

1 **Whole Plants Regeneration of Cassava Cultivars (*Manihot*** 2 ***esculenta* Crantz) Originated From Côte d'Ivoire Via** 3 **Somatic Embryogenesis**

4 **ABSTRACT**

5 **Aims:** Study the capacity of cassava genotypes in Côte d'Ivoire to induce somatic embryos
6 and to regenerate plants from immature leaves

7 **Study Design:** In-vitro, laboratory-based study.

8 **Place and Duration of Study:** National Center for Agronomic Research (CNRA), between
9 January 2017 and April 2018.

10 **Methodology:** An efficient protocol to regenerate by somatic embryogenesis (SE) cassava
11 (*Manihot esculenta* Crantz) plants cultivated in Côte d'Ivoire was achieved. Immature leaf
12 lobes were used as explants on Murashige and Skoog (MS) basal medium supplemented with
13 different concentrations (16; 33; 50; 66 and 83 μM) of the auxins Picloram (Pic) and 2,4-
14 Dichlorophenoxyacetic acid (2,4-D).

15 **Results:** The results obtained showed that the frequency of primary somatic embryogenesis
16 (PSE) and the mean number of somatic embryos varied significantly with the genotype, the
17 type of auxin and the tested concentrations. The highest frequencies and numbers of somatic
18 embryos per explant were observed with cv. TMS 60444 (81.66 %; 190.8) on 50 μM Pic,
19 followed by Local XX1 (90 %; 180) on 66 μM Pic, To (100 %; 145.8) on 50 μM Pic, I (80
20 %; 125,6) on 66 μM 2,4D and M (100 %; 112) on 50 μM 2,4D. Shoot bud induction from
21 green cotyledons varied across cultivars and benzylaminopurine combined with 1-
22 Naphthalene acetic acid was shown to outperform benzylaminopurine associated with Indole-
23 3-butyric acid in the ability to induce organogenesis.

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

26 **Keywords:** Cassava, Organogenesis, Plant regeneration, Plant growth regulators, Somatic
27 embryogenesis

28 **1. INTRODUCTION**

29 Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae ($2n = 36$), and it is
30 a plant grown for its tuberous roots and leaves. The crop is adapted to a wide range of
31 environments and has good resistance to drought and soil acidity [1]. It ranks fifth among

32 food crops behind maize, rice, wheat and potatoes [2]. The plant is grown throughout the
33 country in Côte d'Ivoire and is represented by nearly a hundred local cultivars [3]. It is one of
34 the most important staple food crops in Africa. Its starchy tuberous roots provide a valuable
35 source of cheap calories for about 500 million people in the developing world commonly
36 plagued by chronic food deficiency and malnutrition [4]. World production was estimated at
37 250 million tons in 2011 [5]. In Africa, the continent with the largest production (53 % of
38 world production), the crop plays an important role as famine-reserve crop, rural staple food,
39 cash crop for both rural and urban households and, to a lesser extent, raw material for feed
40 and chemical industries [6]. Cassava is consumed in many forms. The tubers are eaten raw or
41 boiled for so-called "sweet" varieties and prepared according to a complex process of
42 detoxification for so-called "bitter" varieties. This process has resulted in many derived
43 products, the most consumed of which are 'tapioca', 'attiéké', 'gari', 'agou (fufu)' and
44 various types of pasta. Leaves are eaten as a vegetable in most of the countries across Africa
45 [7].

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

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

62 There are nearly 1500 cassava cultivars worldwide [16], and today all of the research
63 efforts on cassava regeneration and processing are devoted to South American varieties [13,
64 17], but the largest cassava production is in Africa. Few studies have focused on the process
65 of genetic transformation of African cassava varieties or a study to show that African

66 cultivars respond differently as compared to those in South America [4]. In Côte d'Ivoire, the
67 ability of somatic embryos to induce the characteristics necessary for the successful genetic
68 transformation of most local cassava cultivars is virtually non-existent in the literature. It is
69 therefore necessary and imperative to carry out an effective regeneration protocol for
70 successful genetic transformation via somatic embryogenesis of cassava cultivars in Côte
71 d'Ivoire in particular and in general for Africa.

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

74

75 **2. MATERIALS AND METHODS**

76 **2.1 Plant materials**

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

84

85 **2.2 Callus induction and primary somatic embryogenesis**

86 Immature leaf lobes (2-6 mm long) excised from *in vitro*-grown plants were cultured on MS
87 basal medium supplemented with 20 g/L sucrose, B5 vitamins, 0.5 mg/L CuSO₄ [23] and
88 various concentrations (16; 33 ; 50 ; 66 and 83 µM) of 2,4-D. The same set of immature leaf
89 lobes was transferred on the same media substituted with Picloram. The media pH was
90 adjusted to 5.7 and solidified with 8 g/L noble plant agar. The cultures were maintained at a
91 temperature of 25 ± 2 °C. The explants were left in the induction medium for 4-6 weeks. The
92 type of calli was observed at each step and the frequency of embryogenic calli formation was
93 recorded after four weeks of culture on callus induction medium (CIM). Each treatment
94 consisted of 10 Petri dishes and each Petri dish containing ten explants (100 explants per
95 treatment).

96

97

98 **2.3 Secondary somatic embryogenesis**

99 Green cotyledon pieces (5 mm²) were excised from the primary cotyledon embryos and
100 transferred to CIM supplemented with 50 µM NAA. Green cotyledon pieces obtained from 2
101 week-old secondary cotyledon embryos were placed on CIM supplemented with 50 µM NAA
102 for the induction of cyclic somatic embryogenesis. Somatic embryogenesis was carried out in
103 a growth chamber set at 25 ± 2 °C in continuous dark. Each treatment contained 10 Petri
104 dishes with ten explants (100 explants per treatment). The frequency of somatic
105 embryogenesis and average number of somatic embryos produced at each stage per
106 embryogenic callus were recorded after 4 weeks of culture.

107 **2.4 Maturation of somatic embryos**

108 This entailed the development of globular stage embryos into green cotyledonary embryos
109 with defined shoot and root axes [13]. The globular stage somatic embryos were subcultured
110 on CMML consisted of MS medium containing 20 g/L sucrose and supplemented with 0,1
111 mg/L BAP as described by [19]. The media pH was solidified with 8 g/l noble plant agar. The
112 embryos were maintained in the maturation medium in the dark for 4 weeks.

113

114 **2.5 Effect of BAP and Auxin (NAA and IBA) on organogenesis under light** 115 **and dark conditions**

116 The effect of the combination 1mg/L BAP with auxins (0.5 mg/L of NAA or AIB) on
117 adventitious bud formation of the cassava cultivars were assessed after three and four cycles
118 of somatic embryogenesis. Matured green cotyledon embryos were divided into 0.5 cm²
119 pieces and transferred on cassava organogenesis medium (COM) [MS basal medium, B5
120 vitamins, 20 g/L sucrose and 2 µM CuSO₄, supplemented with 1 mg/L BAP and 0.5 mg/L
121 IBA or 1 mg/L BAP and 0.5 mg/L NAA, pH 5.7 and noble agar (8 g/L)]. Each treatment
122 contained 10 explants in each of five Petri dishes (50 explants per treatment). Cultures were
123 incubated under continuous dark or under a photoperiod cycle of 16 h light to determine the
124 effect of light on bud formation. After 1 month in culture, the frequency of callus and bud
125 induction, the number of buds per explant and the shoot bud length were recorded.

126 **2.6 Elongation and rooting of shoot buds, and acclimatization of** 127 **regenerated plantlets**

128 Shoot primordia from maturation medium were transferred onto CEM (CBM supplemented
129 with 0.4 mg/L BAP) for shoot elongation. After 4 weeks, the elongated shoots were

130 transferred onto CRM (CBM without plant growth regulators) for rooting and development.
131 Seedlings with well-developed roots were then removed from the test tubes and rinsed with
132 tap water to remove any trace of the gelling agent. In the greenhouse, these seedlings were
133 transplanted into pots containing a sterile substrate composed of black soil. The percentage of
134 plantlet survival and their heights were recorded 4 weeks after being transferred to the
135 greenhouse.

136 **2.7 Experimental design and statistical analysis**

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

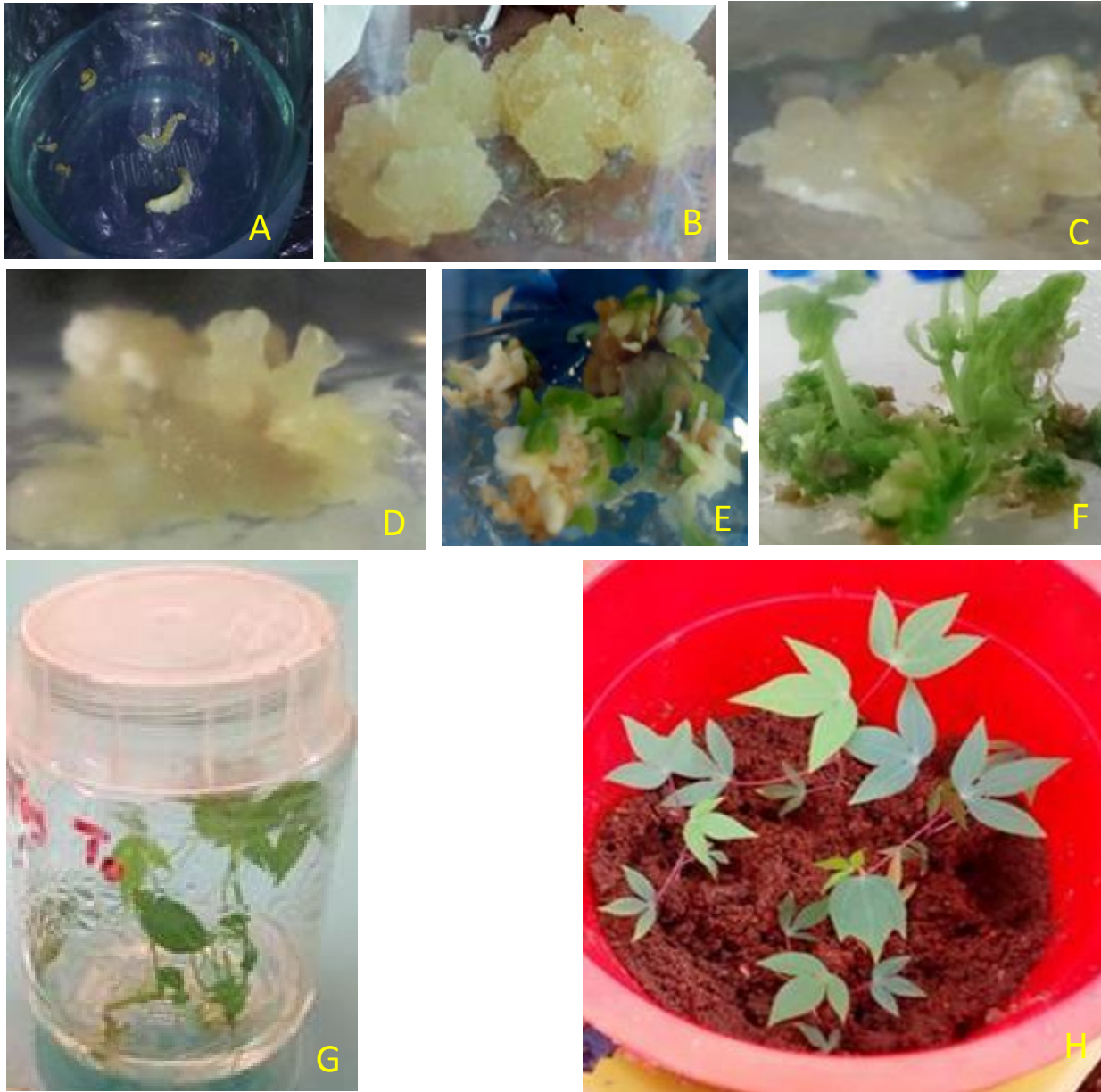
142 **3. RESULTS**

143 **3.1 Effects of 2,4-D and Picloram on callus induction and somatic** 144 **embryogenesis**

145 In this study, seven cassava landraces from Côte d'Ivoire and the control TMS 60444 were
146 tested for their ability to induce calli and somatic embryogenesis on MS basal medium
147 containing five concentrations (16 ; 33 ; 50 ; 66 and 83 μM) of 2,4-D and Picloram. The
148 immature leaf lobe explants (Fig. 1A) developed into a swollen callus mass on callus
149 induction medium (CIM) within 5 days. After 3 to 4 weeks of culture, a compact non-
150 embryogenic callus (Fig. 1B) and a translucent gelatinous callus with proembryogenic masses
151 (Fig. 1C) were observed in all cultivars (Cvs). These proembryogenic masses produced
152 globular somatic embryos (Fig. 1C), which developed through the characteristic somatic
153 embryogenesis stages of, trumpet and cotyledonary (Fig. 1D–E).

154 All seven cassava landraces and the control TMS60444 were able to induce callus. Seven out
155 of eight cassava were amenable to attain cotyledonary stage. Only cultivar (Dr) produced no
156 cotyledonary embryos on medium supplemented with all concentration (Table 2). Time
157 required to induce somatic embryos and to attain cotyledonary stage varied among the
158 genotypes. The potential of calli and somatic embryogenesis, as indicated by the frequency of
159 calli and somatic embryo production and the number of somatic embryos per explant, was
160 assessed in each cultivar (Tables 1 and 2). Results showed that both parameters varied widely

161 across varieties, auxin type and concentration. Formation of embryogenic calli was consistent
 162 with the frequency of callus induction in all the cassava varieties. For both callus induction
 163 and somatic embryogenesis, the best auxin concentration was 50 μ M Picloram (Tables 1 and
 164 2). The highest frequencies and number of somatic embryos per explant were observed with
 165 the Cv. TMS 60444 (81.66 %; 190.8) on 50 μ M Pic, followed by Local XX1 (90 %; 180) on
 166 66 μ M pic, To (100 %; 145.8) on 50 μ M pic, 85a (88.33 %; 135.66) on 50 μ M pic, PK (80 %;



167 133.16) on 50 μ M pic, I (80 % ; 125.6) on 66 μ M 2,4D, M (100 % ; 112) on 50 μ M 2,4D and
 168 Dr (80 % ; 0).

169



170

171 **Fig. 1:** Regeneration of cassava cultivars from Côte d'Ivoire and the control TMS60444. (A)

172 immature leaf lobes (B) induced compact non-embryogenic callus (C) and callus with proembryogenic masses Clusters of organized

173 embryogenic structures consisting of globular (D) trumpet structures (E) formation of green cotyledon (F) Formation of distinct

174 shoots and Elongated shoot buds rooted and developed into whole plantlets (G) *in vitro* After transferring in boxes, hardened plantlets

175 (H) Cassava plantlets growing in the greenhouse

176

177 **Table 1:** Effects of different concentrations of 2,4-D and Picloram on callus induction.

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

178 Within the same line, mean values followed by the same letter are not significantly different at $\alpha = 5\%$ (Newman-Keuls test) \pm , standard deviation;

179

180 **Table 1: continued**

Plant growth regulators and frequency (%) of callus				
Varieties	66 μ M		83 μ M	
	2,4 D	Picl	2,4 D	Picl
XX1	80 \pm 0bcde	90 \pm 0abcde	86,66 \pm 0,06acde	100 \pm 0a
PK	83,33 \pm 0,03bcde	86,66 \pm 0,03abcde	83,33 \pm 0,03bcde	96,66 \pm 0,03abc
DR	93,33 \pm 0,06abc	86,66 \pm 0,03abcde	86,66 \pm 0,06abcde	86,66 \pm 0,06abcde
TMS60444	93,33 \pm 0,06abc	100 \pm 0a	100 \pm 0a	93,33 \pm 0,06abc
TO	100 \pm 0a	100 \pm 0a	100 \pm 0a	100 \pm 0a
M	7 ² 1,66 \pm 0,05de	77,33 \pm 0,05bcde	83 \pm 0bcde	66 \pm 0e
I	77,33 \pm 0,05bcde	71,66 \pm 0,05de	83 \pm 0bcde	71,66 \pm 0,05de
85a	66 \pm 0e	80,66 \pm 0,03abcde	66 \pm 0e	66 \pm 0e

181

182 Within the same line, mean values followed by the same letter are not significantly different at $\alpha = 5\%$ (Newman-Keuls test) \pm , standard deviation;

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

184

Plant growth regulators μM		Varieties							
		TMS 604444		XX1		PK		M	
		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
	Picl	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
	Picl	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
	Picl	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
	Picl	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
	Picl	43.33±0.03f	21.5±0.5v	56.66±0.02e	82.5±1.17l	0±0p	0±0y	0±0p	0±0y

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

186 Within the same line, mean values followed by the same letter are not significantly different at $\alpha = 5\%$ (Newman-Keuls test) \pm , standard deviation;

187 **Table 2: continued**

	Plant growth regulators μM	Varieties							
		I		85a		To		DR	
		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
	Picl	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
	Picl	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
	Picl	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
	Picl	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
	Picl	0±0p	0±0y	0±0p	0±0y	63.33±0.03	41.66±1.33r	0±0p	0±0y

188

189 Within the same line, mean values followed by the same letter are not significantly different at $\alpha = 5\%$ (Newman-Keuls test) \pm , standard deviation

190 3.2 Secondary embryogenesis

191 Secondary somatic embryogenesis has the same embryonic developmental stages as primary
 192 embryogenesis. As the Dr variety did not induce cotyledonary embryos, the secondary
 193 embryogenesis test was not performed with this variety. Results for secondary
 194 embryogenesis responses are shown in Table 3. Regarding the secondary embryogenesis rate
 195 and the number of embryos, a significant difference was noted. The highest frequencies and
 196 the number of somatic embryos per explant were observed in Cvs. TMS 60444 (99 %; 206.1),
 197 To (96 %; 186.8), XX1 and 85a (93 %; 186.80), Pk (92 %; 178.40), M (95 %; 185.50) and I
 198 (94 %; 177.70). The mean frequency and the number of somatic embryos have been
 199 markedly improved during secondary somatic embryogenesis.

200 **Table 3:** Evaluation of secondary somatic embryogenesis induced from primary embryo
 201 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±0.41bc
XX1	93±0.01ab	186.8±0.32bc
85a	93±0.01ab	168±0b
Pk	92±0.01b	178.4±0.26
M	95±0.01ab	185.5±0.5c
I	94±0.01ab	177.7±0.15d

202

203 Within the same line, mean values followed by the same letter are not significantly different at $\alpha = 5\%$ (Newman-Keuls test) \pm , standard
 204 deviation;

205

206 3.3 Effect of BAP and Auxin (NAA and IBA) on organogenesis under light 207 and dark conditions

208 After four weeks of culture on the various organogenesis media, the induction and the
 209 development of buds were observed under the two conditions: light and dark conditions
 210 (figure 1F). Frequencies of bud formation as well as number of buds produced per explant are
 211 presented in Table 4. As for shoot regeneration, seven cultivars (TMS 60444, To, PK, XX1,
 212 85a, M and I) produced shoots. Overall, the frequencies of bud formation were similar under

213 light and dark conditions with higher values recorded in medium supplemented with BAP (1
 214 mg/L) + IBA (0.5 mg/L) (70- 83 %) than in medium containing BAP (1 mg/L) + NAA (0. 5
 215 mg/L) (75– 81 %) where the frequency of budding tended to be higher under light (53– 81 %)
 216 than under dark (13–37 %) (Table4). As for the number of buds , medium supplemented with
 217 BAP (1 mg/L) + IBA (0.5 mg/L), performed better than BAP (1 mg/L) + NAA (0.5 mg/L)
 218 supplemented medium (Table 4). Organogenesis was higher in Cvs. TMS60444 (83 %; 35),
 219 XX1 (81% ; 31), M (80 % ; 25.4), PK (70% ; 23.5), To (70% ; 19.6), 85a (81 % ; 15.5) and I
 220 (75%; 17)

221 **Table 4:** Responses to organogenesis of cassava varieties produced from embryonic callus
 222 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) +	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
NAA (0.5 mg/l)	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) +	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

IBA(0.5 mg/l)	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

223

224 Within the same line, mean values followed by the same letter are not significantly different at $\alpha = 5\%$ (Newman–Keuls test) \pm , standard
225 deviation;

226

227 3.4 Elongation and rooting of leafy shoots

228

229 Prior to transplanting to the greenhouse, lengths of shoots regenerated on maturation medium
230 were measured; values ranged from 0.8 to 1.08 cm and showed no statistical differences.
231 Shoots of all cultivars developed roots efficiently on elongation medium supplemented with
232 0.4 mg/L BAP (figure 1G).

233

234 3.5 Acclimatization of regenerated plantlets

235 The ability of regenerated plantlets to acclimatize and grow in the greenhouse was assessed
236 by measuring the proportion of plantlets recovered as well as plantlet height. Cultivars TMS
237 60444, To, 85a, M and XX1 showed a significantly higher regeneration rate than cvs PK and
238 I. The regenerated plants were morphologically normal and grew rapidly and after 6 weeks
239 under greenhouse conditions, plantlets height ranged from 18 to 27 cm. The regenerated
240 plants from the seven varieties were adapted to growing conditions in the greenhouse with a
241 success rate ranging from 90 to 100 % for all cultivars tested.

242

243 4. DISCUSSION

244 In this study, various factors known to have an effect on the cassava somatic
245 embryogenesis and regeneration were evaluated. The source and concentration of auxin play
246 a role in the regeneration of various plants. This study determined higher levels of 2, 4-D and
247 Picloram as best inducers of somatic embryos. The results of this study are on line with
248 previous study achieved by [20] who evaluated the effect of 2, 4-D, dicamba, picloram and
249 ANA on the somatic embryogenesis of seven Cameroon cassava cultivars and found picloram
250 to be the best inducer at 12 mg/l. Contrary, [4] determined also higher number of cassava

251 somatic embryos produced under 12 mg/l of 2, 4-D. However, [23] reported 8 mg/l of 2, 4-D
252 as the best concentration for callus induction in four Ghanaian cassava cultivars. Other
253 factors like the source and health status of the explants used in this study may have
254 contributed to results obtained.

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

264 Although the frequency of bud induction was found in this study to be similar under light and
265 dark conditions, the number of buds formed per explant were significantly higher when green
266 cotyledons were incubated under 16 h light. The photoperiod has consistently been shown to
267 be genotype-dependent for shoot formation. For example, a photoperiod of 16 h light was
268 reported to be more efficient in inducing shoot formation from green cotyledons [15], while
269 [26] obtained better results under continuous dark. We found that Cv. To was efficient in
270 embryogenesis but less proficient in organogenesis, suggesting that the ability to produce
271 somatic embryos does not necessarily translate to shoot regeneration proficiency. This result
272 indicates that somatic embryogenesis and organogenesis may be controlled by different and
273 independently inherited traits. Taken together, this study shows that the Côte d'Ivoire
274 cultivars investigated here contain sufficient genetic variability for somatic embryogenesis
275 and adventitious shoot formation and can likely be improved using the *Agrobacterium*-
276 mediated approach. [17] also observed a similar phenomenon. It is important to indicate that
277 whereas some cassava cultivars from Colombia [27, 28], Argentina [29] and Côte d'Ivoire
278 [17] exhibit regeneration efficiencies similar to those reported here, others showed very low
279 efficiencies.

280

281 **5. CONCLUSIONS**

282 Côte d'Ivoire farmer-preferred cassava landraces tested in this study demonstrated good
283 ability in producing somatic embryos and plant regeneration potential. Response to somatic

284 embryogenesis and regeneration ability was genotype dependent as reported in the literature.
285 Some of the landraces could be converted to plantlets while one could not. However, other
286 factors like source and age of explants, culture conditions, sub-culturing cycles, age and
287 brand of the media used might have contributed to the regeneration ability and variations of
288 the tested cassava landraces in this work. Although all cassava landraces will be targeted for
289 genetic engineering programs, results obtained from this study are enlightening potential
290 candidate landraces amenable to transformation protocols. There is a need to develop
291 efficient, genotype-independent regeneration and transformation protocols that will overcome
292 a challenge of varying in vitro response of cassava between closely related cassava cultivars.

293 **ABBREVIATIONS**

294 2,4-D: 2,4-Dichlorophenoxyacetic acid; BAP: benzylaminopurine; CBM: cassava basal
295 medium; CEM : cassava elongation medium; CIM: callus induction medium; CMML:
296 cassava maturation medium; COM: cassava organogenesis medium; CRM: cassava rooting
297 medium; CSE: cyclic somatic embryogenesis; NAA: α -Naphthalene acetic acid; P-CIM:
298 callus induction medium supplemented with Pic; Pic: Picloram; PSE: primary somatic
299 embryogenesis; SE: somatic embryogenesis; SSE: secondary somatic embryogenesis; Var :
300 Varieties ; F.S.E.: Frequency of somatic embryos ; N.E.S: Number of Somatic Embryos.

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