J. Agric. Sci. Mansoura Univ., 30 (10): 6197 - 6209, 2005

INDUCTION OF SOMATIC EMBRYOGENESIS AND SHOOT ORGANOGENESIS FROM IMMATURE LEAVES OF CASSAVA (*Manihot esculenta*, crantz) AND FIELD ESTABLISHMENT OF REGENERANTS.

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ABSTRACT

Direct somatic embryogenesis was successfully obtained from young leaves of cassava cultured on induction medium. The addition of 2,4-D and picloram at 4 or 6 mg/l to induction medium was significantly effective in induction of somatic embryogenesis and in increasing number of embryogenic explants. The presence of cytokinins in induction medium, except kinetin, significantly reduced frequency of embryogenesis and number of embryogenic explants. Increasing carbon source from 20 g/l to 40 g/l was associated with a significant reduction in number of embryogenic explants and in frequency of embryogenesis. Maltose at 20g/l was the most effective carbon source for the induction of somatic embryogenesis and for increasing number of embryogenic explants. Supplementing somatic embryo induction medium with amino acids did not improve frequency of embryogenesis or number of embryogenic explants. As for the extended effect of auxins, cytokinins, carbon sources and amino acids on plantlet regeneration from somatic cotyledons, results pointed out that 2,4-D and picloram at 4 mg/l, BA at 1 mg/l and zeatin at 0.5 mg/l and maltose at 20 g/l had a positive extended effect, which led to significant increase in frequency of regeneration and number of regenerated plantlets /explant. However, amino acids had no positive effect on frequency of regeneration or number of regenerants /explant. Adding silver nitrate at 4 mg/l to plantlet regeneration medium was very effective in increasing percentage-regenerating explant, but did not affect number of regenerated plantlets per explant. Field experiments, during summer seasons of 2002/03 and 2003/04 proved that both tissue culture propagated cassava or those propagated by stem cuttings did not significantly differ in average root length, diameter and weight, number of tuber roots/plant and tuber root yield/ plant.

INTRODUCTION

Cassava, a popular industrial root crop, is a major staple food crop in Africa. It provides more than half of the daily dietary calories and essential nutrients for more than 200 million people in Africa, through consumption of storage starchy roots and leaves (Nweke *et al.*, 2002). In fact, it is the fourth most important source of calories in human diet in tropical regions of the world (Wheatly and Chuzel, 1993). In 1999, 52.5% of the world's cassava output was produced in Africa (FAO, 2000). Because of its hardiness and tolerance to adverse environmental conditions, it is a reliable crop, giving adequate yields even when grown on marginal soils unable to support other crop plants. In spite of cassava's high importance to food security in the third world countries, it has been neglected in plant breeding programs; in addition, the improvement by traditional breeding of cassava is constrained by the high heterozygosity, highly outcrossing nature and low natural fertility of plants

(Li *et al.*, 1998). Plant tissue culture and genetic engineering, together could provide an efficient tool to complement traditional breeding, e.g. by genetically engineer disease and resistance, improving protein content of cassava roots and reducing the cyanogenic glucoside content of plants. The development of efficient regeneration systems compatible with *Agrobacterium* and gene gun, act as an important breakthrough in application of genetic engineering techniques, and so far, the availability of an efficient regeneration system has been a limiting factor in cassava biotechnology. Regeneration of cassava plants from germinating somatic embryos induced on cotyledons of zygotic embryo, immature leaves or primary somatic embryo has been achieved (Mathews *et al.*, 1993 and Roemakers, *et al.*, 1993 a & b). Axillary buds have been used to regenerate multiple shoots in cassava (Konan *et al.*, 1994). Regeneration of cassava plants through shoot organogenesis has been successfully done using immature leaf lobe and somatic cotyledons as explants for direct shoot induction (Hankoua et al., 2000 and Hankoua, 2003). However, these systems require extended tissue culture periods or they are poorly compatible with transformation protocols. Therefore, the purpose of this research is to establish an efficient and repeatable protocol for regeneration of cassava plants using aseptic immature leaf lobes excised from *in vitro* induced somatic embryos, and to test the effect of carbon source, auxins, cytokinins and amino acids on somatic embryo induction and their extended effects on plantlet regeneration frequency on regeneration medium. The effect of silver nitrate on regeneration frequency and number of regenerated shoots/ explant was also investigated. In addition, field performance of regenerants was evaluated in terms of their root characters and root yield/plant.

MATERIAL AND METHODS

Plant materials

Shoot cuttings, 15-20 cm long, were excised from greenhouse-grown Nigerian cultivar plants, sterilized in 0.52% sodium hypochlorite for 15 min., and then rinsed with sterile distilled water for 3 times, each for 10 min. For shoot proliferation, explants were placed on MS (Murashige and Skoog, 1962) basal medium supplemented with 0.5 mg/l BA (6-benzyladenine), 30 g/l sucrose, 100 mg /l myo-inositol. The pH of the medium was adjusted to 5.8 before the addition of 7g/l purified agar. Cultures were maintained in 24-25°C, under white fluorescent tubes, with 16/8 hrs photoperiod providing 100µmol m^{-2} sec^{-1}, and subcultured every 4 weeks.

Induction of somatic embryogenesis

Young leaves, 3-5 mm long, excised from 15 days old *in vitro* growing shoots (Fig.1-A) were used as explants and cultured on somatic embryogenesis induction medium containing MS salts (Murashige and Skoog, 1962), B5 vitamins (Gamborg *et al.*, 1968), 2% sucrose, 0.1% casein hydrolysate and 0.7% agar. The pH was adjusted to 5.7 prior to autoclaving (121°C, 20 min.). Explants were incubated in dark for 2 weeks, then

transferred to light and were evaluated after 3 weeks for the production of primary somatic embryos (Fig.1-B). The developing embryo were transferred to fresh medium consisting of MS medium supplemented with 2% sucrose and 0.1 mg/l BA as described by Li *et al.*(1998), to develop green cotyledonary stage embryos (Fig.1-C), where cotyledons were used later on in shoot regeneration experiments. All tested chemicals were described separately for each experiment as follow:

1- Effect of auxins:

Five different auxins namely 2,4-D, picloram, 4-CPA, IBA and NAA, were introduced to basal somatic embryo induction medium at 4 concentrations (2, 4, 6 and 8 mg/l), before autoclaving. The effect of these auxins on somatic embryo induction and their extended effect on shoot regeneration on medium devoid any auxins were studied.

2- Effect of cytokinins:

Benzyladenine (BA), kinetin and zeatin, each at 0.1, 0.5 and 5.0 mg/l, were filter sterilized through 0.22 µm membranes (Millipore) and added to cooled (55°C) autoclaved basal somatic embryo induction medium in the presence of 4 mg /l 2,4-D. The effect of these cytokinins on somatic embryo induction and their extended effect on shoot regeneration from green somatic cotyledons were studied.

3- Effect of carbon sources:

Four sources of carbon, i.e., sucrose, maltose, glucose and fructose, at 20, 30 and 40 g/l, were added to basal somatic embryo induction medium containing 4mg /l 2,4-D. The effect of these carbon sources on somatic embryo induction and their extended effect on shoot regeneration were investigated.

4- Effect of amino acids:

Amino acids, i.e., glutamine, proline and alanine were filter sterilized and evaluated at 5, 10, 50 and 100 mM by introducing them to basal somatic embryo induction medium supplemented with 4mg/l 2,4-D. The effect of these amino acids on somatic embryo induction and their extended effect on shoot regeneration using green cotyledons were searched.

5- Effect of silver nitrate:

The effect of silver nitrate, as anti-ethylene, on shoot regeneration frequency was studied by supplementing shoot regeneration medium with 0.0, 1, 2, 3, 4 or 8 mg/l of filter sterilized $AgNO_3$ after autoclaving, using green cotyledons of somatic embryos as explants.

Shoot organogenesis:

Green cotyledons of somatic embryos were collected and cut into 5 mm^2 pieces and transferred to medium supplemented with 1 mg/l BA and devoid any auxin for shoot organogenesis at 25°C, in the dark. Shoot primordia developed on explants, were transferred after 3 weeks to shoot elongation medium containing the same components of basal somatic embryo induction, but supplemented with 0.4 mg/l BA, and kept in light. Elongated plants were

easily rooted on the same basal medium, but free of BA and supplemented with 0.5 mg/l NAA.

6- Field performance:

Rooted regenerated plantlets were collected and acclimatized in greenhouse (Fig.1-F), then established in soil, following the method described by Ng *et al.* (1994), to evaluate cassava tuber root characteristics and yield/plant, in comparison with plants propagated by stem cuttings. Adopted *in vitro* propagated plants and plants propagated by stem cuttings of Nigerian cassava cv. were planted in field in summer, March 28, of 2002 and 2003 seasons, at the experimental research station of the Faulty of Agriculture, Suez Canal Univ. Field growing plants (Fig.1-G) were fertilized and irrigated using the standard procedures recommended by the Ministry of Agriculture for cassava production.

Data collection and statistical analysis

The variables recorded included number of explants formed primary embryogenic structure, frequency of somatic embryos (%), Percentage regenerating explants and number of plantlets regenerated per explant. All experiments were arranged in a completely randomized design with four replications, performed twice and expressed as exp1 and exp2. Ten 200 ml glass bottles, each contained 25 ml medium and 5 explants, were used per treatment. Data were analyzed by Analysis of Variance using SAS program (SAS, 1985), with means separated by Duncan Multiple Range Test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Effect of auxins:

Data in Table (1) indicate that 2,4-D followed by picloram at 4 and 6 mg/l, were the most efficient treatments for the induction of somatic embryos, where they significantly increased number of explant formed somatic embryos and frequency of somatic embryo induction (%), in both experiments, compared with IBA, NAA, and 4-CPA at any tested concentration. As for the extended effect of auxin on frequency of plantlet regeneration (%) and number of regenerated plantlets/ explant, data in Table (1) show that 2,4-D at 4 mg/l significantly increased percentage of regenerating explants, followed by 2,4-D at 6 mg/l and then picloram at 4 mg/l, in both experiments, compared with other tested auxins. Also, number of regenerated plantlets/ explant significantly increased by using 2,4-D at 6 mg/l followed by picloram at 6 mg/l, in both experiments, compared with other auxins. The efficiency of 2,4-D and picloram on induction of somatic embryogenesis had been reported by Li *et al.* (1998); Ma and Xu 92002) and Hankoua *et al.* (2005). Similarly, several investigators reported that NAA and 4-CPA were not able to induce somatic embryogenesis efficiently (Stejskal and Griga, 1999 and Loiseau *et al.*, 1995). Extended effect of auxins on plant regeneration efficiency studied by Loiseau *et al.* (1995) on pea and by Ma and Xu (2002) on cassava, and their results were in harmony with the obtained results.

Table (1): Effect of auxins on somatic embryo induction and their extended effect on plant regeneration frequency in Nigerian cassava cultivar.

Auxin Conc. (mg/l)		No. of embryogenic explant[1]		Frequency of somatic embryo induction (%)		(%) regenerating explants		No. of regenerated plantlets/ explant	
		Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
2,4-D	2	12.8 g	13.0 g	25.5 g	26.0 g	53.5 g	51.0 h	2.0 gh	2.5 gh
	4	23.3 b	24.5 b	46.5 b	49.0 b	94.0 a	98.0 a	9.3 c	11.5 c
	6	24.0 a	25.3 a	48.0 a	50.5 a	91.5 b	93.5 c	16.6 a	14.3 a
	8	17.8 e	16.5 e	35.5 e	33.0 e	63.8 e	59.5 e	3.5 f	2.8 g
Picloram	2	8.0 h	9.3 h	16.0 h	18.5 h	50.0 h	56.5 f	1.5 h	2.0 h
	4	23.5 b	22.3 c	47.0 b	44.5 c	90.3 c	94.8 b	6.4 d	8.3 d
	6	23.3 b	21.5 d	46.5 b	43.0 d	78.4 d	80.3 d	11.3 b	12.8 b
	8	13.5 f	13.8 f	27.0 f	27.5 f	57.5 f	54.0 g	5.8 d	4.5 e
IBA	2	0.8 no	1.0 m	1.5 no	2.0 l	2.5 m	1.5 p	1.2 h	2.0 h
	4	7.3 i	6.0 i	14.5 i	12.0 i	28.9 j	32.8 j	4.6 e	3.8 ef
	6	18.5 d	14.3 f	37.0 d	28.5 f	29.5 j	24.2 l	6.7 d	8.1 d
	8	19.3 c	21.5 d	38.5 c	43.0 d	38.6 i	41.5 i	3.2 f	2.9 g
NAA	2	0.0 p	0.0 n	0.0 b	0.0 m	0.0 b	0.0 q	0.0 i	0.0 j
	4	1.0 n	1.0 m	2.0 n	2.0 l	15.2 m	17.9 m	1.5 h	1.8 h
	6	2.3 l	1.8 l	4.6 l	3.5 k	22.5 k	26.4 k	1.6 h	1.0 i
	8	5.3 j	4.5 j	10.5 j	9.0 j	23.1 k	24.6 l	2.1 gh	2.9 g
4-CPA	2	0.5 o	0.0 n	1.0 o	0.0 m	0.0 b	0.0 q	0.0 i	0.0 j
	4	0.8 no	1.0 m	1.5 no	2.0 l	1.0 o	1.5 p	1.6 h	1.8 h
	6	1.5 m	2.3 k	3.0 m	4.5 k	3.2 nm	2.8 o	2.0 gh	2.0 h
	8	2.8 k	4.5 j	5.5 k	9.o j	4.6 n	5.2 n	2.8 fg	3.2 fg

-Means followed by different letters in the same column are significantly different at p≤ 0.05 by DMRT. 1- Based on a total of 50 explants per treatment.

Effect of cytokinins:

Data in Table (2) show that basal medium containing only 2,4-D and devoid any cytokinin (control) was more efficient in increasing number of embryogenic explants and frequency of somatic embryo induction (%), than basal medium containing cytokinins, except kinetin at low concentration. Increasing cytokinin concentration associated with strong and gradual reduction in somatic embryogenesis and embryo development, especially with BA and zeatin, where callus greening and compactness increased clearly. Only low concentration of kinetin gave results that were significantly similar to control, in both experiments. Significantly the lowest number of embryogenic explants and (%) of somatic embryo induction obtained as a result of adding 5 mg/l BA to the basal medium. Similar results described by Loiseau *et al.* (1995) on pea, where they reported that the addition of cytokinins to auxin-containing medium reduced embryo production. As for the extended effect of cytokinins on plant regeneration, data in Table (2) indicate that cytokinins, especially BA and zeatin significantly increased plantlet regeneration percentage from cotyledons of somatic embryos. Percentage of regenerated explants was the lowest on medium supplemented with kinetin at 0.1 mg/l. The use of BA at 1.0 mg/l also resulted in a significant increase in

number of regenerated plantlets/ explant, with an average of 9.5 and 10.5 transplantable shoots/ explant in exp1 and exp2, respectively. Other cytokinins did not enhance number of regenerated plantlets/ explant, compared with control. Ma and Xu (2002) concluded similar results by reporting that if the embryonic cells were induced, the auxin enhanced formation of somatic embryos, whereas 6-benzylaminopurine stimulated development of adventitious shoots.

Table (2): Effect of cytokinins, in the presence of 4.0mg/l 2,4-D, on somatic embryo induction and their extended effect on plant regeneration frequency in Nigerian cassava cultivar.

Cytokinin Conc. (mg/l)		No. of embryogenic explant[1]		Frequency of somatic embryo induction (%)		(%) regenerating explants		No. of regenerated plantlets/ explant	
		Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Control		13.3 a	11.0 a	26.5 a	22.0 a	37.2 def	41.5 ce	7.8 b	8.5 b
BA	0.1	8.9 c	7.0 d	17.8 c	14.0 d	44.9 bcd	45.8 bce	4.1 de	3.9 c
	0.5	3.7 e	3.1 fg	7.3 ef	6.1 fg	56.5 ab	51.7 a	6.5 c	7.2 b
	1.0	2.4 fg	2.0 hi	4.8 gh	3.9 hi	60.3 a	58.4 a	9.5 a	10.8 a
	5.0	1.5 g	1.2 i	2.9 h	2.3 i	41.2 cde	40.6 cef	3.9 def	3.4 cde
Zeatin	0.1	8.0 c	8.2 c	15.9 c	16.4 c	53.7 abc	49.8 abc	1.5 h	2.0 ef
	0.5	8.2 c	6.5 d	6.4 c	12.9 d	59.6 a	57.1 a	3.1 efg	3.6 cd
	1.0	5.6 d	4.3 e	11.1 d	8.6 e	43.2 cde	40.9 ce	4.3 d	3.8 c
	5.0	4.9 de	3.8 ef	9.8 de	7.5 ef	33.4 def	37.6 ef	2.8 fg	2.3 def
Kinetin	0.1	12.8 a	10.4 ab	25.6 a	20.7 ab	18.7 h	20.8 g	2.1 gh	1.9 ef
	0.5	11.7 b	9.8 bc	23.4 b	19.6 b	27.3 fgh	23.6 eg	4.2 de	3.6 cd
	1.0	11.1 b	9.4 c	22.1 b	18.7 b	31.5 efg	29.4 fg	4.6 d	4.2 c
	5.0	3.1 ef	2.5 gh	6.1 fg	5.0 gh	19.6 gh	21.5 g	1.5 h	1.7 f

-Means followed by different letters in the same column are significantly different at p≤ 0.05 by DMRT. 1- Based on a total of 50 explants per treatment.

Effect of carbon sources:

The effect of four carbon sources (sucrose, maltose, glucose and fructose) on induction of somatic embryogenesis and their extended effect on plantlet regeneration are presented in Table (3). Data point out that increasing the concentration of carbon source from 20 to 40 g/l resulted in a significant decrease in number of embryogenic explants and frequency of somatic embryo induction (%). Also, data indicate that maltose increased significantly number of explants formed somatic embryos and percentage of somatic embryo induction, compared with other tested carbon sources, at the same concentration, in both experiments. The highest number of embryogenic explant resulted from using 2% maltose in basal somatic embryo induction medium. As for the extended effect of carbon sources on plant regeneration, data in table (3) also illustrate that increasing level of carbohydrates in medium was associated with a significant reduction in percentage regenerating explants and number of regenerated plantlets/ explant. Maltose was found to have a significant positive effect on percentage of regenerating explants and number of regenerated plantlets/ explant, compared with sucrose, glucose and fructose, in both experiments. The highest number of regenerated plantlets/ explant was obtained on 2% maltose-containing medium. Similar results reported by Loiseau *et al.* (1995) who indicated that

increasing carbon source in pea embryo induction medium caused a reduction in water content of calli and somatic embryos, resulting in a decrease in number of explants formed somatic embryos and percentage of plantlet regeneration from somatic embryos. At the same trend, Li *et al.* (1998) reported that replacing sucrose with maltose increased somatic embryo induction and plant regeneration efficiencies. They also added that glucose, lactose and 4% sucrose inhibited somatic embryo formation and plant regeneration.

Table (3): Effect of carbon sources on somatic embryo induction and their extended effect on plant regeneration frequency in Nigerian cassava cultivar.

C source Conc. (g/l)	No. of embryogenic explant[1]		Frequency of somatic embryo induction (%)		(%) regenerating explants		No. of regenerated plantlets/ explant	
	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Sucrose 20	42.0 a	39.8 c	84.0 a	79.5 b	41.5 c	38.3 c	12.8 d	10.7 d
30	36.3 b	31.8 d	72.5 b	63.5 c	23.0 de	24.0 def	7.5 e	6.8 e
40	29.8 c	27.4 e	59.5 c	54.8 d	18.5 e	20.5 ef	5.3 f	4.9 ef
Maltose 20	43.8 a	46.0 a	87.5 a	92.0 a	81.5 a	79.5 a	25.2 a	23.1 a
30	40.8 a	41.7 b	81.5 a	83.3 b	78.3 a	75.0 a	18.7 b	19.4 b
40	2.7 c	28.0 e	54.0 cd	56.0 d	56.5 b	59.0 b	15.6 c	13.3 c
Glucose 20	13.3 d	14.7 g	26.5 e	29.3 f	30.0 de	32.3 d	2.9 gh	2.3 hi
30	8.3 e	7.4 ij	16.5 f	14.8 h	22.5 de	21.5 def	2.1 hij	1.9 i
40	5.3 e	4.8 j	10.3 f	9.5 h	19.5 e	18.3 f	1.6 ij	1.4 i
Fructose 20	23.3 c	21.7 f	46.5 d	43.3 e	43.5 bc	40.3 c	3.8 g	4.1 f
30	14.2 d	13.0 gh	28.3 e	26.0 fg	34.2 d	31.5 cde	2.3 hi	3.0 h
40	8.9 e	9.7 hi	17.8 f	19.3 gh	26.8 de	22.5 def	1.2 j	1.4 i

-Means followed by different letters in the same column are significantly different at p≤ 0.05 by DMRT. 1- Based on a total of 50 explants per treatment.

Effect of amino acids:

Data in Table (4) show that the addition of the amino acids namely glutamine, proline and alanine to somatic embryo induction medium, at any tested concentration, did not improve somatic embryogenesis efficiency or development, compared with control treatment, in both experiments. The only difference noticed was that medium containing glutamine had greener calli than other medium. As for amino acid extended effect on plantlet regeneration, data in Table (4) indicated that all tested amino acids at any used concentration did not improve percentage of regenerating explants or number of regenerated plantlets per explant, in both experiments. The obtained results are contradictory with several investigator results, where glutamine found to be essential for the embryogenesis of soybean (Tetu *et al.*, 1987), whereas alanine can have a stimulatory effect on carrot embryogenic suspension cultures (Kamada and Harada, 1979). Also proline is known to promote somatic embryogenesis in alfalfa (Shetty and McKerise, 1993). However, the obtained results are in agreement with those of Loiseau *et al.* (1995) on pea who reported that amino acid supply in induction medium

improved neither yield nor embryo quality although alanine and glutamine are the most prominent constituents of free amino acids in embryo sac liquid (Rochat and Boutin, 1991). Loiseau *et al.* (1995) speculated that the effect of amino acid supply could be concealed by auxin inhibition.

Table (4): Effect of amino acids, in the presence of 4.0mg/l 2,4-D, on somatic embryo induction and their extended effect on plant regeneration frequency in Nigerian cassava cultivar.

Amino acid	Conc. mg/l	No. of embryogenic explant[1]		Frequency of somatic embryo induction (%)		(%) regenerating explants		No. of regenerated plantlets/ explant	
		Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Control		15.8 a	14.3 a	31.5 ab	28.5 ab	35.2 a	38.5 a	11.2 a	12.8 a
Glutamine	5.0	15.4 a	12.9 ab	30.8 ab	25.8 abc	24.9 bc	26.7 b	5.8 bcd	6.4 c
	10.0	13.9 abcd	12.2 abc	27.8 abcd	24.3 abcd	23.1 bc	24.0 bc	7.2 b	8.9 b
	50.0	10.5 cde	9.3 cd	21.0 def	18.5 de	18.6 cde	15.3 efg	4.2 efg	3.7 def
	100.0	4.2 f	3.7 e	8.3 g	7.3 f	7.8 f	7.2 h	2.8 gh	3.0 ef
Proline	5.0	12.5 abcde	10.9 ab	25.0 bcdef	21.8 bcde	20.6 cde	17.3 def	3.4 fg	3.9 def
	10.0	14.4 abc	12.7 abc	28.8 ab	25.3 abc	22.4 cd	19.8 cdef	4.6 def	5.2 cd
	50.0	11.0 bcde	10.9 abcd	22.0 cdef	21.8 bcde	16.3 de	13.9 fg	3.5 fg	4.1 de
	100.0	9.4 e	8.5 d	18.8 f	17.0 e	14.6 e	9.8 gh	1.8 h	2.3 f
Alanine	5.0	13.5 abcde	12.3 abc	27.0 abcde	24.5 abcd	20.4 cde	22.3 bcd	5.6 cde	4.9 cd
	10.0	14.8 ab	13.8 ab	32.5 a	29.5 a	30.9 ab	37.6 a	10.8 a	11.6 a
	50.0	12.2 abcde	10.9 bcd	24.3 bcdef	21.5 cde	19.1 cde	20.8 cde	6.1 bc	4.7 d
	100.0	10.0 de	9.3 cd	20.5 ef	18.5 de	15.8 e	13.4 fgh	4.2 efg	3.6 def

-Means followed by different letters in the same column are significantly different at p≤ 0.05 by DMRT. 1- Based on a total of 50 explants per treatment.

Effect of silver nitrate on plantlet regeneration:

Effect of silver nitrate frequency of plantlet regeneration (%) and number of regenerated plantlets per explant is illustrated in Table (5). Data show that the frequency of regeneration increased with increasing $AgNO_3$ concentrations of up to 4 mg/l. On a medium containing 4 mg/l $AgNO_3$, 78.7 and 80.9% of explant produced shoots, in the first and second experiments, respectively, whereas in the control, the regeneration frequency was 64.5 and 60.8%, in the first and second experiments, respectively. Data also indicate that using a concentration higher than 4 mg/l had a slightly inhibitory effect on regeneration frequency. However, obtained results indicate that the addition of silver nitrate to regeneration medium did not significantly affect number of regenerated plantlets/ explant, at any tested concentration, compared with control, in both experiments. It was noticeable, in both experiments, that

supplementing regeneration medium with $AgNO_3$ altered the regeneration mode from organogenesis involving a callus phase (Fig.1-E) to that without an intervening callus phase (Fig1-D). Similarly Mussio *et al.* (1998) and Zhang *et al.* (2001) assumed that the increased capacity of shoot organogenesis is at least partially related to the inhibition of callus formation. The mode of action of $AgNO_3$ in plant tissue culture is associated with the physiological effects of ethylene. Silver ions acting as a competitive inhibitor of ethylene action rather than inhibiting ethylene synthesis per se (Zhang *et al.*, 1998). Ethylene may act as inhibitor in several plant regeneration system (Chraibi *et al.*, 1991; Kong and Yeung, 1994). Other studies showed that silver ions may interact with polyamines, which have been shown to promote organogenesis and embryogenesis (Pua *et al.*, 1999) since ethylene and polyamines compete for the same precursor, SAM (S-adenosyl methionine).

Table (5): Effect of silver nitrate on plant regeneration frequency (%) and number of regenerated plantlets per explant

$AgNO_3$ (mg/l)	(%) Regenerating Explants		No. of regenerated plantlets/ explant	
	Exp1	Exp2	Exp1	Exp2
0.0	64.5 bc	60.8 c	13.4 a	11.6 a
1.0	63.3 bc	61.2 c	14.2 a	12.5 a
2.0	69.1 b	71.3 b	12.7 a	11.9 a
4.0	78.7 a	80.9 a	15.2 a	10.9 a
8.0	61.3 c	62.5 bc	13.7 a	10.6 a

-Means followed by different letters in the same column are significantly different at p≤ 0.05 by DMRT.

Effect of propagation method on field performance:

Data of field experiments are presented in Table (6). Results indicate that method of propagation; tissue culture or stem cuttings, neither significantly affects tuber root (Fig.1-H) characters, represented by root length, root diameter and root weight, nor number of tuber roots and yield/ plant, in both seasons. Similar results reported by Donnelly *et al.* (2003) on potato and by Hankoua *et al.* (2005) who indicated that there were no differences between cassava plants propagated by stem cutting or those propagated through *in vitro* plantlet regeneration technique, suggesting a potential for using plants produced by tissue culture for field planting.

Table (6): Effect of propagation methods on cassava tuber root characters, number of tuber roots and yield per plant, during 2002/ 2003 and 2003/2004 seasons.

Propagation methods	Root characters						Yield			
	Root length (cm)		Root diameter (cm)		Tuber root weight (g)		No. of tuber roots/ plant		Yield/plant (kg)	
	I*	II**	I	II	I	II	I	II	I	II
Tissue culture	21.3a	18.7a	6.7a	7.6a	443.1a	396.7a	6.2a	7.1a	2.75a	2.82a
Stem cuttings	23.4a	19.6a	6.9a	7.4a	461.6a	411.3a	5.6a	7.0a	2.58a	2.98a

-Means followed by different letters in the same column are significantly different at p≤ 0.05 by DMRT.

* I= First season (2002/03). **II= second season (2003/04).

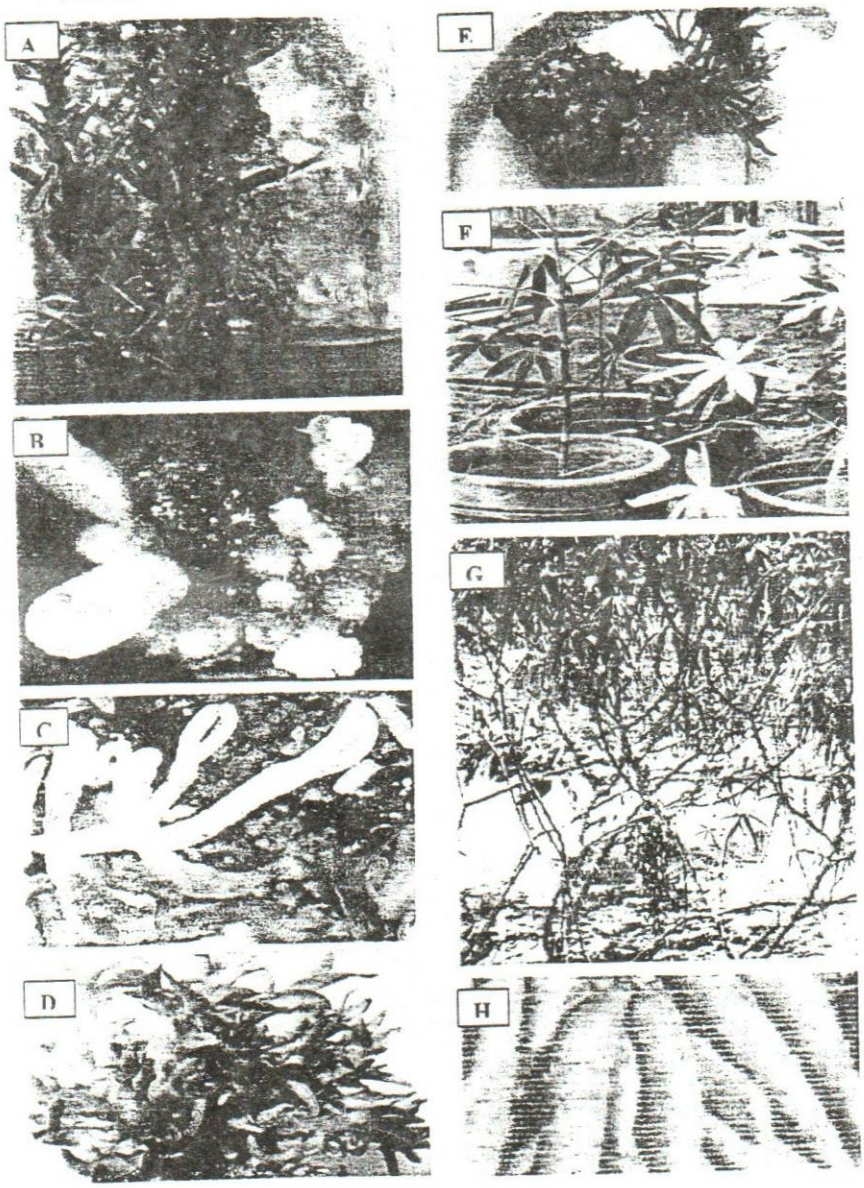

Fig. 1: Scheme of somatic embryo induction and plant regeneration of *cassava. A: In vitro shoot proliferation. B: Somatic embryos at* different maturation stages; globular, heart and torpedo stages. C: Mature somatic embryos at cotyledonary stage. D: Direct shoot organogenesis without an intervening callus phase. E: Shoot organogenesis involving a callus phase. F: Acclimatized plants grown in glasshouse. G: Field established cassava plants. H: Harvested cassava tuber roots.

REFERENCES

Chraibi, B.K.M., A. Latch, J.P. Roustan and J.Fallot.1991. Stimulation of shoot regeneration from cotyledons of *Helianthus annuus* by ethylene inhibitor, silver and cobalt. Plant cell Rep. 10:204-207.

Donnelly, D.J., W.K. Coleman and S.E. Coleman.2003. Potato microtuber production and performance: A review. Amer. J. of Potato Res. 80:103-115.

FAO. 2000. Production year book, Food and Agricultural Organization. Rome, Italy.

Gamborg. O.L., R.A. Miller and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151-181.

Gomez, K.A. and A.A.Gomez.1984. Statistical procedures for agricultural research. John Wiley and Sons. NY, USA.

Hankoua, B.B. 2003. Regeneration and transformation of African cassava (*Manihot esculenta* Crantz) germplasm. Ph.D. Thesis (P. 197). Univ. of Ibadan, Nigeria.

Hankoua, B.B., J. Puonti-Kaerlas, S.Y.C. Ng, I. Fawole, A.G.O. Dixon and I. potrykus. 2000. Plant regeneration of African cassava (*Manihot esculenta* Crantz) germplasm via somatic embryogenesis and organogenesis. In: Abstracts of International Symposium on Tropical Root and Tuber Crops. (p. 127). 19-20, Jan. 2000,Thiruvananthapuram, Kerala, India.

Hankoua, B.B., S.Y.C. Ng, I. Fawole , J. Puonti-Kaerlas, M.Pillay and A.G.O.Dixon. 2005. Regeneration of a wide range of African cassava genotypes via shoot organogenesis from cotyledons of maturing somatic embryos and conformity of field-established regenerants. Plant Cell, Tissue and Organ Culture. 82: 221-231.

Kamada, H.and H. Harada.1979. Studies on the organogenesis in carrot tissue cultures. II. Effects of amino acids and inorganic nitrogenous compounds on somatic embryogenesis. Z.Pflanzenphysiol. 91:453-463.

Konan, N.K, R.S. Sangwan and B.S. Sangwan. 1994. Somatic embryogenesis from cultured mature cotyledons of cassava (manihot esculenta Crantz). Plant Cell,Tissue and Organ Culture. 37:91-102.

Kong, L. and E.C. Yeung. 1994. Effects of ethylene and ethylene inhibitions on white spruce somatic embryo maturation. Plant Sci .104:71-80.

Li, H.Q., J.Y. Guo, Y.W. Huang, C.Y. Liang, H.X.Liu , I. Potrykus and J. Puonti-Kaerlas. 1998. Regeneration of cassava via shoot organogenesis. Plant Cell Rep. 17: 410- 414.

Loiseau,J., C. Marche and Y.L. Deunff. 1995. Effect of auxins, cytokinins, carbohydrates and amino acids on somatic embryogenesis induction from shoot apices of pea. Plant Cell,Tissue and Organ Culture. 41:267-275.

Ma, G. and Q. XU. 2002. Induction of somatic embryogenesis and adventitious shoot from immature leaves of cassava. Plant Cell, Tissue and Organ culture.70: 281-288.

Mathew, H., C. Schopke, R. Carcano, P. Chavarriage, C. Fauquet and R.N. Beachy. 1993. Improvement of somatic embryogenesis and plant recovery in cassava. Plant Cell Rep. 12: 328-333.

Mussio, I., M.H. Chaput, G. Serraf, G. Ducreux and D. Sihachakr.1998. Adventitious shoot regeneration from leaf explants of an African clone of cassava (*Manihot esculenta* Crantz) and analysis of the conformity of the regenerated plant. Plant Cell, Tissue and Organ culture. 53: 205-211

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

Ng, S.Y.C., P. Ilona and O.J. Adeniyi.1994. Post flask management of cassava and Yams. Trop. Root and Tuber Crop Bull. 8 (1): 6-7.

Nweke, F.I., S.C.D. Spencer and K.J Lynam. 2002. The cassava transformation. Africa best kept secret. 48823-5202. (p.273).

Pua, E.C., X. Deng and A.T.C. Koh. 1999. Genotypic variability of de novo shoot morphogenesis of *Brassica oleraceae in vitro* in response to ethylene inhibitor and putrescine. J. Plant physiol. 155: 598-605.

Raemakers, C.J.J.M., M. Amati, G. Staritsky, E. Jacobsen, R.G.F. Visser. 1993a. Cyclic somatic embryogenesis and plant regeneration in cassava. Ann. Bot. 71: 289-294.

Raemakers, C.J.J.M., C.M. Schavemaker, E. Jacobsen, R.G.F. Visser. 1993b Improvement of cyclic somatic embryogenesis of cassava (*Manihot esculenta* Crantz). Plant Cell Rep. 12:226-229.

Rochat, C. and J.P. Boutin. 1991. Metabolism of phloem-borne amino acids in maternal tissues of fruit of nodulation or nitrate fed pea plants (*Pissum sativum* L.). J. Exp. Bot. 42: 207-214.

SAS Institute. 1985. SAS/STAT guide for personal computer, 5th ed. SAS Institute Inc., NC, USA.

Shetty, K. and B.D. McKersie. 1993. Proline, thioproline and potassium mediated stimulation of somatic embryogenesis in alfalfa (*Medicago sativa* L.). Plant Sci. 88:185-193.

Stejskal, J. and M. Griga. 1992. Somatic embryogenesis and plant regeneration in pea (*Pissum sativum*, L.). Biol. Plant. 34:15-22.

Tetu, T., B. Sangwan-Norreel and R. Sangwan. 1987. Embryogenese somatique et regeneration *in vitro* chez trios varieties precoces de soja. C.R. Acad. Sci. Paris. Serie III, 305: 613-617.

Wheatly, C.C. and G. Chuzel. 1993. Cassava in Encyclopaedia of Food Science, Food Technology and Nutrition, by Macrae, R., R.K. Robinson and M.T. Sadler, eds. Academic Press, London, UK.

Zhang, F.L., Y. Takahata and J.B. Xu. 1998. Medium and genotype factors influencing shoot regeneration from cotyledonary explants of Chinese cabbage (*Brassica campestris,* L. ssp. *Pekinensis*). Plant Cell Rep. 17: 780-786.

Zhang, P., S. Phansiri and J. Puonti-Kaerlas. 2001. Improvement cassava shoot organogenesis by the use of silver nitrate. Plant Cell, Tissue and Organ Culture. 67: 47-54.

تكوين أجنة جسدية ونباتات جديدة من أوراق الكسافا الغير ناضجة وزراعة النباتات الجديدة الناتجة فى الحقل

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تم الحصول على أجنة جسدية بطريقة مباشرة بإستخدام أوراق الكسافا الصغيرة (٠,٣ ، ٠,٥ مم فى الطول) على بيئة معدة للحث على تكوين الأجنة الجسدية وذلك تحت الظروف المعملية المعقمة. وقد أظهرت النتائج أن أضافة الأكسينات 2,4-D أو البيكلورام بتركيز ٤ أو ٦ مجم / لتر لبيئة الزراعة كان ذو تأثيرا فعالا بدرجة معنوية فى تشجيع تكوين الأجنة الجسدية . وكان لوجود السيتوكينينات فى بيئة الزراعة ، فيما عدا الكينتين ، تأثيرا سلبيا معنويا على عملية تكوين الأجنة الجسدية. وقد صاحب زيادة تركيز مصدر الكربون (السكروز – المالتوز – الجلوكوز – الفركتوز) فى البيئة من ٢٠ جم الى ٤٠ جم / لتر الى انخفاض معنوى فى النسبة المئوية لتكوين الأجنة الجسدية وفى عدد الأوراق المزروعة المكونة لأجنة جسدية وكان سكر المالتوز هو أكثر مصادر الكربون كفاءة على تشجيع تكوين الأجنة الجسدية وفىزيادة عدد الأوراق المزروعة المكونة لأجنة جسدية . ولم يكن لأضافة الأحماض الأمينية (الجلوتامين والبرولين والألانين) لبيئة الزراعة تأثير إيجابى على نسبة تكوين الأجنة الجسدية ولا على عدد الأجزاء النباتية المكونة لأجنة جسدية . وفيما يتعلق بالتأثير الممتد للأكسينات والسيتوكينينات ومصادر الكربون والأحماض الأمينية المستخدمة فى بيئة الحث لتكوين الأجنة الجسدية على تكوين نباتات جديدة Regeneration من فلقات الأجنة الجسدية والمزروعة على بيئة لأنتاج نباتات جديدة فأن النتائج أوضحت أن 2,4-D أو البيكلورام بتركيز ٤ مجم / لتر ، والبنزيل أدنين بتركيز ١ مجم / لتر والزيتانين بتركيز ٠,٥ مجم/ لتر ، وسكر المالتوز بتركيز ٢٠ جم / لتر كان لهم تأثيرا ممتدا إيجابيا أدى لزيادة النسبة المئوية لتكوين نباتات جديدة والى زيادة عدد النباتات الجديدة لكل جزء مزروع ولم يكن للأحماض الأمينية أى تأثير ممتد على عملية تكوين النباتات الجديدة من فلقات الأجنة الجسدية . أما إضافة نترات الفضة بتركيز ٤ مجم / لتر الى بيئة تكوين النباتات الجديدة فكانت فعالة فى زيادة النسبة المئوية لتكوين النباتات الجديدة ولكن لم تؤثر على عدد النباتات الجديدة المتكونة على فلقة الجنين الجسدى. أثبتت التجارب الحقلية خلال موسمى ٢٠٠٢/٢٠٠٣ ، ٢٠٠٣ / ٢٠٠٤ أن نباتات الكسافا المكاثرة بواسطة زراعة الأنسجة أو بالطريقة التقليدية وهى العقل الساقية لم تختلف معنويا فى المحصول الكلى / نبات أو فى عدد الجذور / نبات أو فى صفات الجذر المتمثلة فى متوسط طول وقطر ووزن الجذر المتدرن.