VOL. 37 NO. 1 (2003) ISSN 0029-0114

Nigerian Journal Of Science

Published by the Science Association of Nigeria

TABLE OF CONTENTS

Nigerian Journal of Science Vol. 37 No. 1 (2003) 69-78

Cassava Micropropagation in a Developing Economy: Efficacy of the use of Alternative Sources of Water, Macro and Micro-Nutrients

Balogun, M. O., Ng, S. Y. C. and Fawole, I.
Institute of Agricultural Research and Training, Moor Plantation, P.M.B.5029, Ibadan, Nigeria.
Tissue Culture Unit, International Institute of Tropical Agriculture, P.M.B. 5320, Ibadan, Nigeria.
Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria.

Abstract

The effect of water, micro- and macro-nutrient source on growth and development of cassava plantlets was investigated. Two varieties of cassava (TME 2 and TMS 4(2) 1425) were used. The double-distilled water currently being used was substituted with well water, IITA tap water and water from air conditioner (a/c); while the Murashige and Skoog (MS) basal medium popularly adopted as the source of macro-nutrients, micro-nutrients and vitamins was also substituted with fertilizers and multimineral tablet. Percentage root formation, plantlet formation and number of roots per plantlet were considerably reduced when tap water was used in TMS 4(2) 1425 but not in TME 2. Well water and a/c water performed equally well as double-distilled water in supporting plantlet growth and development. Almost all the plantlets grown in medium with fertilizer as macro-nutrient source did not survive while those grown in medium with multimineral tablet as micro-nutrient source appeared stunted. Most of those grown in medium with both fertilizer and multimineral tablet as macro- and micro-nutrient sources also did not survive. MS basal medium still proved to be the best in supporting plantlet growth and development, it was however the most expensive.

Introduction

Cassava (*Manihot esculenta* Crantz) is a very important food and industrial crop. Disease incidence however is high and this often result in yield losses of up to 100% (Lazano and Booth, 1974). In Nigeria, yield losses from cassava bacterial blight have been estimated at 75%, while in Columbia, super-elongation disease and *Cercospora* leaf spot can reduce the yield by 3t/ha or more (Cock, 1985). In Africa, cassava mosaic virus can cause root yield losses of up to 95% in susceptible genotypes (Byrne, 1984). The multiplication rate of cassava is also low. Breeding for increased yield, increased protein content, reduced cyanogenic potential and resistance to diseases and pests and conservation of valuable germplasm however constitute high priorities in cassava national improvement programme. The techniques of tissue culture thus becomes relevant through disease elimination by meristem and/or shoot tip culture, higher rates of multiplication of plants by micropropagation and for various genetic manipulations. A wide collection of valuable germplasm can also be conserved in minimal space in culture and can easily be transported across national boundaries without fear of attack by and spread of pests and diseases (Thro *et al*, 1998).

The prohibitively high cost of chemicals and the expenses involved in water distillation however limits the adoption of tissue culture techniques by national and private research laboratories in developing countries. Hence, most farmers can not afford the use of plantlets as clean planting materials on their farms due to high production costs.

The overall goal of this study was therefore to reduce the cost of micropropagation of cassava relative to the current cost. This will make the benefits of tissue culture more accessible to local scientists and farmers. One way of doing this is to use cheaper, easily obtainable substitutes for water, macro- and micro-nutrients in the medium for cassava micropropagation.

The specific objective of this study therefore was to assess the efficacy of alternative sources of water, macro- and micro-nutrients on growth and development of cassava plantlets.

Materials and Methods

The study, which was conducted in the Tissue Culture Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria was in two stages viz. (1) Effects of water source on growth and development of cassava plantlets and (2) Effect of micro- and macro- nutrient source on growth and development of cassava plantlets. In both studies, the factorial design was used, with four replicates and ten units per replicate. Two cassava genotypes (TME 2 and TMS 4(2) 1425) were used.

Experiment 1: Effects of water source on growth and development of cassava plantlets

The four water sources used were:

i) IITA tap water (T_1)
ii) Air- conditioner (a/c) water (T_2), collected from the central drain-off pipe of the a/c of the Biotechnology Research and Tissue Culture units of the IITA.
iii) Well water from Number 7, Sariyu Adebisi Road, New Bodija, Ibadan Nigeria (T_3).
iv) Double-distilled water (T_4).

The hydrogen ion concentration (pH), electrical conductivity and amounts of nitrate, phosphate, potassium, iron and calcium ions in each of the water samples were determined at the analytical services laboratory of the IITA (Five hundred millilitres each of four types of media which differed only in the water source were used in the study. The culture medium used is as described by Ng (1998a; T_1 of Table 2). The pH of the media was set at 5.7. Agar was used as the solidifying agent, the medium was melted in the microwave oven, dispensed into test tubes in 5ml quantities and autoclaved for 15minutes at 121°C. On cooling, single node cuttings of about one centimetre long, excised from full-grown plantlets were introduced into each test tube in sterile hoods in the transfer room. The test tubes, which were sealed with parafilms, were labelled and arranged on growth cabinets in culture rooms at a light intensity of 3000lux and 16hours photoperiod at 25+ 2°C. Each replicate consisted of ten test tubes per treatment combination. Treatments were randomized in each replicate while data were taken on % survival, shoot formation, root formation, plantlet formation, number of roots, nodes and leaves per plantlet at three and five weeks after culturing. The height of each plantlet was also recorded at five weeks after culturing.

Experiment 2: Effects of macro- and micro- nutrient source on growth and development of cassava plantlets

The macro- and micro- nutrient source in use at the Tissue Culture Laboratory is the Murashige and Skoog (MS) basal medium. The fertilizers used as alternative source of macro- nutrient include :

(i) Urea ($CO(NH_2)_2$: Nitrogen source, 46% N.
(ii) Potash (KCl) : Potassium source, 49.8% K.
(iii) Single superphosphate(P_2O_5): 43.66% P.

The amount of fertilizer added was:

$$\frac{\text{100 x amount of element in MS}}{\text{Amount of ingredient in 100g fertilizer}}$$

The micro- nutrient and vitamin source used as substitute was multivitamin/multimineral tablet with the trademark 'Centrum'. The constituent elements of the 'Centrum' tablet are listed in Table 1. Sodium (Na), Boron (B) and Cobalt (Co) which were present in MS medium but missing in the multivitamin tablet were therefore replaced by stock solutions of boric acid, sodium chloride and cobalt chloride as sources of these missing ions. In the trial, six types of media were tested:

(i) T_1 (control): MS basal medium (no substitution),
(ii) T_2: complete substitution of both micro- and macro-nutrient source with multivitamin/ multimineral tablet and fertilizers respectively.
(iii)T_3: As in T_2, but stock solutions of nutrient ions missing in tablet but present in MS basal medium were added.

(iv) T_4: Only the micro-nutrient source was substituted with multivitamin/multimineral tablets, but stock solutions of the ions missing in the tablet were added.
(v) T_5: As in T_4, but without stock solutions of the missing ions.
(vi) T_6: Only macro-nutrient source was substituted with fertilizers.

Water from air-conditioner was used in all the media since the result of experiment 1 justified its use. Media preparation, culture conditions and data taken were as described in experiment 1.

Cost Analysis of the media

The 1998 edition of the SIGMA catalogue book of price listings of laboratory reagents and chemicals was used to obtain the cost of each medium in the experiment on micro- and macro-nutrient source substitution (Table 6).

Statistical Analysis.

Data collected in the two experiments were subjected to statistical analysis using the SAS software. Analysis of variance was done at p=0.05 level of probability. Means were also separated at alpha=0.05.

Results

Well water had the highest concentration of calcium and nitrate ions (54.74 and 18.67ppm respectively). This was followed by IITA tap water (0.34 and 0.0ppm), a/c water and double-distilled water respectively. Highest potassium ion concentration (17.77ppm) was however obtained in IITA tap water, while none of the water samples contained any phosphate ions. IITA tap water had the highest electrical conductivity (0.49) followed by well water, a/c water and double-distilled water respectively (Table 3).

Significant differences in % survival among the four water sources were only obtained at five weeks after culture initiation while treatment effects were not significant with respect to % shoot formation at both three and five weeks after culturing. Differences in % root formation was significant among the four water sources, with tap water having significantly low % root formation relative to others (Table 4). At five weeks after culturing, a/c water was higher than, and not significantly different from double-distilled water in % root formation.

At both three and five weeks after culture initiation, differences in number of leaves per plantlet was not significant among water sources while at five weeks after culture initiation, differences in number of nodes per plantlet were significant among water sources. Well water had the highest number of nodes per plantlet, but not significantly different from a/c and double-distilled water. Tap water had significantly lower number of nodes per plantlet relative to the others. Differences in number of roots per plantlet was only significant at five weeks, with a/c water having the highest, although not significantly different from well and double-distilled water. Tap water still had the lowest number of roots per plantlet relative to the others. The same trend was obtained with respect to % plantlet formation. Plantlet height was significantly low in tap water relative to the others only at five weeks after culture initiation.

Varietal differences were only observed in % root formation, % plantlet formation and plantlet height at five weeks after culture initiation. TMS 4(2) 1425 had significantly low % shoot formation relative to TME 2. In contrast, TME 2 had significantly high % root and plantlet formation relative to TMS 4(2) 1425. Plantlets of the latter variety were however taller than TME 2 plantlets. These results are shown in Tables 4 and 5 and Figures 1 and 2.

Among the six types of media, each of which differed in micro- and macro-nutrient sources, differences in all the observed parameters were found to be significant (Table 5). By five weeks after culture initiation, more than 60% of the macro-nutrient and completely substituted media (T_2, T_3, T_6) had died, without producing new leaves or nodes. Plantlets grown in micro-nutrient-substituted medium had stunted growth while the best performance was observed in the non-substituted medium (control) with respect to all observed parameters (Figures 3 and 4).

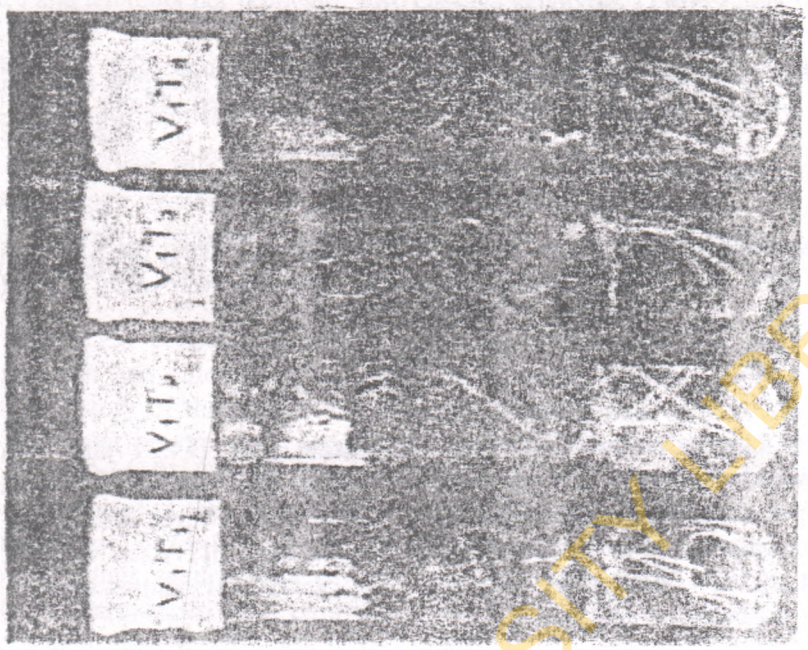

Fig. 1: Plantlets of TME-2 in different Media with respect to water source
T_1: IITA Tap Water
T_2: Air Conditioner Water
T_3: Well Water
T_4: Double-Distilled Water

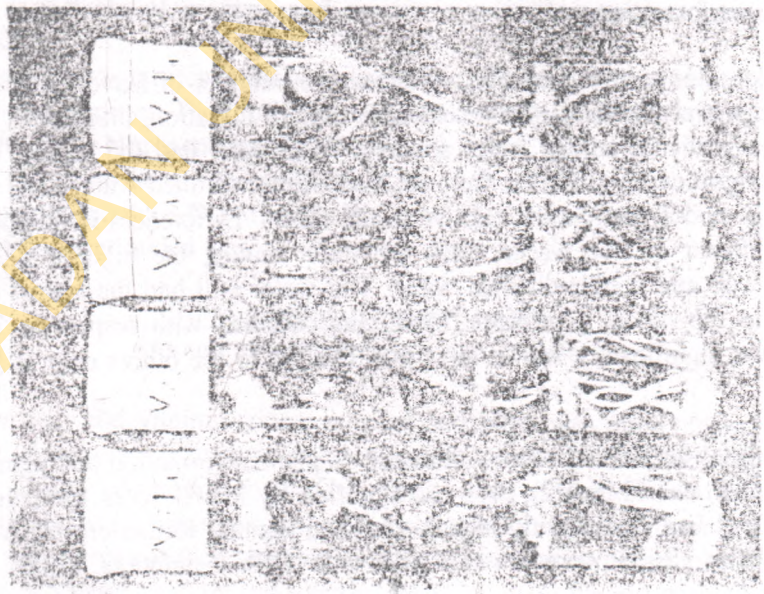

Fig. 2: Cassava Plantlets of TMS 4(2) 1425 grown in media differing in water source
T_1: IITA Tap Water
T_2: Air Conditioner Water
T_3: Well Water
T_4: Double-Distilled Water

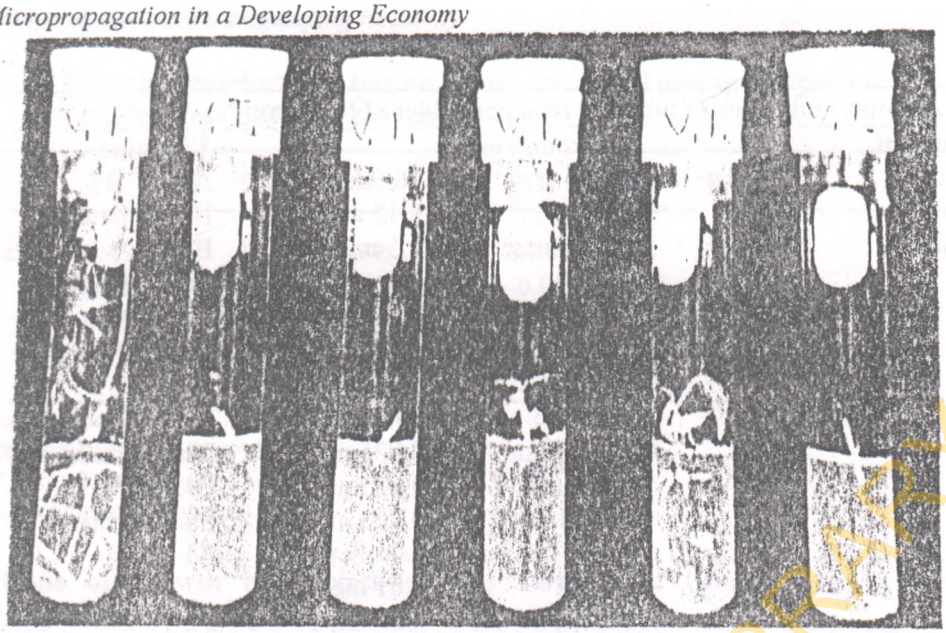

Fig. 3: Cassava Plantlets of TME-2 grown in media differing in macro – and micronutrient source
T_1: Murashige and Skoog Basalt Salt Medium (Control)
T_2: Fertilizers as Macronutrients, Multimineral tablet as Micronutrient source
T_3: As in T2, but the ions missing in multimineral table but present in MS salts were added
T_4: Multimineral tablet as micronutrient source, missing ions added
T_5: As in T4, missing ions not added
T_6: Fertilizers as macronutrient source, MS as micronutrient source

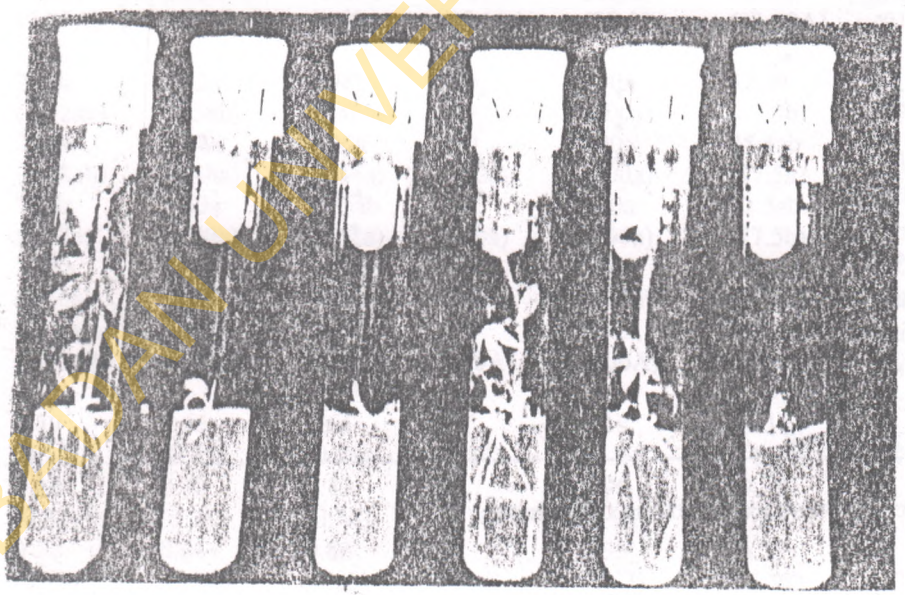

Fig. 4: Cassava Plantlets of TMS 4(2) 1425 grown in media with different sources of micro – and macro – nutrients. $T_1 – T_6$ are as in Fig. 3

Table 1: Amount and types of nutrient ions per tablet of 'Centrum'

Macronutrient		Micronutrient		Vitamins	
Phosphorus	125mg	Iron	18.0mg	A	5000iu
Potassium	40mg	Iodine	0.15 mg	B1 (Thiamine)	1.5 mg
Magnesium	100mg	Manganese	2.5 mg	B2	1.7 mg
		Zinc	15 mg	B6 (Pyridoxine)	2.0 mg
		Molybdenum	0.025 mg	B12	0.006 mg
		Copper	2.0 mg	C	60 mg
		Folic acid	0.4 mg	D	400iu
		Chloride	36.3 mg	E	30iu
		Chromium	0.025 mg	Niacinamide	20 mg
		Selenium	0.025 mg	Biotin	0.03 mg
		Nickel	0.005 mg	Panthotenic acid	10 mg
		Tin	0.01 mg	KI	0.025 mg
		Silicon	0.01 mg		
		Vanadium	0.01 mg		

Table 2: Nutrient composition of 500ml of six types of media , each with different sources of macro- and micro-nutrients

Constituent	T1	T2	T3	T4	T5	T6
Sucrose 15g	*	*	*	*	*	*
Inositol 50mg	*	*	*	*	*	*
BAP 0.025mg	*	*	*	*	*	*
NAA 0.005mg	*	*	*	*	*	*
Agar 3.5g	*	*	*	*	*	*
MS 2.22g	*					
Potash 0.745g		*	*			*
Urea 0.92g		*	*			*
Tablet (half)		*	*	*	*	
Boric acid 3mg			*	*		
NaCl 13mg			*	*		
CoCl2 0.005mg			*	*		
Stock I 25ml				*	*	
Stock II 2.5ml						*
Stock III 5ml						*
Vitamin stock 2.5ml						*
Single superphosphate						*

* indicates presence of media constituent.

Table 3: Electrical conductivity ($msmm^{-1}$) and amount of ions (ppm) present in water samples from different sources.

Water source	EC	pH	Ca	K	Fe	PO_4	NO_3	NH_4
IITA tap water	0.49	7.6	19.87	17.77	0.00	0.00	0.20	0.00
a/c water	0.021	6.3	1.11	0.72	0.2	0.00	0.02	0.70
Well water	0.39	7.5	54.74	6.66	0.00	0.00	18.67	0.00
D-D water	0.004	6.0	0.34	0.11	0.00	0.00	0.00	0.00

EC : Electrical conductivity.
pH : Hydrogen ion concentration.
A/c water: Air-conditioner water.
D-D water: Double-distilled water.

Table 4: Means of growth parameters of two varieties of cassava grown in four types of micropropagation media differing only in water source.

	No.leaves/ plt.	No.nodes/ plt.	No.roots/ plt.	% survival.	% Shoot formation	% Root formation	%Plt. formation	Plt. Height.
V_1 Tap	2.60a	3.38a	1.92a	100	97.5a	72.5ab	72.5ab	
	(3.22ab)	(4.08ab)	(3.15a)	(97.5a)	(97.5a)	(87.5)	(87.5)	1.94b
A/C	2.25b	2.90b	1.68ab	100	100a	85.0a	85.0a	
	(2.80b)	(3.75ab	(3.00a)	(100a)	(100a)	(97.5a)	(97.5a)	1.64b
Well	2.58a	3.58a	1.58ab	100	100a	87.5a	87.5a	
	(3.40ab)	(4.40a)	(2.92a)	(100a)	(100a)	(95.0a)	(95.0a)	1.85b
Distilled	2.40b	3.45a	1.92a	100	95.0a	85.0a	80.0ab	
	(2.85b)	(4.18ab)	(2.92a)	(100a)	(95.0a)	(92.5ab)	(87.5ab)	1.75b
Mean V_1	2.46A	3.32A	1.78A	100	98.12A	82.5A	81.25A	
	(3.07A)	(4.10A)	(3.00A)	(99.38A)	(98.12A)	(93.12A)	(91.88A)	1.79B
V_2 Tap	2.60a	3.12a	0.98b	100	100a	52.5b	52.5b	
	(3.12ab)	(3.58b)	(1.48b)	(100a)	(100a)	(67.5b)	(67.5b)	1.71b
A/C	2.82a	3.48a	1.96a	100	100a	75.4ab	75.4ab	
	(3.48ab)	(4.48a)	(3.44a)	(100a)	(100a)	(97.5a)	(97.5a)	3.47a
Well	3.08a	3.60a	1.75ab	100	100a	72.5ab	72.5ab	
	(3.62a)	(4.40a)	(2.62a)	(100a)	(100a)	(77.5b)	(77.5b)	2.95a
Distilled	2.88a	3.39a	1.98a	100	100a	84.17a	84.17a	
	(3.66a)	(4.37a)	(2.94a)	(100a)	(100a)	(89.4ab)	(89.4ab)	2.96a
Mean V_2	2.84A	3.40A	1.66A	100	100A	71.14A	71.14A	
	(3.47A)	(4.21A)	2.62A)	(100A)	(100A)	(82.99B)	(82.99B)	2.77A
Lsd Media	0.39	0.45	0.61	Ns	4.19	18.91	20.09	
	(0.50)	(0.53)	(0.71)	(1.84)	(4.19)	(11.29)	(12.26)	0.69
Genotype	0.28	0.32	0.43	Ns	2.96	13.37	14.21	
	(0.35)	(0.38)	(0.50)	(1.30)	(2.96)	(7.99)	(8.67)	0.49
Interaction	0.56	0.64	0.86	Ns	5.93	26.75	28.41	
	(0.70)	(0.76)	(1.00)	(2.60)	(5.93)	(15.97)	(17.34)	0.97
CV	40.65	34.02	86.90	0.0	9.76	55.04	56.01	
	(43.65)	(34.15)	(70.52)	(5.63)	(9.79)	(36.96)	(38.06)	79.62

V_1: TME 2, V_2: TMS 4(2) 1425. Values in bracket were recorded at 5 weeks after culture initiation, those not in bracket were recorded at 3 weeks after culturing. For each age group, means within each column followed by the same low case letters are not significantly different at the 5% probability level. Varietal means within each column followed by the same upper case letter are not significantly different.

Table 5: Means of growth parameters of two varieties of cassava grown in four types of micropropagation media differing only in macro- and micro-nutrient source.

Macro. source	Micro. Source	Trt	No.leaves/ plt.	No.nodes/ plt.	No.roots/ plt.	% survival	% Shoot formation	% Root formation	%Plt. formation	Plt. Height
V_1										
MS	MS	T_1	2.25ab	2.75a	1.15a	100a	95.0a	62.5a	57.5a	
			(3.1a)	(3.42a)	(2.92b)	(97.5a)	(95.0a)	(87.5a)	(87.5a)	2.27b
Fertilizer	Tablet	T_2	0.3c	0.58cd	0.0c	30b	22.5bc	0.0d	0.0d	
			(0.08d)	(0.125d)	(0.0e)	(5.0c)	(5.0c)	(0.0f)	(0.0f)	0.07c
Fertilizer	Tablet	T_3	0.78c	1.00c	0.0c	45b	37.5b	0.0d	0.0d	
			(0.78cd)	(0.925c)	(0.02e)	(37.5b)	(35.0b)	(2.5f)	(2.5f)	0.44d
MS	Tablet	T_4	1.82b	2.27ab	0.64b	92.2a	92.2a	35.83b	35.83b	
			(2.48b)	(2.76b)	(1.39c)	(97.5a)	(94.72a)	(64.4b)	(66.94b)	1.15c
MS	Tablet	T_5	1.58b	2.12b	0.25bc	97.5a	87.5a	15.0cd	17.5c	
			(2.30b)	(2.30b)	(0.97cd)	(94.72a)	(89.72a)	(48.89c)	(48.89c)	1.10c
Fertilizer	MS	T_6	0.45c	0.98c	0.05c	50.0b	25.0bc	2.5d	2.5d	
			(0.38cd)	(0.40cd)	(0.05e)	(20bc)	(15.0c)	(2.5f)	(2.5f)	0.20de
V_1 Mean			1.2A	1.62A	0.35A	69.12A	59.95A	19.31A	18.89A	
			(1.52A)	(1.66A)	(0.89A)	(58.7A)	(55.74A)	(34.31A)	(34.72A)	0.89A
V_2										
MS	MS	T_1	2.48a	2.72a	1.52a	97.5a	95.0a	65.0a	62.5a	
			(3.38a)	(3.45a)	(3.52a)	(97.5a)	(95.0a)	(87.5a)	(87.5a)	2.86a
Fertilizer	Tablet	T_2	0.10d	0.12d	0.0c	7.5c	7.5c	0.0d	0.0d	
			(0.03d)	(0.06d)	(0.0e)	(3.12c)	(3.12c)	(0.0f)	(0.0f)	0.03e
Fertilizer	Tablet	T_3	0.15d	0.18d	0.0c	7.5c	7.5c	0.0d	0.0d	
			(0.00d)	(0.025d)	(0.0e)	(2.5c)	(0.0c)	(0.0f)	(0.0f)	0.05e
MS	Tablet	T_4	1.85b	2.40ab	0.05c	95.0a	90.0a	5.0cd	5.0cd	
			(2.42b)	(2.23b)	(0.37de)	(89.38a)	(86.25a)	(20.0e)	(20.0e)	1.10c
MS	Tablet	T_5	2.08ab	2.18b	0.30bc	95.0a	92.5a	17.5c	17.5c	
			(2.35b)	(2.25b)	(0.72d)	(95.0a)	(90.0a)	(40.0d)	(40.0d)	1.14c
Fertilizer	MS	T_6	0.32c	0.45cd	0.0c	32.5b	25.0bc	0.0d	0.0d	
			(0.20d)	(0.225d)	(0.0e)	(15.0c)	(15.0c)	(0.0f)	(0.0f)	0.16de
V_2 Mean			1.16A	1.34B	0.31A	55.83B	52.92A	14.58A	14.17A	
			(1.40A)	(1.37B)	(0.77A)	(50.42B)	(48.23B)	(24.58B)	(24.58B)	0.87A
Lsd										
Media			0.34	0.39	0.33	15.56	14.16	8.99	9.25	
			(0.42)	(0.45)	(0.41)	(12.78)	(12.42)	(6.24)	(6.14)	0.20
Genotype			0.19	0.22	0.19	8.98	8.18	5.19	5.34	
			(0.24)	(0.26)	(0.24)	(7.38)	(7.17)	(3.6)	(3.55)	0.12
Interaction			0.48	0.55	0.47	22.01	20.03	12.72	13.09	
			(0.60)	(0.64)	(0.58)	(18.08)	(17.57)	(8.83)	(8.68)	0.29
CV			104.24	96.03	263.88	77.24	87.65	221.34	224.69	
			(115.57)	(110.16)	(205.61)	(91.95)	(96.77)	(154.94)	(155.72)	145.29

V_1: TME 2, V_2: TMS 4(2) 1425. Values in bracket were recorded at 5 weeks after culture initiation, those not in bracket were recorded at 3 weeks after culturing. For each age group, means within each column followed by the same low case letters are not significantly different at the 5% probability level. Varietal means followed by the same upper case letter are not significantly different. T_3 and T_4 composition are as in T_2 and T_5, but with stock solutions of ions missing in tablet added

Table 6: Costs (£) per 500ml of the six types of media.

Reagent	T1	T2	T3	T4	T5	T6
Sucrose 15g	0.345	0.345	0.345	0.345	0.345	0.345
Inositol 50mg	0.00495	0.00495	0.00495	0.00495	0.00495	0.00495
BAP 0.025mg	$1.6x10^{-4}$	$1.6x10^{-4}$	$1.6x10^{-4}$	$1.6x10^{-4}$	$1.6x10^{-4}$	$1.6x10^{-4}$
NAA 0.005mg	$1.08x10^{-6}$	$1.08x10^{-6}$	$1.08x10^{-6}$	$1.08x10^{-6}$	$1.08x10^{-6}$	$1.08x10^{-6}$
Agar 3.5g	0.91	0.91	0.91	0.91	0.91	0.91
MS 2.22g	0.75					
Potash 0.745g		$9.68x10^{-5}$	$9.68x10^{-5}$			$9.68x10^{-5}$
Urea 0.92g		$2.39x10^{-4}$	$2.39x10^{-4}$			$2.39x10^{-4}$
Tablet (half)		0.052	0.052	0.052	0.052	
Boric acid 3mg			$2.16x10^{-4}$	$2.16x10^{-4}$		
NaCl 13mg			$1.4x10^{-4}$	$1.4x10^{-4}$		
$CoCl_2$ 0.005mg			$1.5x10^{-6}$	$1.5x10^{-6}$		
Stock I 25ml				0.0621	0.0621	
Stock II 2.5ml						$1.49x10^{-3}$
Stock III 5ml						$3.86x10^{-3}$
Vitamin stock 2.5ml						$1.97x10^{-4}$
Single superphosphate (97.1mg)						0.02
Totals	**2.01**	**1.31**	**1.31**	**1.38**	**1.37**	**1.29**

T_1: Control (MS basal medium as macro- and micro-nutrient source); T_2, T_3: both micro-and macro-nutrient sources were substituted with fertilizer and multi-mineral tablet; T_4, T_5: only micronutrient source was substituted with multi-mineral tablet; T_6: only macronutrient source was substituted with fertilizers.

Discussion

In general, high electrical conductivity of IITA tap water (0.49) shows high overall ionic concentration, this is followed by well water, a/c water and double-distilled water (Table 3). Reports from earlier studies have shown that water source did not have negative effects on development of coconut embryos *in vitro* (Areza et al, 1994). That significantly lower values were recorded for percent root formation, plantlet formation and number of roots per plantlet with IITA tap water relative to other water sources (Table 4), may be attributable to the osmotic role of the different ions present in each water sample in influencing the ion transport mechanism. The latter is in turn closely related to the uptake of nutrients by roots. A low- salt medium was found satisfactory for rooting of shoots in a large number of plant species (Bhojwani and Razdan, 1983). Better rooting ability of TME (2) relative to TMS 4 2 1425 in IITA tap water suggests that TME 2 may be more tolerant to high-salt medium than TMS 4(2) 1425 showing genotypic difference in response to different water sources in this trait. Significantly reduced height of plantlets grown in IITA tap water is expected because poor root formation reduces the capacity of nutrient uptake by plantlets which consequently becomes stunted. A/C water may therefore be recommended for use in tissue culture media. It supports plantlet growth and development, is relatively cheaper and easily affordable. Well water also was equally good, but requires laboratory analysis for the determination of the type and concentration of ions it contains as this may vary from well to well.

The very low survival obtained in the macro-nutrient and completely substituted media may be attributable to inability of the plantlet to make use of urea using the enzyme urease which is produced by putrefying bacteria in the soil. Putrefying bacteria were however absent due to the sterile conditions of the tissue culture medium. Nitrogen is an essential constituent of proteins, chlorophyll, protoplasm and nucleic acids. Growth is adversely affected in the absence of this vital element. In addition to nitrogen deficiency, absence of phosphorus could have led to the death of plantlets grown on the completely substituted medium. This is because the form in which phosphorus exists in the multimineral tablet may not be absorbable by the plantlets.

The stunted growth recorded for plantlets grown in micro-nutrient source-substituted medium (T_4, T_5) suggests that the micro-nutrients and even phosphorus in the multivitamin tablet used may not be in a form easily absorbable by plantlets. Information on the chemical form of the micro-nutrients was not indicated on the pack of the tablet. The optimum performance observed in the non-substituted medium (control) is expected because it contained all the required nutrients in the right proportions.

Although all the substituted media were cheaper and easily affordable compared to the control (Table 6), their performances were not as good as the non-substituted medium. Use of multimineral tablets containing the required micronutrients in the desired forms can be explored in micropropagation of cassava plantlets. Ammonium fertilizers may also be tested in subsequent studies as sources of nitrogen for easy absorption by plantlets in culture. Soil/water solutions of different concentrations can also be used as alternative sources of micro- and macro-nutrients. To further reduce the cost of medium, agar can be substituted with corn starch as earlier reported in studies of Ng (1998b) or cassava starch. This will not only promote tissue culture research in developing countries, but will enhance increased production of food and raw materials for agro-based industries.

References

Areza, M. B. B; Rillo, E. P; Cueto, C. A; Ebert, A.W. and Orenze, O. D. (1994): Effects of water source, pH and state of medium on growth and development of coconut embryo *in vitro*. In: Abstracts, 10th International Congress of Plant Tissue and Organ culture, Firenze. June 12-17, 1994. Pp. 17.

Bhojwani, S. S. and Razdan, M. K. (1983): Tissue Culture, Theory and Practice. In: Developments In Crop Science (5): Elsevier pp330-331.

Byrne, D. (1984): Breeding Cassava. In: Janick (ed). Plant Breeding Reviews, Vol. 2. AVI Publishers, Westport, Connecticut, Pp. 73-134.

Cock, J.H. (1985): Cassava: New Potentials for a neglected crop. Westview Press, Boulder.

Lazano, J. C. and Booth R. H. (1974): Diseases of Cassava (*Manihot esculenta* Crantz). PANS 20:30-54.

Ng, S.Y.C. (1998a): IITA training manual for trainees in Tissue Culture Practicals.

Ng, S.Y.C. (1998b): Local substitutes for some ingredients in Tissue Culture media. In: Book of Abstracts, 7th International Symposium of the ISTRC-AB, Cotonou, Rep. of Benin. Pp34.

Thro, A. M; Roca, W; Iglesias, C; Henry, G. and Ng, S. Y. C. (1998): Contributions of *in vitro* biology to Cassava Improvement. African Crop Science Journal 6(3) :303-315.