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Whole Plants Regeneration of Cassava Cultivars (*Manihot esculenta* Crantz) Originated from Côte d'Ivoire via Somatic Embryogenesis

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Authors' contributions

This work was carried out in collaboration between all authors. Author Kouassi Konan Marius designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Kouassi Kan Modeste, KKE, GYG and KKL managed the analyses of the study. Author KM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the capacity of cassava genotypes in Côte d'Ivoire to induce somatic embryos and to regenerate plants from immature leaves
Study Design: *In-vitro*, laboratory-based study.
Place and Duration of Study: National Center for Agronomic Research (CNRA), between January 2017 and April 2018.
Methodology: An efficient protocol to regenerate somatic embryogenesis (SE) from cassava (*Manihot esculenta* Crantz) plants cultivated in Côte d'Ivoire was achieved. Immature leaf lobes were used as explants on Murashige and Skoog (MS) basal medium supplemented with different

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concentrations (16; 33; 50; 66 and 83 µM) of the auxins Picloram (Pic) and 2,4-Dichlorophenoxyacetic acid (2,4-D).

Results: The results showed that the frequency of primary somatic embryogenesis (PSE) and the mean number of somatic embryos varied significantly with the genotype, the type of auxin and the tested concentrations. The highest frequencies and numbers of somatic embryos per explant were observed with cv. TMS 60444 (81.66%; 190.8) on 50 µM Pic, followed by Local XX1 (90%; 180) on 66 µM Pic, To (100%; 145.8) on 50 µM Pic, I (80%; 125.6) on 66 µM 2,4D and M (100%; 112) on 50 µM 2,4D. Shoot bud induction from green cotyledons varied across cultivars and benzylaminopurine combined with 1-Naphthalene acetic acid outperformed benzylaminopurine associated with Indole-3-butyric acid regarding induce of organogenesis.

Conclusion: Regenerated plants grew easily in the greenhouse with 90 – 100% survival rate and did not display detectable variation in morphology.

Keywords: Cassava; organogenesis; plant regeneration; plant growth regulators; somatic embryogenesis.

ABBREVIATIONS

2,4-D: 2,4-Dichlorophenoxyacetic acid; BAP: Benzylaminopurine; CBM: cassava basal medium; CEM: Cassava elongation medium; CIM: Callus induction medium; CMML: Cassava maturation medium; COM: Cassava organogenesis medium; CRM: Cassava rooting medium; CSE: Cyclic somatic embryogenesis; NAA: α-Naphthalene acetic acid; Pic: Picloram; PSE: Primary somatic embryogenesis; SE: Somatic embryogenesis; SSE: Secondary somatic embryogenesis; Var: Varieties; F.S.E.: Frequency of somatic embryos; N.E.S: Number of somatic embryos.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae (2n = 36), and is a plant grown for its tuberous roots and leaves. The crop is adapted to a wide range of environments and has a good resistance to drought and soil acidity [1]. It ranks fifth among food crops behind maize, rice, wheat and potatoes [2]. The plant is grown throughout the country in Côte d'Ivoire and is represented by nearly a hundred local cultivars [3]. It is one of the most important staple food crops in Africa. Its starchy tuberous roots provide a valuable source of cheap calories for about 500 million people in the developing world commonly plagued by chronic food deficiency and malnutrition [4]. World production was estimated of about 250 million tons in 2011 [5]. In Africa, the continent with the largest production (53% of world production), the crop plays an important role as famine-reserve crop, rural staple food, cash crop for both rural and urban households and, to a lesser extent, raw material for feed and chemical industries [6]. Cassava is consumed in many forms. The tubers are eaten raw or boiled for so-called "sweet" varieties and prepared according to a complex process of detoxification for so-called "bitter" varieties. This process has resulted in many derived products, which are ‘’tapioca’’, ‘’attiéké’’, ‘’gari’’, ‘’agou (fufu)’’ and various types of pasta. Leaves are eaten as a vegetable in most of the countries across Africa [7].

Despite its significant importance in ensuring food security in developing countries, biotic and abiotic constraints such as disease, insect attack and drought severely limit cassava production [8]. Cassava is heterozygous, and some varieties do not flower [9]. The low protein content (1-2%), the presence of toxic compounds (cyanogens) and the low storage time of tubers (1-3 days after harvest) are also other constraints to cassava cultivation [10].

To overcome the cultural constraints that significantly affect cassava production, several studies have been conducted for the creation of high-performing and/or disease-resistant varieties [11]. For this, the classic selection has been adopted. However, the high rate of heterozygosity and the long time required to fix a new variety are increasingly orienting research towards the use of an alternative or complementary pathway to conventional breeding, namely, genetic transformation [12]. Application of this pathway, however, requires the development of an effective whole plant regeneration protocol in cassava [13]. The protocol for plant regeneration frequently in cassava is via the process of somatic embryogenesis [14]. Responses to somatic embryogenesis, regeneration, and/or transformation vary greatly among genotypes, and not all varieties of cassava can be amenable to this morphogenesis pathway [15].

There are nearly 1500 cassava cultivars worldwide [16], and today all of the research efforts on cassava regeneration and processing are devoted to South American varieties [13,17], while the largest cassava production is in Africa. Few studies have focused on the process of genetic transformation of African cassava varieties or a study to show that African cultivars respond differently as compared to those in South America [4]. In Côte d'Ivoire, the ability of somatic embryos to induce the characteristics necessary for the successful genetic transformation of most local cassava cultivars is virtually non-existent in the literature. It is, therefore, necessary and imperative to carry out an effective regeneration protocol for successful genetic transformation via somatic embryogenesis of cassava cultivars in Côte d'Ivoire in particular and in general for Africa.

The present research aims to study the capacity of cassava genotypes in Côte d'Ivoire to induce somatic embryos and to regenerate plants from immature leaves

2. MATERIALS AND METHODS

2.1 Plant Materials

Eight cassava cultivars namely: To, XX1, Pk, Dr, 85a, M, I and TMS60444 (control) were collected from the ex-situ conservation plots of cassava germplasm at the University of Nangui Abrogoua, Côte d'Ivoire. Apart from TMS 60444 as control, the seven other cultivars are landraces from Côte d'Ivoire. The plantlets were grown *in vitro* on Murashige and Skoog media [18] supplemented with 20 g/L sucrose, Murashige and Skoog Vitamins (Duchefa, Germany) and 8 g/L of noble agar. All media used for *in vitro* propagation of cassava was sterilised through autoclaving. The growth chamber conditions were set at a temperature of 25°± 2°C and a 16 hr day/8-night cycle.

2.2 Callus Induction and Primary Somatic Embryogenesis

Immature leaf lobes (2-6 mm long) excised from *in vitro*-grown plants were cultured on Murashige and Skoog basal medium supplemented with 20 g/L sucrose, Gamborg B5 vitamins, 0.5 mg/L CuSO4 [19] and various concentrations (16; 33; 50; 66 and 83 µM) of 2,4-D. The same set of immature leaf lobes was transferred on the same media substituted with Pic. The media pH was adjusted to 5.7 and solidified with 8 g/L noble plant agar. The cultures were maintained at a temperature of 25 ± 2°C. The explants were left in the induction medium for 6 weeks. The type of calli was observed at each step, and the frequency of embryogenic calli formation was recorded after four weeks of culture on callus induction medium (CIM). Each treatment consisted of 10 Petri dishes and each Petri dish containing 10 explants (100 explants per treatment).

2.3 Secondary Somatic Embryogenesis

Green cotyledon pieces (5 mm^2) were excised from the primary cotyledon embryos and transferred to CIM supplemented with 50 µM NAA. Green cotyledon pieces obtained from 2 week-old secondary cotyledon embryos were placed on CIM supplemented with 50 µM NAA for the induction of cyclic somatic embryogenesis. Somatic embryogenesis was carried out in a growth chamber set at 25 ± 2°C in the continuous dark. Each treatment contained 10 Petri dishes with ten explants (100 explants per treatment). The frequency of somatic embryogenesis and an average number of somatic embryos produced at each stage per embryogenic callus were recorded after 4 weeks of culture.

2.4 Maturation of Somatic Embryos

This entailed the development of globular stage embryos into green cotyledonary embryos with the defined shoot and root axes [13]. The globular stage somatic embryos were subcultured on cassava maturation medium (CMML) consisted of MS medium containing 20 g/L sucrose and supplemented with 0.1 mg/L BAP as described by Li et al. [20]. The media pH was solidified with 8 g/l noble plant agar. The embryos were maintained in the maturation medium in the dark for 4 weeks.

2.5 Effect of BAP and Auxin (NAA and IBA) on Organogenesis under Light and Dark Conditions

The effect of the combination 1mg/L BAP with auxins (0.5 mg/L of NAA or IBA) on adventitious bud formation of the cassava cultivars were assessed after three and four cycles of somatic embryogenesis. Matured green cotyledon embryos were divided into 0.5 cm^2 pieces and transferred on cassava organogenesis medium (COM) [MS basal medium, B5 vitamins, 20 g/L sucrose and 2 µM CuSO4, supplemented with 1 mg/L BAP and 0.5 mg/L IBA or 1 mg/L BAP and

0.5 mg/L NAA, pH 5.7 and noble agar (8 g/L)]. Each treatment contained 10 explants in each of five Petri dishes (50 explants per treatment). Cultures were incubated under continuous dark or under a photoperiod cycle of 16 h light to determine the effect of light on bud formation. After 1 month, the frequency of callus and bud induction, the number of buds per explant and the shoot bud length were recorded.

2.6 Elongation and Rooting of Shoot Buds, and Acclimatisation of Regenerated Plantlets

Shoot primordia from maturation medium were transferred onto cassava elongation medium (CEM: CBM supplemented with 0.4 mg/L BAP) for shoot elongation. After 4 weeks, the elongated shoots were transferred onto cassava rooting medium (CRM: CBM without plant growth regulators) for rooting and development. Seedlings with well-developed roots were then removed from the test tubes and rinsed with tap water to remove any trace of the gelling agent. In the greenhouse, these seedlings were transplanted into pots containing a sterile substrate composed of black soil. The percentage of plantlet survival and their heights were recorded 4 weeks after being transferred to the greenhouse.

2.7 Experimental Design and Statistical Analysis

All experiments were carried out in a completely randomised design. The treatments were repeated three times (100 explants per treatment). Samples were evaluated using Analysis of Variance (ANOVA). Newman–Keuls multiple range tests were used to separate treatment means found significantly different by ANOVA. All analyses were at $p \leq 0.05$ confidence level. The analysis was performed with the Statistica 7.1 software.

3. RESULTS

3.1 Effects of 2,4-D and Pic on Callus Induction and Somatic Embryogenesis

In this study, seven cassava landraces from Côte d'Ivoire and the control TMS 60444 were tested for their ability to induce calli and somatic embryogenesis on MS basal medium containing five concentrations (16; 33; 50; 66 and 83 µM) of 2,4-D and Pic. The immature leaf lobe explants (Fig. 1A) developed into a swollen callus mass on callus induction medium (CIM) within 5 days. After 4 weeks of culture, a compact non-embryogenic callus (Fig. 1B) and a translucent gelatinous callus with proembryogenic masses (Fig. 1C) were observed in all cultivars (Cvs). These proembryogenic masses produced globular somatic embryos (Fig. 1C), which developed through the characteristic somatic embryogenesis stages of, trumpet and cotyledonary (Fig. 1D–E).

All seven cassava landraces and the control TMS60444 were able to induce callus. Seven out of eight were amenable to attain cotyledonary stage. Only cultivar (Dr) produced no cotyledonary embryos on medium supplemented with all concentration (Table 2). The time required to induce somatic embryos and to attain cotyledonary stage varied among the genotypes. The potential of calli and somatic embryogenesis, as indicated by the frequency of calli and somatic embryo production and the number of somatic embryos per explant, was assessed in each cultivar (Tables 1 and 2). Results showed that both parameters varied widely across varieties, auxin type and concentration. Formation of embryogenic calli was consistent with the frequency of callus induction in all the cassava varieties. For both callus induction and somatic embryogenesis, the best auxin concentration was 50 µM Pic (Tables 1 and 2). The highest frequencies and number of somatic embryos per explant were observed with the Cv. TMS 60444 (81.66%; 190.8) on 50 µM Pic, followed by Local XX1 (90%; 180) on 66 µM pic, To (100%; 145.8) on 50 µM pic, 85a (88.33%; 135.66) on 50 µM pic, PK (80%; 133.16) on 50 µM pic, I (80%; 125.6) on 66 µM 2,4D, M (100%; 112) on 50 µM 2,4D and Dr (80%; 0).

3.2 Secondary Embryogenesis

Secondary somatic embryogenesis has the same embryonic developmental stages as primary embryogenesis. As the Dr variety did not induce cotyledonary embryos, the secondary embryogenesis test was not performed with this variety. Results for secondary embryogenesis responses are shown in Table 3. Regarding the secondary embryogenesis rate and the number of embryos, a significant difference was noted. The highest frequencies and the number of somatic embryos per explant were observed in Cvs. TMS 60444 (99%; 206.1), To (96%; 186.8), XX1 and 85a (93%; 186.80), Pk (92%; 178.40), M (95%; 185.50) and I (94%; 177.70). The mean

frequency and the number of somatic embryos have been markedly improved during secondary somatic embryogenesis.

3.3 Effect of BAP and Auxin (NAA and IBA) on Organogenesis under Light and Dark Conditions

After four weeks of culture on the various organogenesis media, the induction and the development of buds were observed under the two conditions: light and dark conditions (Fig. 1F). Frequencies of bud formation, as well as number of buds produced per explants, are presented in Table 4. As for shoot regeneration, seven cultivars (TMS 60444, To, PK, XX1, 85a, M and I) produced shoots. Overall, the frequencies of bud formation were similar under light and dark conditions with higher values recorded in medium supplemented with BAP (1 mg/L) + IBA (0.5 mg/L) (70- 83%) than in medium containing BAP (1 mg/L) + NAA (0. 5 mg/L) (75– 81%) where the frequency of budding tended to be higher under light (53– 81%) than under dark (13–37%) (Table 4). As for the number of buds , medium supplemented with BAP (1 mg/L) + IBA (0.5 mg/L), performed better than BAP (1 mg/L) + NAA (0.5 mg/L) supplemented medium (Table 4). Organogenesis was higher in Cvs. TMS60444 (83%; 35), XX1 (81%; 31), M (80%; 25.4), PK (70%; 23.5), To (70%; 19.6), 85a (81%; 15.5) and I (75%; 17).

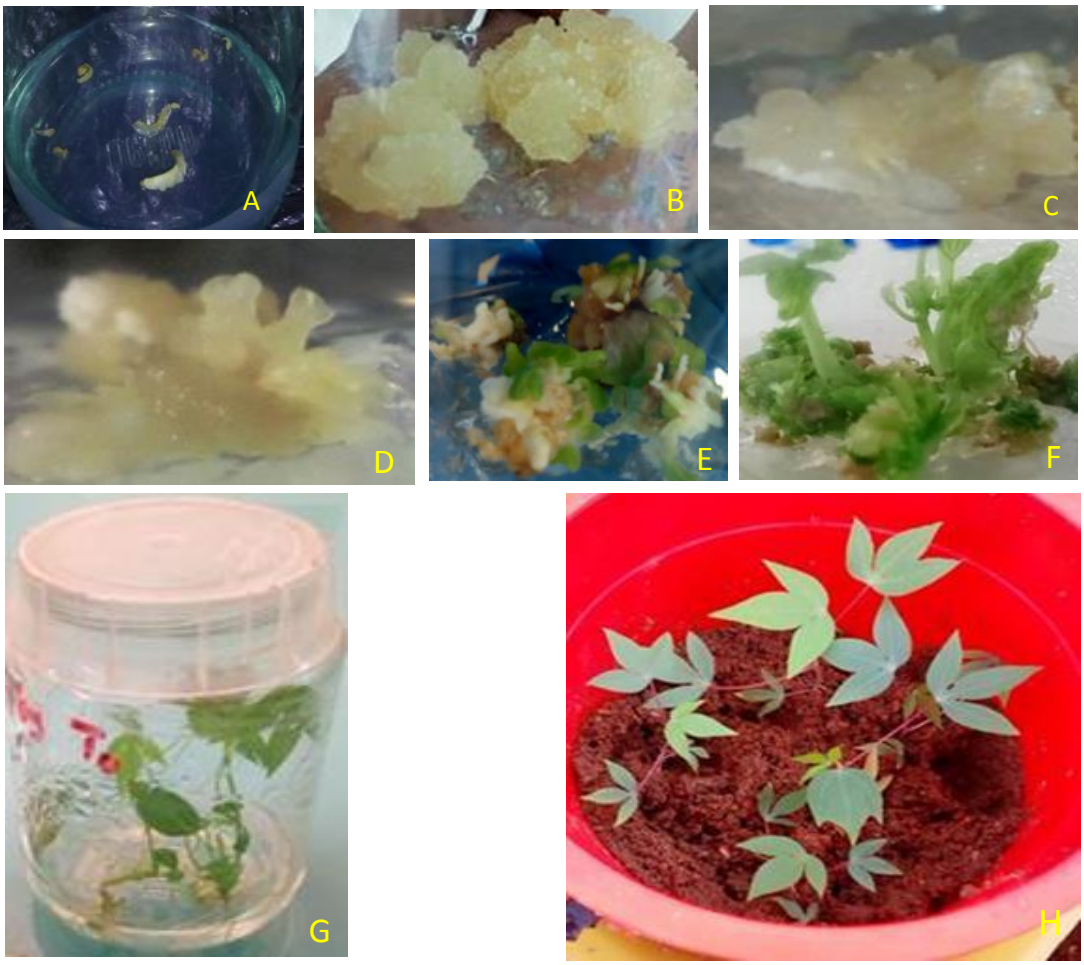

Fig. 1. Regeneration of cassava cultivars from Côte d'Ivoire and the control TMS60444. (A) immature leaf lobes (B) induced compact non-embryogenic callus (C) and callus with proembryogenic masses Clusters of organised embryogenic structures consisting of globular (D) trumpet structures (E) formation of green cotyledon (F) Formation of distinct shoots and Elongated shoot buds rooted and developed into whole plantlets (G) *in vitro* After transferring in boxes, hardened plantlets (H) Cassava plantlets growing in the greenhouse

Table 1. Effects of different concentrations of 2,4-D and Pic on callus induction

	Plant growth regulators and frequency (%) of callus					
	16 µM		33 µM		50 Mm	
Varieties	**2,4 D**	**Pic**	**2,4 D**	**Pic**	**2,4 D**	**Pic**
XX1	86,66±0,06abcde	80±0bcde	93,33±0,06abc	93,33±0,03abcd	96,66±0,03abc	90±0,05abcde
PK	93,33±0,03abcd	86,66±0,03abcde	86,66±0,06abcde	83,33±0,03bcde	83,33±0,03bcde	90±0,05abcde
DR	96,66±0,03abc	90±0abcde	86,66±0,06abcde	86,66±0,03abcde	86,66±0,03abcde	93,33±0,03abcd
TMS60444	0±0f	0±0f	83,33±0,08bcde	86,66±0,03abcde	96,66±0,03abc	80±0bcde
TO	0±0f	0±0f	100±0a	100±0a	100±0a	100±0a
M	0±0f	0±0f	83,33±0bcde	77,33±0,05cde	100±0a	86,66±0,05abcde
I	0±0f	0±0f	83,33±0bcde	77,33±0,05cde	100±0a	100±0a
85a	0±0f	0±0f	100±0a	97,66±ab	77,33±0,05	71,66±0,05de

Within the same line, mean values followed by the same letter are not significantly different at α = 5 % (Newman–Keuls test) ±, standard deviation

Table 1. Continued

	Plant growth regulators and frequency (%) of callus			
	66 µM		83 µM	
Varieties	**2,4 D**	**Pic**	**2,4 D**	**Pic**
XX1	80±0bcde	90±0abcde	86,66±0,06acde	100±0a
PK	83,33±0,03bcde	86,66±0,03abcde	83,33±0,03bcde	96,66±0,03abc
DR	93,33±0,06abc	86,66±0,03abcde	86,66±0,06abcde	86,66±0,06abcde
TMS60444	93,33±0,06abc	100±0a	100±0a	93,33±0,06abc
TO	100±0a	100±0a	100±0a	100±0a
M	7²1,66±0,05de	77,33±0,05bcde	83±0bcde	66±0e
I	77,33±0,05bcde	71,66±0,05de	83±0bcde	71,66±0,05de
85a	66±0e	80,66±0,03abcde	66±0e	66±0e

Within the same line, mean values followed by the same letter are not significantly different at α = 5 % (Newman–Keuls test) ±, standard deviation

Table 2. Effect of plant growth regulators on somatic embryogenesis derived from immature leaf lobe of cassava cultivars from Côte d'Ivoire

Plant growth regulators		Varieties							
		TMS 604444		XX1		PK		M	
µM		F.S.E	N.SE	F.S.E	N.SE	F.S.E	N.SE	F.S.E	N.SE
16	2,4D	0±0p	0±0y	0±0p	0±0y	0±0p	0±0y	0±0p	0±0y
	Pic	0±0p	0±0y	0±0p	14.66±1.17w	10±0o	24.16±1.01u	0±0p	0±0y
33	2,4D	0±0p	0±0y	0±0p	0±0y	0±0p	0±0y	76.66±0.05c	68.66±1.94m
	Pic	31.66±0.04ijk	90±1.06k	38.33±0.04fghi	56.16±0.6p	80±0c	96.33±0.61j	0±0p	0±0y
50	2,4D	10±0o	10±0.51x	40±0fgh	14.33±0.71w	10±0o	8.13±1.30x	100±0a	112.66±0.84a
	Pic	81.66±0.01c	190.83±1,10a	58.33±0.04de	113.66±0.80h	80±0c	133.16±0.4f	33.33±0.03ghij	12.33±0.84w
66	2,4D	0±0p	0±0y	10±0o	12.66±0.55w	31.66±0.01ijk	31.66±0.79	80±0c	137.83±2.16d
	Pic	45±0.02f	90±1.48k	90±0b	180±1.71b	40±0fghi	55.5±0.22p	20±0l	8.83±0.98x
83	2,4D	0±0p	0±0y	0±0p	0±0y	0±0p	0±0y	40±0fghi	64±0.51o
	Pic	43.33±0.03f	21.5±0.5v	56.66±0.02e	82.5±1.17l	0±0p	0±0y	0±0p	0±0y

FSE = frequency of somatic embryogenesis; NSE = number of somatic embryos per explant

Within the same line, mean values followed by the same letter are not significantly different at α = 5 % (Newman–Keuls test) ±, standard deviation

Table 2. Continued

Plant growth regulators		Varieties							
		I		85a		To		DR	
µM		F.S.E	N.SE	F.S.E	N.SE	F.S.E	N.SE	F.S.E	N.SE
16	2,4D	0±0p	0±0y	0±0p	0±0y	0±0p	0±0p	80±0c	0±0y
	Pic	0±0p	0±0y	0±0p	0±0y	0±0p	0±0p	0±0p	0±0y
33	2,4D	65±0.05d	38.5±2.21s	15±0.08mno	0±0y	29.16±0.08jk	0±0y	60±0de	0±0y
	Pic	0±0p	0±0y	100±0a	104±0.93i	81.66±0.04c	56.66±0.61p	0±0p	0±0y
50	2,4D	100±0a	97.16±1.30j	16.66±0.03lmn	0±0y	40±0fgh	14.33±0.71w	40±0fgh	0±0y
	Pic	26.66±0.02jk	8.5±0.8x	88.33±0.01b	135.66±0.49e	100±0a	145.83±0.47c	26.66±0.1k	0±0y
66	2,4D	80±0c	125.66±0.42g	0±0p	0±0y	90±1.48k	0±0y	80±0c	0±0y
	Pic	11.66±0.04no	0±0i	40±0fghi	46.83±1.30q	81.66±0.04c	66.33±0,42n	0±0p	0±0y
83	2,4D	40±0fghi	32±0.93t	0±0p	0±0y	0±0p	0±0y	36.66±0.08fghi	0±0y
	Pic	0±0p	0±0y	0±0p	0±0y	63.33±0.03	41.66±1.33r	0±0p	0±0y

Within the same line, mean values followed by the same letter are not significantly different at α = 5 % (Newman–Keuls test) ±, standard deviation

Table 3. Evaluation of secondary somatic embryogenesis induced from primary embryo explants of seven cassava varieties

Varieties	Frequency (%) of somatic embryos	Number of somatic embryos
TMS60444	99±0.02a	206.1±0.88a
To	96±0.01ab	186.8±0.41b
XX1	93±0.01ab	186.8±0.32b
85a	93±0.01ab	168±0e
Pk	92±0.01b	178.4±0.26d
M	95±0.01ab	185.5±0.5c
I	94±0.01ab	177.7±0.15d

Within the same line, mean values followed by the same letter are not significantly different at α = 5 % (Newman–Keuls test) ±, standard deviation

Table 4. Responses to organogenesis of cassava varieties produced from embryonic callus derived from immature leaf explants under 16h photoperiod and continued darkness

Hormonal combination	incubation conditions	Varieties	Frequency (%) bud induction	Number of buds/ explant
BAP (1 mg/l) + NAA (0.5 mg/l)	16 hr day/8 night	TMS 60444	60±0d	23.3±0.57c
		To	70±0c	19.6±2.16d
		XX1	53±0.01e	15.6±1.21ef
		Pk	50±0ef	16.2±0.2e
		85a	81±0.01a	15.5±0.76ef
		M	80±0a	25.4±1.30c
		I	75±0.02b	17±0.33e
		Dr	0±0n	0±0n
	Darkness	TMS 60444	20.2±0.02jkl	13.6±0.26fh
		To	37±0.01g	7.5±0.83ij
		XX1	16.4±0.03l	8.5±0.76ij
		Pk	13±0m	11.8±0.24h
		85a	18±0.01kl	6.1±0.45j
		M	27±0.01hi	11.8±0.24gh
		I	25±0.01hij	6.3±0.47j
		Dr	0±0n	0±0n
BAP (1 mg/l) + IBA(0.5 mg/l)	16 hr day/8 night	TMS 60444	30±0h	20.2±0.46d
		To	59±0.01d	11.6±0.37h
		XX1	24.9±0.01hij	11.8±0.96h
		Pk	21±0jk	12.6±0.22h
		85a	38±0.04	9.2±0.44gi
		M	47±0.01f	12.7±0.26h
		I	58±0.01d	12±0.73gh
		Dr	0±0n	0±0n
	Darkness	TMS 60444	83±0.02a	35±0a
		To	24±0.01ij	6.2±0.32j
		XX1	81±0.02a	31±1.24b
		Pk	70±0.02c	23.5±0.87c
		85a	35±0.01g	6.8±0.48ij
		M	36±0.01g	6.2±0.41j
		I	27±0.02	6.3±0.44j
		Dr	0±0n	0±0n

Within the same line, mean values followed by the same letter are not significantly different at α = 5 % (Newman–Keuls test) ±, standard deviation

3.4 Elongation and Rooting of Leafy Shoots

Prior to transplanting to the greenhouse, lengths of shoots regenerated on maturation medium were measured; values ranged from 0.8 to 1.08 cm and showed no statistical differences. Shoots of all cultivars developed roots efficiently on elongation medium supplemented with 0.4 mg/L BAP (Fig. 1G).

3.5 Acclimatization of Regenerated Plantlets

The ability of regenerated plantlets to acclimatize and grow in the greenhouse was assessed by measuring the proportion of plantlets recovered as well as plantlet height. Cultivars TMS 60444, To, 85a, M and XX1 showed a significantly higher regeneration rate than cvs PK and I. The regenerated plants were morphologically normal and grew rapidly and after 6 weeks under greenhouse conditions, plantlets height ranged from 18 to 27 cm. The regenerated plants from the seven varieties were adapted to growing conditions in the greenhouse with a success rate ranging from 90 to 100 % for all cultivars tested (Fig. 1H) [21,22].

4. DISCUSSION

In this study, various factors known to have an effect on the cassava somatic embryogenesis and regeneration were evaluated. The source and concentration of auxin play a role in the regeneration of various plants. This study determined higher levels of 2, 4-D and Picloram as the best inducers of somatic embryos. The results of this study are in accordance with the previous study achieved by Raemakers et al. [23] who evaluated the effects of 2, 4-D, dicamba, picloram and NAA on the somatic embryogenesis of seven Cameroon cassava cultivars and found Picloram to be the best inducer at 12 mg/l. On the contrary, Ihemere et al. [4] also determined a higher number of cassava somatic embryos produced under 12 mg/l of 2, 4-D. However, Fletcher et al. [19] reported 8 mg/l of 2, 4-D as the best concentration for callus induction in four Ghanaian cassava cultivars. Other factors like the source and health status of the explants used in this study may have contributed to results obtained.

Organogenesis from cotyledons of maturing somatic embryos is the most commonly used regeneration method for cassava [24]. In the medium supplemented BAP (1 mg/L) + NAA (0.5 mg/L) and BAP (1 mg/L) + IBA (0.5 mg/L), callus induction was observed. It is obvious that the auxin IBA and NAA combined with BAP might be responsible for this callus induction. The present results showed that BAP treatment gave the best organogenesis responses and thus in agreement with others [25,15]. Even though it is not clear why BAP + IBA was less efficient in inducing organogenesis from maturing somatic embryos, it is possible that the concentration and nature of auxin may be an important factor and subsequent studies will need to assess different levels.

Although the frequency of bud induction was found in this study to be similar under light and dark conditions, the number of buds formed per explant was significantly higher when green cotyledons were incubated under 16 h light. The photoperiod has consistently been shown to be genotype-dependent for shoot formation. For example, a photoperiod of 16 h light was reported to be more efficient in inducing shoot formation from green cotyledons [15], while Li et al. [26] obtained better results under continuous dark. The study found that Cv. to was efficient in embryogenesis but less proficient in organogenesis, suggesting that the ability to produce somatic embryos does not necessarily translate to shoot regeneration proficiency. This result indicates that somatic embryogenesis and organogenesis may be controlled by different and independently inherited traits. Taken together, this study shows that the Côte d'Ivoire cultivars investigated here contain sufficient genetic variability for somatic embryogenesis and adventitious shoot formation and can likely be improved using the *Agrobacterium*- mediated approach. Konan et al. [17] also observed a similar phenomenon. It is important to indicate that whereas some cassava cultivars from Colombia [27,28], Argentina [29] and Côte d'Ivoire [17] exhibited regeneration efficiencies similar to those reported here, others showed very low efficiencies.

5. CONCLUSIONS

Côte d'Ivoire farmer-preferred cassava landraces tested in this study demonstrated good ability in producing somatic embryos and plant regeneration potential. Response to somatic embryogenesis and regeneration ability was genotype dependent as reported in the literature. Some of the landraces could be converted to plantlets while one could not. However, other

factors like source and age of explants, culture conditions, sub-culturing cycles, age and brand of the media used might have contributed to the regeneration ability and variations of the tested cassava landraces in this work. Although all cassava landraces will be targeted for genetic engineering programs, results obtained from this study are enlightening potential candidate landraces amenable to transformation protocols. There is a need to develop efficient, genotype-independent regeneration and transformation protocols that will overcome a challenge of varying in vitro response of cassava between closely related cassava cultivars.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Coulibaly N, Sery Z. Situation de la culture et de la recherche sur le manioc en Côte d'Ivoire. IDESSA. 1992;6.
2. FAO Food and agriculture organization of United Nations. Statistical databases. Rome (Italy) ; 2010.
   Available:http://www.fao.org
   (Consulté le 12-01-2014)
3. Kouakou NI. Le manioc, programme de vulgarisation de nouvelles variétés. Edition, Compagnie Ivoirienne Pour le Développement des Cultures Vivrières, Côte d'Ivoire. 1990;135.
4. Ihemere U, Arias-Garzon D, Lawrence S, Sayre R. Genetic modification of cassava for enhanced starch production. Plant Biotechnology Journal. 2006;4(4):453-65.
   DOI: 10.1111/j.1467-7652.2006.00195.x
5. FAO Agricultural Statistics. Food and Agricultural Organization of the United Nations. Rome.
   Available:http://faostat.fao.org
   (Accessed July 2008)
6. Nweke IF. Cassava: A cash crop in Africa. Cosca Working Paper n°14- Ibadan, Nigeria. 1996;77.
7. Manusset S. Projet culturel et scientifique pour la création d'une Maison du Manioc, expertise réalisée pour la Mission du Patrimoine Ethnologique/DRAC, Cayenne, Rapport Final + Diaporama. 2004;135.
8. Bull SE, Ndunguru J, Beeching JR, Gruissem W, Vanderschuren H. Cassava: Constraints to production and the transfer of biotechnology to African laboratories. Plant Cell Reproduction. 2011;30:779–788.
   DOI: 10.1007/s00299-010-0986-6
9. Jennings DL, Iglesias C. Breeding for crop improvement. In: Cassava Biology, Production, Utilization. Hillocks RJ, Thresh JM and Bellotti AC, Editors. CABI Publishing, Oxon, New York; 2002.
10. Westby A. Cassava utilization, storage and small-scale processing. In: Hillocks RJ, Thresh JM, Bellotti AC, (Eds) Cassava biology, production and utilization. CABI Publishing, Wallingford. 2002;281–300.
11. Okogbenin E, Porto MCM, Egesi C, Mba C, Espinosa E, Santos LG et al. Marker-assisted introgression of resistance to cassava mosaic disease into Latin American germplasm for the genetic improvement of cassava in Africa. Crop Sci. 2007;47:1895–1904.
    DOI: 10.2135/cropci2006.10.0688
12. Sayre R, Beeching JR, Cahoon E, Egesi C, Fauquet C, Fellman J, et al. The BioCassava Plus Program: Biofortification of cassava for sub-Saharan Africa. Annual Review Plant Biology. 2011;62:251-272.
13. Taylor NJ, Makwarela M, Fauquet CM, Rey M. Screening of four selected South African cassava (*Manihot esculenta* Crantz) cultivars for production of embryogenic tissues. Euphytica, Chapter 4; 2006.
14. Osorio M, Gamez E, Molina S, Infante D. Evaluation of cassava plants generated by somatic embryogenesis at different stages of development using molecular markers. Electron J Biotechnol. 2012;15:3.
    DOI: 10.2225/vol15-issue4-fulltext-3
15. Hankoua BB, Ng SYC, Fawole I, Puonti-Kaerlas J, Pillay M, Dixon AGO. Regeneration of a wide range of African cassava genotypes via shoot organogenesis from cotyledons of maturing somatic embryos and conformity of the field-established regenerants. Plant Cell, Tissue and Organ Culture. 2005;82: 221–231.
    DOI: 10.1007/s11240-005-0514-5
16. Alves AAC. Cassava botany and physiology. In: Cassava Biology, Production and Utilization. Hillocks RJ, Thresh JM, and Bellotti AC, Editors. CABI Publishing Oxon, New York; 2002.
17. Konan NK, Sangwan RS, Sangwan-Norreel BS. Efficient *In vitro* shoot regeneration systems in cassava (*Manihot*

esculenta Crantz). Plant Breed. 1994;113: 227–236.
DOI: 10.1111/j.1439-0523.1994.tb00727.x

18. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plant. 1962;15:473-497.
19. Fletcher EKA, Amoako TNE, Twumasi P. Effect of 2, 4-D, explants type and cultivar on the callogenesis expression of cassava (*Manihot esculenta* Crantz) in Ghana. Afr.J. Biotechnol. 2011;10(46):9396-9401.
20. Li HQ, Sautter C, Potrykus I, Puonti-Kaerlas J. Genetic transformation of cassava (*Manihot esculenta* Crantz). Nat Biotechnol. 1996;14:736–740.
DOI: 10.1038/nbt0696-736
21. Hankoua BB, Taylor NJ, Ng SYC, Fawole I, Puonti-Kaerlas J, Padmanabhan C, et al. Production of the first transgenic cassava in Africa via direct shoot organogenesis from friable embryogenic calli and germination of maturing somatic embryos. Afr. J. Biotechnol. 2006;5:1700-1712.
22. Mongomake K, Oumar D, Behnam K, Vincent NF. Somatic embryogenesis and plant regeneration of cassava (*Manihot esculenta* Crantz) landraces from Cameroon. Springer Plus. 2015;4:477.
DOI: 10.1186/s40064-015-1272-4
23. Raemakers CJJM, Sofiari E, Jacobsen E, Visser RGF. Regeneration and transformation of cassava. Euphytica. 1997;96:153-161.
24. Puonti-Kaerlas J. Cassava biotechnology. In: Hillocks RJ, Thresh JM, Bellotti AC, (Eds). Cassava: Biology, Production and Utilization. CAB International, Wallingford, Oxon. 2002;179–207.
25. Guohua M, Qiusheng X. Induction of somatic embryogenesis and adventitious shoots from immature leaves of cassava. Plant Cell, Tissue and Organ Culture. 2002;70:281-288.
26. Li HQ, Huang YW, Liang CY, Guo JY, LIU HX, Potrykus I, et al. Regeneration of cassava plants via shoot organogenesis. Plant Cell Rep. 1998;17:410–414.
27. Szabados L, Hoyos R, Roca W. *In vitro* somatic embryogenesis and plant regeneration in cassava. Plant Cell Rep. 1987;6:248–251.
28. Mathews H, Schopke C, Carcamo R, Chavarriaga P, Fauquet C, Beachy RN. Improvement of somatic embryogenesis and plant recovery in cassava. Plant Cell Rep. 1993;12:328–333.
29. Medina RD, Faloci MM, Solis Neffa V, Mroginski LA. Embriogénesis somática y regeneración de plantas de mandioca (*Manihot esculenta* Crantz) de cultivares de interés para Argentina. Revista de Investigaciones Agropecuarias. 2003;32: 143–160.

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