 to 9% proteins, according to the varieties of *I. batatas* (Adelia, 2007). The high amount of β-carotene found in many cultivars make sweet potato an alimentary complement rich in pro-vitamin A (Issa et al., 2004).

In Togo, among the tubers, sweet potato is the fourth by its production and the second by its yield behind yam (DSID, 2010), but it is still a minor culture (Akpavi, 2008). Grown essentially for food, it is a source of income for Togo's population; however the lack of valorization of the great potentialities of this tuber is regrettable. The culture of sweet potato in Togo, as in many African countries, faces various obstacles such as difficult climatic conditions, that is, high temperature which favors the growth of pathogenic organisms and also inadequate storage conditions (ITRA, 2006). The creation of new varieties

*Corresponding author. E-mail: glatokodjo@gmail.com.

Abbreviations: MS, Murashige and Skoog; **WPM,** wood plant medium; **IBA,** indole-3-butyric acid; **NAA,** naphthalene acetic acid; **GA_3,** gibberellic acid; **IAA,** indole-3-acetic acid; **ABA,** abscisic acid.

a b c

Figure 1. Sections in three most common cultivars of sweet potato in Togo. a, *Tombolo* cultivar: yellow skin, yellow flesh; b, *Nagohé* cultivar: white skin, white flesh; c, *Damadoami* cultivar: red skin, white flesh.

capable of overcoming these hurdles will be a major biotechnological advance from the economical view point.

Sweet potato is an hexaploide plant which is not capable for auto-fecundation. The vegetative multiplication remains the principal means of producing this plant. Biotechnological tools are efficacious ways that allow betterment, selection and variety creation. So to overcome one of the great problem of sweet potato, the viral disease, the meristem culture is used (Iftekhar and al., 2013). But the multiplication of meristem- plantlet is under control not only for the medium but also the cultivars. Triqui et al. (2007) said that better results of micropropagation, somatic embryogenesis, organogenesis, gene transfers, cell cultures and other techniques depended on the choice of the cultivar, or even of the clone.

This study was focused on the comparison of morphogenetic capacities of the different clones derived from tree cultivars of sweet potato from Togo and on the analysis of the own characteristics of the three cultivars (*Damadoami, Tombolo and Nagohé*) using biotechnological approaches. To our knowledge, this investigation achieved from the *in vitro* cultures and acclimation of three *I. batatas* cultivars/clones of Togo is the first to be reported.

MATERIALS AND METHODS

Plant material

The experiments were carried out on the three most common cultivars of sweet potato in Togo which can be distinguished from each other by the colour of the skin and the flesh of the storage roots. Their local name are *Damadoami* (red skin, white flesh), *Tombolo*, (yellow skin, yellow flesh) and *Nagohé* (white skin, white flesh) (Figure 1).

Methods

Establishment of in vitro culture of each cultivar

Ten pieces of root of each cultivar were buried in fine marine sand in perforated trays and watered every 48 h. After six weeks, the healthiest stems were collected for the *in vitro* culture.

Explant disinfection

The leaves were removed leaving the petiole on the stem which was cut into pieces of two or three nodes. Pieces of stem from the same tuber wre called clones. Four clones from each cultivar were used. After washing with tap water, the explants were placed in water with 0.2% tween 80 and taken to the laboratory. They were surface sterilized, four times each, with 80% betadine solutions for 2 min, in 60% sodium hypochlorite for 5 min, in 80% sodium hypochlorite for 2 min and washed four times in sterilized water for 2 min.

Test run

Culture establishment

The pieces of disinfected stems were cut into single nodal fragment of 1.5 to 2 cm in length and planted, respecting the normal stem polarity, on the basis of MS medium (Murashige and Skoog,1962) solidified with agar-agar 0.8% (w/v) without any exogenous phytohormones. The top of the tubes were flamed up and covered. The tubes were stored in a culture room at 27 ± 2°C. The light was provided by cool- white fluorescent lamp with an intensity of 120 $\mu Em^{-2}s^{-1}$ and a photoperiod of 16 h/days.

Micropropagation

After six weeks, the plantlets were cut into single nodal explant of 1 to 1.5 cm in length, planted, according to normal polarity, in tubes containing the MS medium (Murashige and Skoog, 1962) solidified with agar-agar 0.8% (w/v) without any exogenous phytohormones and stored in a culture room as previously described. During the multiplication, the root production, the stem growth and the survival ratio were considered after six weeks. Thirty six (36) single nodal explants were used for each clone.

Acclimatization

Seven-eight weeks old plantlets were taken out of the culture medium. The gel was removed and the roots washed down under tap water. The plantlets were transferred into acclimatization pots containing wet sterile sand with humus (1v/1v). For each cultivar, 30 plantlets were tested and then were regularly watered with tap water.

Organogenesis induction

Two clones of each cultivar were cut into single nodal explants of 1

Table 1. Root, bud and survival ratio (%) during initiation phase of clones derived from different cultivars after six weeks.

Parameter	Cultivar											
	Nagohé				*Damadoami*				*Tombolo*			
	Clone1	Clone2	Clone3	Clone4	Clone1	Clone2	Clone3	Clone4	Clone1	Clone2	Clone3	Clone4
TE	100	100	100	100	66.66	100	83.33	83.33	83.33	66.66	100	100
TD	100	100	100	100	100	100	100	100	100	100	100	100
TS	100	100	100	100	100	100	100	100	100	100	100	100

TE, Rate of rooting; TD, rate of sprouting; TS, rate of survival.

to 1.5 cm in length which were grown on the MS medium solidified with agar-agar 0.8% (w/v) containing 0, 2, 4, 5 and 8 mg/L of indole-3-butyric acid (IBA).

Evaluation of different parameters

Every week, the size and number of roots, leaves and shoots were assessed in order to calculate the ratio of survival as well as the stem and root growth. The observation of the cultures, during the organogenesis phase, was regularly made every three days and the new formations were recorded.

Statistical analysis

Data were subjected to the variance analysis (one-way ANOVA) and means were classified in homogenous groups according to Newman and Keuls's range test (α = 0.05) using Statistica version 10 (Statsoft Inc; Tulsa, USA: 2011) program. Survival ratio was calculated as follows:

$$\text{Survival ratio} = \frac{\text{Number of survival plantlets}}{\text{Number of total explant}} \times 100$$

RESULTS

Cultivar disinfection

Most of the residual contaminations showed up within the first week. The disinfection process was more effective on some of the cultivars than in others. In fact, after six weeks, *Nagohé* showed only 4% contamination whereas *Tombolo* and *Damadoami* developed 12 and 16% contamination, respectively.

In vitro culture establishment

Comparison of root and, stem growth ratio and survival ratio

The various clones had a survival ratio and a stem growth ratio up to 100%. The root growth ratio was 100% for the *Nagohé* clones and ranged from 66 to 100% for the *Damadoami* and *Tombolo* clones (Table 1). The root growth ratio was less than 100% for some clones.

Morphogenesis of the clones during initiation phase

Nagohé clones

After six weeks of culture on the MS medium, morphological characters of the different *Nagohé* clones were studied (Table 2). The characters of the clones varied with regard to the leaf and root production and the size of the plantlets. The clones derived from *Nagohé* cultivars showed long main roots with many lateral roots within two weeks. They had an average of five roots per plantlets. The leaves were large with long petioles. They were light or deep green. The leaves were alternated but sometime they were very next and seem opposed. No significant variation appeared within clones. The shoots appeared the first week and had regular growth. After six weeks, the clone 2 presented the best growth with an average size of 4.13 cm in length. But the clones did not show significant variation. The four *Nagohé* clones growing *in vitr*o on the MS medium showed the same morphogenetic characteristics.

Damadoami clones

All the clones derived from the *Damadoami* cultivar increased easily during the first week. After six weeks, the average number of roots per plantlet varied from 7 to 14. Clone 2 presented the best rhizogenesis (Table 2) while clone 4 showed the highest number of shoots with an average of 1.80 shoot per plantlet. The production near to 10 leaves per plantlet was similar for the four clones. The stems attained about 10 cm in length. The four clones did not show any significant variation with regard to the leaf and shoot production and stem growth. The only noticeable fact was that the plantlets of the clone 2 developed twice as many roots as the other plantlets.

Tombolo clones

The *Tombolo* plantlets of clones 1, 2, 3 produced only one stem while the plantlets of clone 4 produced axillary shoots for the sixth week and had an average of 1.33 shoot per plantlet. After six weeks, the plantlets of the clones 1, 2 and 3 produced 3 to 4 roots whereas half was produced by clone 4. The plantlets of clone 4 demonstra-

Table 2. Morphogenesis of clones derived from three cultivars during the initiation phase.

Cultivar	Clone	Parameter			
		Shoot	Root	Leaf	Size (cm)
Damadoami	1	1.33 ± 0.81^{ns}	8.83 ± 0.81^{a}	10.67 ± 0.05^{ns}	10 ± 0.17^{ns}
	2	1.5 ± 0.83^{ns}	14 ± 0.41^{b}	11.67 ± 1.21^{ns}	8.25 ± 0.18^{ns}
	3	1 ± 0.00^{ns}	7.33 ± 0.29^{a}	10.33 ± 0.24^{ns}	8.33 ± 0.32^{ns}
	4	1.8 ± 0.00^{ns}	7.83 ± 0.03^{a}	11.66 ± 0.22^{ns}	8.41 ± 0.08^{ns}
Tombolo	1	1 ± 0.00^{ns}	3.83 ± 0.11^{a}	7.16 ±0.19^{ns}	4.18a ±0.05
	2	1 ± 0.00^{ns}	3 ± 0.10^{a}	7.16 ± 0.09^{ns}	3.08a ± 0.03
	3	1 ± 0.00^{ns}	3.5 ± 0.10^{a}	8.5 ± 0.29.^{ns}	3.91a ±0.14
	4	1.33 ± 0.05^{ns}	8b ± 0.2	9.16 ± 0.29^{ns}	7.28b ± 0.25
Nagohé	1	1 ± 0.00^{ns}	4 ± 0.35^{ns}	4 ± 0.20^{ns}	1.97 ± 0.11^{ns}
	2	1 ± 0.00^{ns}	5.66 ± 0.23^{ns}	6.83 ± 0.18^{ns}	4.13 ± 0.36^{ns}
	3	1 ± 0.00^{ns}	5.5 ± 0.28^{ns}	5.83 ± 0.13^{ns}	3 ± 0.17 ^{ns}
	4	1 ± 0.00^{ns}	5.33 ± 0.31^{ns}	5.66 ± 0.30^{ns}	3.17 ± 0.18^{ns}

Each value represents mean ± 1/10 SE. Means within each column followed by the same latter are not significantly different by Newman and Keul at 0.05%. ns, not significantly different at probability level (p = 0.05).

Table 3. Morphogenesis of the cultivars during the initiation phase.

Cultivar	Shoot	Root	Leaf	Size (cm)
Damadoami	1.41 ± 0.08 b	9.54 ±0.37 b	11.08 ± 0.17^{c}	8.75 ± 0.20^{c}
Tombolo	1.12± 0.03 ab	4.55 ± 0.24 a	8 ± 0.20^{b}	4.61 ± 0.21^{b}
Nagohé	1 ± 0.00 a	5.12 ± 0.28 a	5.62 ± 0.22^{a}	3.06± 0.22^{a}

Each value represents mean ± 1/10 se. Means within each column followed by the same letter are not significantly different by Newman and Keul at the 0.05% probability level (p = 0.05). The mean of each cultivar were calculated from the four clones considered as replicates.

ted a more important stem growth (average of 7.22 cm in length) than clone 1, 2 and 3 with 4.18, 3.08 and 3.91 cm in length, respectively. The numbers of leaves (average 7.16 to 9.16) were comparable. Statistical analyses did not show any significant differences for the number of shoots, roots, of leaves and stem growth, except for the clone 4 which presented a faster stem growth and a higher number of roots (Table 2).

Morphogenesis of the cultivars during initiative phase

Root, shoot and leaf production and the size of the plantlets were recorded during six weeks (Table 3). The number of roots was different for each cultivar. During the first week, *Damadoami* produced an average of 7.83, *Tombolo* 3.79 and Nagohé 4.45 roots. After six weeks, the average number of roots was 9.54 for *Damadoami*, 4.55 for *Tombolo* and 5.12 for *Nagohé*. The difference was significant between *Damadoami* and the two other cultivars. Almost all the shoots appeared during the first week, each explant produced one shoot during the first week but the explants of *Damadoami* and *Tombolo* produced other shoots around the sixth week. The average of shoot per plantlet after six weeks was 1.41 for *Damadoami,* 1.16 for *Tombolo* and 1.00 for *Nagohé.*

After six weeks, *Damadoami* produced more leaves, 11.80 per plantlets then *Tombolo* 8 and *Nagohé* 5.62. *Damadoami* grew faster. The average size was 8.75 cm for *Damadoami*, 4.61 cm for *Tombolo* and 3.06 cm in length for *Nagohé*. During the third week, the slow growth of stem of the *Tombolo* plantlets could be due to the appearance of new shoots. Statistical analysis shows significant differences in the number of leaves and shoots and the stem growth from one cultivar to the other.

Multiplication phase

Rooting, stem growth and plantlet survival

During multiplication, the percentage of plantlets that grew root, changed according to the clones (Table 4). The lowest ratio was of 66.66% for clone 1 of *Nagohé* and the highest ratio was of 100% for clone 4 of *Damadoami*. In all cases, the ratio was higher than 60%. The ratio of stem growth was higher than 75% but varied with the clone. It reached 100% or so with two clones (1 and 3) of *Tombolo*. The *Nagohé* cultivar presented the lowest ratio of stem growth; the clone 1 had less than 50%. The ratio of survival was above 75% for each of the clones. This ratio reached 100% with *Damadoami* and *Tombolo*. The stem of some clones grew normally

Table 4. Percentages of rooting, sprouting and survival of clones in the multiplication phase for six weeks.

Parameter	Cultivar											
	Nagohé				***Damadoami***				***Tombolo***			
	Clone1	**Clone2**	**Clone3**	**Clone4**	**Clone1**	**Clone2**	**Clone3**	**Clone4**	**Clone1**	**Clone2**	**Clone3**	**Clone4**
TE	66.66	75	91.66	94	80.55	66.88	72.22	100	88.88	80.5	83.33	77.77
TD	50	77	77.77	83.83	77.77	72.22	83.33	83.33	94.44	86.11	100	83.33
TS	75	86.11	91.66	83.33	100	100	86.61	100	100	86.11	100	88.88

TE, Rooting %; TD, sprouting %; TS, survival %.

Table 5. Morphogenesis of clones of the cultivars during the multiplication phase.

Cultivar	Clone	Parameter			
		Shoot	**Root**	**Leaf**	**Size (cm)**
Damadoami	1	0.77a ± 0.04	1.25*b* ± 0.08	4.8a ± 0.28	2.42a ± 0.13
	2	0.72a ± 0.04	0.83a ± 0.08	5.52a ± 0.38	3.35a ± 0.28
	3	0.83a ± 0.03	0.91a ± 0.04	6.25a ± 0.3	2.86a ± 0.16
	4	0.83a ± 0.03	0.75a ± 0.04	6.33a ± 0.34	3.31a ± 0.23
Tombolo	1	0.58a ± 0.05	0.80a ±0.05	2.38a ± 0.38	0.87a ± 0.08
	2	0.61a ± 0.04	0.77a ± 0.06	3.50a ± 0.3	1.17a ± 0.09
	3	0.66a ± 0.04	1.05a ± 0.09	3.25a ± 0.27	1.09a ± 0.09
	4	0.58a ± 0.05	0.69a ±0.06	2.44a ± 0.44	0.85a ± 0.08
Nagohé	1	0.55a ±0.05	0.69a ±0.05	1.66a ±0.19	0.70a ±0.07
	2	0.80a ± 0.03	1.61b± 0.14	4.75c ±0.26	2.08c ± 0.11
	3	0.75a ± 0.02	1.16b ± 0.05	2.86b ±0.22	0.87ab ± 0.07
	4	0.83a ± 0.03	1.36b ± 0.06	3.13b ± 0.16	1.24b ±0.07

Each value represents mean ± 1/10 SE. Means within each column followed by the same latter are not significantly different by Newman and Keul at 0.05% probability level (p = 0.05).

without any rooting. On the other hand, some other clones produced roots and survived for six weeks of culture without any stem development.

Morphogenesis of the clones after six weeks of culture

Nagohé

About the number of shoots per clone, we noticed that some of the clones presented a more important stem growth. For instance, the clone 4 presented 0.83 shoot per plantlet while the clone 1 had an average number of only 0.55 shoot per plantlet. However, the analyses did not show any significant difference. The values are lower than 1 because some of the explants did not show any stem growth during the six weeks.

Clone 1 and 2 had extreme values for number of root per plantlet 0.69 and 1.61, respectively. Statistical analysis revealed two groups (clone 1) and (clone 2, 3, 4) (Table 5). The number of leaves depended on the clone. Clone 2 had the highest number of leaves 4.75 per plantlet. After six weeks clone1 had a size of 0.70 cm, and clone 2 had a size of 2.08 cm. The number of leaves and the size showed important differences according to the clones.

Damadoami

The number of root changed from one clone to the other. Clone 1 with 1.25 root per plantlet was from far better rooted. The number of shoots and leaves and their sizes were not very different. The clones of *Damadoami* were similar with regard to leaves production, shoots production and stem growth.

Tombolo

Shoot production was similar for all the clones; clone 3 had the highest number of shoot per plantlet, 0.66. The number of roots was low. Clone 1 had the highest number of roots with 1.05 root per *in vitro* plant. Leaves production was similar with extreme value of 2.38 and 3.50, respectively for clone 1 and clone 2. The plantlets of clone 2 reached the size of 1.17cm. The four clones of the cultivar *Tombolo*, had similar morphogenetic aspect during the multiplication stage.

Table 6. Morphogenesis of the cultivar during multiplication phase.

Cultivar	Shoot	Root	Leaf	Size (cm)
Damadoami	0.60ab ± 0.49	0.93a ± 0.05	5.73a ± 0.23	2.98a ± 0.21
Tombolo	0.8b ± 0,41	0.83a ± 0.06	2.90b ± 0.26	0.99b ± 0.08
Nagohé	0.70a ± 0.47	1.2b ± 0.09	3.09a ± 0.24	1.19a ± 0.10

Each value represents mean ± 1/10 SE. Means within each column followed by the same latter are not significantly different by Newman and Keul at 0.05% probability level (p = 0.05) The mean of each cultivar were calculated from the four clones considered as replicates.

Figure 2. Plantlets acclimatized in greenhouse (4 weeks in the greenhouse).

Morphogenesis of the cultivars

The number of roots, leaves, shoots and the size of 144 (4 X 36 explants) plantlets for each cultivar were compared. After six weeks, *Nagohé* had an average of 1.20 root/plantlet, *Damadoami* 0.93 root/plantlet and *Tombolo* 0.83 root/plantlet (Table 6). There were significant differences between *Nagohé* and the other cultivars. The ratio of the plantlets which produced root and the total number of roots were important. The ratio was always higher than 75%. The plantlets of *Damadoami* gave stem faster than the rest. After six weeks, the number of shoot per explants was lower than 1 with important disparities. This was due to explants that did not grow stem within the six weeks.

Damadoami produced more leaves and faster than the two other cultivars. It had 5.73 leaves per plantlet, *Nagohé* 3.09 and *Tombolo* 2.90. The leaves were small with a green colour that gets lighter with multiplication. The size depended on the cultivar. *Damadoami* was the tallest with 2.98 cm in average. *Nagohé* had 1.19 cm, and *Tombolo* 0.99 cm. Statistical analysis showed a large difference between *Damadoami* and the other cultivars. In terms of stem growth, even between clones of same cultivar, the results were not similar.

Acclimatization

In the greenhouse, the acclimated plantlets were rooted, grew and produced leaves easily (Figure 2). All the leaves were green except some that became yellow specially the leaves at the base of the plantlets. The acclimatization rate ranged between 80 and 100%.

Uninodal explants organogenesis

Organogenesis was induced by IBA at various concentrations: 0; 2; 4; 5 and 8 mg/L. The use of an auxin induces callogenesis as well as embryogenesis. With the concentration of 2 m /L, a yellowish callus formation appeared along the explant which emerged a tuft of roots. Explant broke bud and developed plantlet (Figure 3a). For the other concentrations, there was also a callus formation. The explant broke bud but did not develop plantlet (Figure 3d). The calluses were compact and gave roots neoformation via embryogenic (Figures 3b and c). The response revealed that the explants had an important embryogenic capacity while they were exposed to auxin. The somatic embryos developed from calli and proembryogenic zones. After four weeks, the embryos reached the cordiform stage. After eight weeks, short and thick root grew out of the calli (Figure 3)

DISCUSSION

The *in vitro* culture of three sweet potato cultivars from Togo gave plantlets which characteristics varied according to the clone and the cultivar studied. The disinfection method was efficient as demonstrated by the relatively

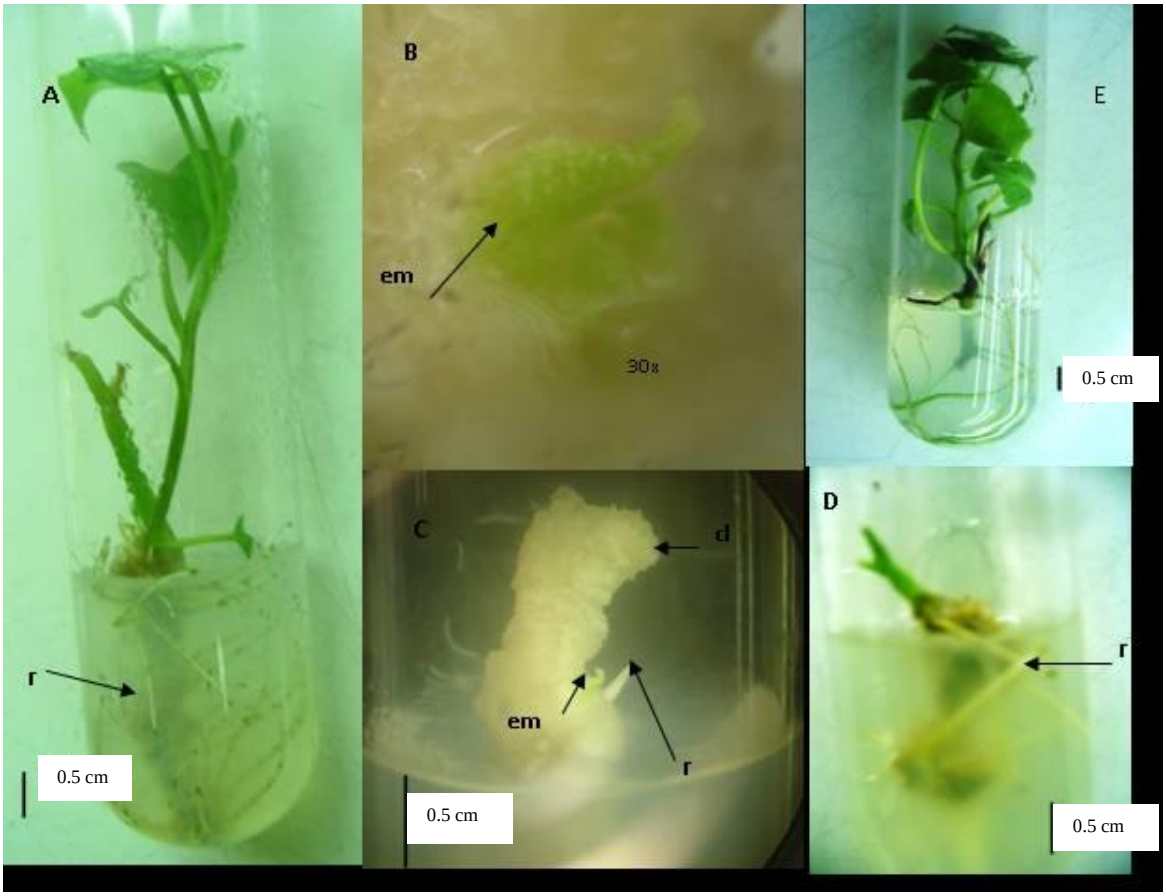

Figure 3. Effect of AIB on *Damadoami* cultivars. **A,** Plantlets cultured in the presence of 2mg/L AIB after eight weeks; **B,** an heart-shape embryo after three weeks on 5mg/l AIB; **C,** roots on 5 mg/l AIB after five weeks; **D,** thick root on 5 mg/l AIB after eight weeks; **E,** control plantlet; em, embryo; cal, callus; r, roots.

low level of contamination of the sweet potato explants. The method could be improved by varying the disinfectant concentrations and the time of exposure as reported by Archana (2002). The level of contamination of *Damadoam*i was higher than that of the two other cultivars.

Derived from the cultivars of sweet potato, the plantlets grew normally during culture establishment. The clones of *Nagohé* exhibited the similar behavior. On the other hand, the clones of *Damadoami* as those of *Tombolo* showed differences relating to the root production and plantlet size. Our observations are in agreement with those of Triqui et al. (2007) and of Sihachackr and Ducreux (1993) on somatic embryogenesis of sweet potato which revealed that characters depended on the genotype. Intra clone variations could be due to variability inherent to the *in vitro* culture, as previously and largely described by Trémouillaux-Guiller et al., (1987). Of the three cultivars studied, *Damadoami* was the most reactive to the *in vitro* culture. It produced more roots and its stems grew faster. Two hypotheses can explain this, as a higher level of endogenous hormones and a culture medium more propitious to the resumption of morphogenetic activities from the explants. The esta-blishment of the *in vitro* culture is a critical stage which depends partly on the mineral medium used (Le Nard et al., 1987).

During the vegetative multiplication, the clones of the different cultivars displayed different morphogenetic activities which could be explained by the genotypic impact and also by variability resulting from the passage of the *in vitro* culture. Some of the explants did not develop, some others did not produced roots. In general, root production was more important during initiation stage than during subcultures. Our observations were different from those of Hou et al., (2010) on *Castanea mollissima* cv. ”yanshanhong” who noticed better rooting of the subculture explants. They showed that the ratio indole-3-acetic acid (IAA)/abscisic acid (ABA) increased with increased rooting rate during successive subculture.

The problem can be solved by adding auxin naphthalene acetic acid (NAA) or IBA necessary for the root production and gibberellic acid (GA_3) for stem growth as it has been suggested by the studies achieved by Kodja et al.(1997) and Iftekhar et al. (2010) on sweet potato micropropagation. The growth is less important for the *Nagohé* and *Tombolo* organs. This could be due to the pH of the

medium which is also an important factor for growth and normal behavior *in vitro* (Auge et al., 1989). Our results showed that *Damadoami* was more reactive during the establishment of *in vitro* cultures and the vegetative multiplication than *Tombolo* and *Nagohé*. This could mean that the MS medium is more propitious to the need of *Damadoami.* Maybe, the culture on another medium as wood plant medium (WPM) could ameliorate the results as reported by Aidam et al. (2008) on *Ocimum gratissimum* root growth.

IBA induced an important callogenesis leading to newly formed roots; however, it is well known that IBA, at low concentration, favors rhizogenesis. We can infer that IBA has a concentration threshold for inducing callogenesis which can lead to organogenesis in sweet potato from Togo.

Plantlet acclimatization was successful. During the first week, the leaves became yellow because of the change of the physico-chemistry conditions. Subsequently, the leaves became larger and green. The success of the acclimatization is probably due to the substrate, leaf-mould, which was efficient for plantlet growth. Our result can be compared with those of Aidam et al. (2008) on *O. gratissimum* and of Sun et al. (2008) on *Zygophyllum xanthoxylon*. Other species were difficult to acclimatize such as *Irvingia gabonensis* (Ndomou et al., 2004) and *Ricinodendron hendoletii* (Oumar et al., 2004).

Conclusion

The three cultivars of sweet potato from Togo, as well as clones derived from each cultivar, showed different morphogenetic capacities during the vegetative micropropagation process. The root, shoot, node and leaf production, but also, the plantlet size depended on the clone and the cultivar tested. During the multiplication phase, the stem growth was slow for some clones. The average number and the size of the roots during multiplication stage decreased considerably compared with the initiation stage and were very heterogeneous. Other culture media could give better results for growth and root production. Sweet potato from Togo can easily answer somatic embryogenesis by combining different medium phytohormones. Acclimatized plantlets grew normally on sand with humus and could be transferred to a field.

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REFERENCES

Adelia CB (2007). Sweet potato a review of its past, present and future role in Human nutrition. J. Agric. Food Chem. 50(6)**:** 56-60.

Aïdam A, Etse K D, Koba K, Raymond C, Sanda K, Chaumont J-P, Trémouillaux**-** Guiller J (2008). Capacités morphogénétiques *in vitro*, performance au champ et production d'huiles essentielles chez *Ocimum gratissimum* L. Acta Bot. Gallica 155(3)**:** 341-354.

Akpavi S (2008) Plantes alimentaires mineurs ou menacées de disparition au Togo: diversités ethnobotaniques et valeurs. Thèse de Doctorat Université de Lomé. pp. 163.

Archana M (2002). Effect of Nacl on *in vitro* propagation of sweet potato (*Ipomoea batatas)*. Appl. Biochem. Biotechnol. 102-103(1-6):431-41.

Auge R, Beauchesne G, Boccon-Gibod J, Decourtye L, Digat B, Jalouzot R (1989) La culture *in vitro* et ses applications horticoles, Ed. J.B. Baillière. Tech.et doc. Lavoisier, Paris, Xpp.

Division Statistique agricole de l'Information et de Documentation (DSID)(2010). 1997 à 2010 : Campagne agricole, production en milieux paysans et autres secteurs. pp. 30.

FAOSTAT(2010). http:// faostat.fao.org

Hattorie T, Nakagawa T, Maeshima M, Nakamura K Asahi T (1985). Molecular cloning and nucleotide sequence of cDNA for sporamin, the major soluble protein of sweet potato tuberous roots. Plant Mol. Biol. 5: 313-398.

Hou J, Guo S, Wang G (2010). Effects of in vitro subculture on the physiological characteristics of adventitious root for formation in microshoots of Cactanea mollissima. cv "yanshanhong". 21(2):155-160

Iftekhar A, Shamina AS, Mst KN, Jahangir MA, Anisuzzaman M, Mohammad FA (2010). Effect of growth regulators on meristem culture and plantlet establishment in sweet potato (Ipomoea batatas (L.) Lam) POJ. 3(2) :35-39

Iftekhar A, Shamina AS, Mst KN, Jahangir MA, Anisuzzaman M, Mohammad FA (2013). Elimination and detection of virus in meristem-derived plantlets of sweet potato as low-cost option toward commercialization. 3 Biotech 3: 153-164

Institut Togolaise de Recherche Agronomique (ITRA)(2006). La patate douce au Togo. pp.12.

Issa TS, Marie-Noël Z, Patrick EK, Mohamed AB, Shawn KB, Hélène D, Pierre IG (2004) Validation d'une méthode de dosage des caracténoïdes par CLHP : Application à la determination de teneur en caracténoïde dans dix variétés de patate douce (Ipomoea batatas) C.R Chimie 7

Kodja H, Robene-Soustrade I, Figier J, (1997). Micro propagation de la patate douce a partir du développement axillaire. 6^{e} journée scientifique du réseau biotechnologie végétale AUPELF- UREF ; Orsay.

Le Nard M, Ducommun C, Weber G, Dorion N, Bigot C, (1987). Observations sur la multiplication in vitro de Tulipe, Tulipa gesneriana L., à partir de hampes florales prélevées chez les bulbes en cours de conservation. Agronomie 7: 321-329.

Murashige T, Skoog FA (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497

Ndomou DO, Oumar F, Mbouna D (2004). Propagation *d'irvingia gabonensis* par microbouturage in vitro. Fruits 59: 31-38.

Oumar F, Donfagsiteli TN, Mbouna D et Ndomou D (2004). Propagation de Ricinodendron heudoletii par bouturage *in vitro*. Fruits 59: 351-358.

Sihachakr D, Ducreux G (1993). Regeneration of plant from protoplasts of sweet potato (*Ipomoea batatas* (L.) Lam) in: Bajaj y. (Ed.). Biotechnology in agriculture and forestry vol.23, Plant protoplast and genetic engineering IV Springer-Verlag. Berlin, Heidelberg. pp. 43-59.

Sun L D, Hou SW, Wu DL, Zhang YC (2008). Rapid clonal propagation of zygophyllum xanthoxylon (Bunge) Maxim, an endangered desert forage species. *In Vitro* Cell. Dev. Biol. Plant 44: 396-400.

Trémouillaux-Guiller J, Andreu F, Crèche J, Chénieux JC, Rideau, M (1987). Variability in tissue culture of *Choisya ternata*. Alkaloid accumulation in protoclones and aggregate clones obtained from established strains. Plant Cell Rep. 6: 375-378.

Triqui Z E A, Abdelkarim G, Averil C, Hassane C, Vingthip S, Robert H, Sihachakr D (2007). Effet du génotype de l'agent gélifiant et de l'auxine sur l'induction de l'embryogenèse Somatique chez la patate douce C.K. Biologies 331.