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A simple and low-cost strategy for micropropagation of cassava (*Manihot esculenta* Crantz)

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With the aim of transferring micropropagation techniques to cassava seed producers, a simple and low-cost medium for *in vitro* micropropagation was developed. CM6740-7 cassava cultivar from CIAT was used as planting material. Commercially available nutrients were used in order to substitute the propagation media components. A Hydro Agri's fertilizer (12-11-18/3 (MgO-EDTA)) was used as a substitute for Murashige and Skoog macro and micronutrients. Additionally, table sugar and molasses were used as a source of carbohydrates and vitamins. Radixone 3AS from Praquim C.A. was used as a source of auxins and activol as a source of gibberellic acid. Numbers of roots and leaves, height and dry weight of plantlets was determined for each treatment and compared with a conventional micropropagation media. For all the parameter measured, the plantlets obtained on the fully substituted media showed a good growth performance. Canonical biplot analysis showed that this media was especially good for the number of nodes (multiplication rate) in 60-day-old cultured plants. The designed medium is simple, easy to prepare and allows a low-cost strategy to obtain a high quality *in vitro* micropropagated cassava plants and its ready availability may have an impact on the production of planting material of cassava.

Key words: Media design, biotechnological transference, canonical biplot analysis.

INTRODUCTION

Plant tissue culture methodologies were developed 5 decades ago and since, they have been used to produce large amounts of high quality seed of many crops. Despite this, to guarantee that the benefits from these technologies reach local farmers in developing countries there is a lot to do. Due to the complexity and cost of the

technological transferences, these technologies are just starting to be broadly used in these countries.

Cassava (*Manihot esculenta* Crantz) is a perennial shrub from the American tropics with a tuberous edible root. It is cultivated throughout the tropical world for its starchy roots, from which cassava breads, flour, tapioca and beverages are derived. In the tropics cassava is the third most important source of calories with more than 600 million people in Africa, Asia and latin America depending on it (Cock, 1982; Ospina and Ceballos, 2002).

Cassava is multiplied mainly by stem cuttings which is a slow process when compared with grain crops. It is mostly grown on small farms and the cuttings are usually planted at the start of the rainy season. The crop cycle depends on whether the early or late varieties are grown, the first type are harvested 8 months after planting,

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Abbreviations: T, Tons; ha, hectare; NAA, α -naphthaleneacetic acid; GA₃, gibberellic acid; IBA, indole-3-butyric acid; IAA, indole-3-acetic acid; BA, 6-benzylaminopurine; CBA, canonical biplot analysis; CIAT, Centro Internacional de Agricultura Tropical.

Table 1. Composition of each media assayed.

Media component		M1	M2	M3	M4	M5	M6	M7	M8	M9
Salts	Murashige and Skoog	x	x	x				x	x	
	Hydro Agri's Fertilizer (12-11-18/3(MgO)-EDTA)				1 g/l	2 g/l	2.5 g/l			2 g/l
Hormones	Plant Tissue Grade	x	x		x	x	x	x	x	
	Agriculture Applications Grade			x						x
Vitamins	Plant Tissue Grade	x	x	x	x	x	x			
	Molasses							1.5 g/l	2.5 g/l	2.5 g/l
Sucrose (2%, w/v)	Analytical Grade	x	x	x	x	x	x	x	x	
	Table Sugar									x
Gelling agent	Phytigel® (0,2%w/v)	x								
	Cassava Starch (10%w/v)		x	x	x	x	x	x	x	x

M1: Contain Murashige and Skoog (MS) (1962) salts complemented with thiamin-HCl (18 µM), myo inositol (555 µM), sucrose ((2%, w/v), α-naphthaleneacetic acid (NAA) (0.027 µM), gibberellic acid (GA₃) (0.023 µM) and Phytigel™ (0.2%, w/v) (Roca, 1980). **M2:** The same as M1 but starch AIM TF351 from Agroindustrial Mandioca was used as gelling agent (Romay et al., 2006). **M3:** The same as M2 but analytical plant hormones were substituted in the case of auxins by Radixone 3AS from Praquim C.A (0.027 µM NAA, 0.011 µM IBA, 0.027 µM IAA) and in the case of gibberellic acid by Activol (0.023 µM GA₃). **M4-M6:** The same as in M2 but different concentrations of a Hydro Agri's Fertilizer (12-11-18/3(MgO)) (0.15, 0.20 and 0.25% (w/v)) plus EDTA were used instead of MS macro and micronutrients. **M7 - M8:** The same as in M2 but 2 different concentrations of local produced molasses (1.5 and 2.5 g/l respectively) were used as a source of carbohydrates and vitamins. **M9:** A fully substituted media containing 2% (w/v) of table sugar, 2 g/l of Hydro Agri's fertilizer 12-11-18/3 (MgO) plus EDTA, 2.5 g/l molasses, auxins (0.027 µM NAA, 0.011 µM IBA, 0.027 µM IAA) from Radixol, 0.023 µM GA₃ from Activol, 10% (w/v) cassava modified starch AIM TF351 and tap water.

whereas the second one are collected 18 months post planting (Ospina and Ceballos, 2002).

Despite the importance of cassava, over the past 30 years its average worldwide productivity has remained at 12 -13 t/ha and this number is far away from its potential crop productivity of 80 t/ha (FAOSTAT, 2006). In Venezuela, cassava is a neglected crop grown primarily by farmers in marginal areas and its large-scale cultivation is constrained by the lack of high quality seed and the low productivity and profit (Montaldo, 1996). Tissue culture methodologies can be used to produce high quality vegetative planting material, which ensures productivity higher than 30 t/ha. A higher increase in productivity may also be obtained by using genetic varieties resistant to pests and diseases or to environmental constrains.

However, to reach a technological transference, a cheap and simplified method to micropropagate cassava *in vitro* plants is required. To achieve this aim, the experiments here discussed allowed the production of a cheap media, made of local ready available components, which facilitates and encourage the transference of tissue culture technology to cassava seed producers.

MATERIALS AND METHODS

Plant material and culture establishment

Previously established cassava *in vitro* plants of variety CM6740-7 obtained from Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia) were used as a source of micropropagation cuttings. The experiment was conducted in a complete randomized design with 9 treatments and 3 replications. Each replication con-

sisted of a glass flask containing 5 stem cuttings (approx. 1 cm long). The cassava micropropagation media containing Murashige and Skoog salts, supplemented with thiamine 10 mg/l, myo-inositol 100 mg/l, 2% (w/v) sucrose, 0.027 µM α-naphthaleneacetic acid (NAA), 0.023 µM gibberellic acid (GA₃) and either 0.18% (w/v) Phytigel™ or 10% (w/v) cassava modified starch AIM TF-351 from Agroindustrial Mandioca C.A., was used as a control medium (Murashige and Skoog, 1962; Romay et al., 2006). Conductivity and pH of MS (M1) media was 5.94 mS cm⁻¹ and 5.7 respectively. On different treatments, Murashige and Skoog (1962) salts were substituted by different concentrations of a Hydro Agri's fertilizer (12-11-18/3(MgO)-EDTA (100 µM) (0.15, 0.20 and 0.25% (w/v)). In addition to table sugar (sucrose) at 2% (w/v), 2 different concentrations of locally produced molasses (0.15 and 0.25% (w/v)) were also assayed as a source of carbohydrates and vitamins. Radixone 3AS from Praquim C.A (1000 mg NAA, 200 mg indole-3-butyric acid (IBA), 500 mg indole-3-acetic acid (IAA)) was used as an alternative source of auxins and Activol (Reg. M.A.C. N° A-0064) as an alternative source of gibberellic acid. A fully substituted media containing 0.2% (w/v) of Hydro Agri's fertilizer (12-11-18/3(MgO))-EDTA, 2% (w/v) table sugar, 0.25% (w/v) molasses, 0.027 µM NAA, 0.011 µM IBA, 0.027 µM IAA, 0.023 µM GA₃ (from Radixone and Activol) and 10% (w/v) cassava modified starch was assayed. Conductivity and pH of the fully substituted media (M9) was 2.74 mS cm⁻¹ and 5.74 respectively. Tap water (conductivity 0.289 mS, pH 7.22) was used in the fully substituted media (M9). The composition of each medium assayed can be observed in Table 1. Before autoclaving at 15 psi and 121°C, pH was adjusted to 5.7 - 5.8 using KOH. For all treatments, 20 ml of medium were dispensed in 5.5 x 10 cm glass flasks (200 ml flask); 5 stem cuttings (approx. 1 cm long) were cultured on each flask. All cultures were incubated at 28 ± 2°C under a 12/12-h photoperiod and fluorescent light intensity of 2500 lux. Growth parameters such as number of nodes, roots, plant height, shoot dry weight and roots dry weight, were recorded at 15 days interval for 2 months after raising the cultures. Validation of the M9 media was performed using the same experimental designed but with 7 different cultivars of the cassava *in vitro* germo-



Figure 1. *In vitro* cassava plants growing under M1 and M9 medium 60 days after sub culturing.

plasm bank of Instituto de Estudios Avanzados (IDEA), including cultivars IDEA-22, IDEA-52, MBRA383 (from CIAT), CM8027-3 (from CIAT), IDEA-85, IDEA-87 and IDEA-98.

Statistical analysis

To establish the differences between treatments and characterise the variables responsible for them, we propose the use of an innovative methodology well-known like canonical biplot analysis (CBA). CBA is a methodology for discrimination among groups, using multiple observed variables and allows deriving linear combinations that successively maximize the ratio of “between-groups” to “pooled within-group” sample variance. The method consider the matrix $\mathbf{Y} = \mathbf{D}_n^{1/2} \bar{\mathbf{X}} \mathbf{W}^{-1/2}$ of the weighted group means and its singular value decomposition (SVD), $\mathbf{Y} = \mathbf{U} \mathbf{D}_\lambda \mathbf{V}'$, where $\bar{\mathbf{X}}$ is a matrix that containing the means of each group (Treatments - culture medium in our case) for the variable DW aerial, DW roots, height, nodes and roots. \mathbf{D}_n the diagonal matrix containing the group sample sizes

and $\mathbf{W} = \frac{1}{n-k} (\mathbf{X}' \mathbf{X} - \bar{\mathbf{X}}' \mathbf{D}_n \bar{\mathbf{X}})$ and $\mathbf{B} = \frac{1}{k-1} \bar{\mathbf{X}}' \mathbf{D}_n \bar{\mathbf{X}}$ the

“within-groups” and “between-groups” covariance matrices, where \mathbf{X} is a data matrix divided into K groups. The first canonical variate derived from the eigenvectors of $\mathbf{W}^{-1} \mathbf{B}$ is interpreted as the linear combination having the highest F ratio in an univariate ANOVA, thus showing the highest differences between groups. The second canonical variate is orthogonal to the first and accounts for the highest part of the remaining differences and so on. The decomposition in $\bar{\mathbf{X}} = \mathbf{D}_n^{-1/2} \mathbf{U} \mathbf{D}_\lambda \mathbf{V}' \mathbf{W}^{1/2}$ allows for the construction of a biplot representation (in dimension r) for the matrix of group means ($\bar{\mathbf{X}} \cong \mathbf{G} \mathbf{H}'$), taking the first r columns $\mathbf{G} = \mathbf{D}_n^{-1/2} \mathbf{U} \mathbf{D}_\lambda$ as row markers and the first r columns of $\mathbf{H} = \mathbf{W}^{1/2} \mathbf{V}$ as column markers. The row markers were represented as points and the column markers as vectors in a scatter diagram. Thus, the similarity between groups can be measured as an inverse function of its distance on the graph. The angle between variables can be interpreted as an approximation of its correlation. Confidence circles are added to the group markers in such a way that the projections of the circles onto the direction representing a variable, approximates a confidence interval. Checking the overlapping of 2 projections allows us to

establish the significance of the difference between groups over a particular variable. The ratio of the circle is calculated as

$$\frac{t_{n-K, \alpha}}{\sqrt{n_K}}$$

where $t_{n-K, \alpha}$ is the critical point of a student's t distribution

with $(n-K)$ degrees of freedom, for a significance level of α . The procedure is conservative in the sense that if no overlap is founded it can be conclude that there is a significant difference but, if there is an overlap, a significant difference along another direction of the multi-dimensional space can be defined. Definitions and algebraic derivations of the canonical biplot analysis are discussed in Amaro et al. (2004). All the analyses were computed with programs written for this purpose using MatLab versión 2006a (The MathWorks Inc, 2006). (Available in: <http://biplot.usal.es>) To detect the differences between M1 and M9 media DGC test was applied to the height and nodes data of different cassava cultivars (Di Rienzo et al., 2002).

RESULTS

The design of plant culture media as a low-cost strategy to produce planting material must guarantee high quality and well developed plants that can easily be adapted to green house and field conditions. Figure 1 shows a picture of *in vitro* cassava plants after growing 60 days in the different media assayed. As observed in the Figure the tested media supported growth of shoots and roots with the same morphology and plant structure as the control plantlets. In general, the longest plants were obtained with the control media (M1) but the best multiplication rate was obtained with the low-cost media M9. Table 2 summarises the results of plant height, number of nodes and roots and dry weight of shoots and roots, of 60-day-old *in vitro* cassava plants grown on different media. The CBA for the different time point observations is presented in Figure 2 (a, b, c and d). The inertia absorption calculated with the 2 first retained dimensions was 77.51, 77.63, 77.13 and 89.72% for each time point. Each vector in these planes is a variable, and each projected star on the plane represents the average of the culture medium analysed. The p-value for the global analysis of the canonical biplot provides a lambda (Wilks) significant ($p < 0.01$) in all the cases, indicating that there are differences among the plant growth due to the culture media. To explain the treatment averages after adjusting the values of canonical biplot, the cumulative goodness-of-fit was calculated with the 2 first dimensions of the variables (Table 3). From these results the best-represented and more homogeneous variables were dry weight of roots and height with goodness of fit greater than 77 and 81% respectively.

Through out 60 days observation, cassava *in vitro* plants grown on M1 medium were significant different from the rest of the media assayed, mainly at the level of plants height. However, according to number of nodes, factor that determines the multiplication rate for the next cycle, 60 days-old plantlets growing in M9 showed the best results. On the other hand, during the first 15 days observation, the fully substituted medium (M9) was different from the rest of the media assayed by having

Table 2. Height, number of nodes, number of roots and dry weight of leaves and roots of 60 days old cassava *in vitro* plants grown on different medium.

Treatment	Height (mm)	Number nodes	Number roots	Shoot DW (mg)	Roots DW (mg)
M1	117.20 ± 8.51	6.33 ± 0.25	6.80 ± 0.33	23.32 ± 2.36	7.65 ± 0.63
M2	105.07 ± 11.03	6.47 ± 0.26	5.40 ± 0.31	24.48 ± 2.05	17.95 ± 1.96
M3	72.00 ± 5.81	6.47 ± 0.22	4.73 ± 0.28	20.49 ± 1.94	8.90 ± 0.83
M4	38.49 ± 3.57	4.20 ± 0.31	3.73 ± 0.24	10.83 ± 1.70	12.59 ± 1.29
M5	65.29 ± 8.51	4.73 ± 0.56	5.13 ± 0.39	17.47 ± 3.19	19.60 ± 3.16
M6	49.92 ± 7.95	4.93 ± 0.77	3.87 ± 0.65	13.66 ± 3.18	13.68 ± 2.88
M7	60.85 ± 7.59	6.00 ± 0.34	4.13 ± 0.40	19.04 ± 3.02	11.13 ± 1.28
M8	75.40 ± 5.90	6.00 ± 0.29	5.07 ± 0.47	22.95 ± 2.71	12.50 ± 1.15
M9	75.17 ± 4.08	7.27 ± 0.21	4.53 ± 0.38	20.23 ± 2.24	7.26 ± 0.55

Values are expressed as the mean ± standard error (n = 15).

greater number of roots. The evaluation of 30 and 45 days-old plantlets regeneration activity detected 3 main groups of growth, showing the greater performance in plants growing on M1 medium. It can also be observed that plants growing in M4 (low concentration of fertilizer) and M6 (high concentration of fertilizer) were the ones with less growth ability in all the parameter measured. Plants growing on M2 (cassava starch as gelling agent) showed generation ability comparable to plants growing on M1 but with less number of roots and higher dry weight.

In order to validate the efficiency of the new medium (M9), different cassava cultivars were grown on M9 and M1. The average of height and number nodes produced in 60 days for each cultivar can be observed in Figures 3 and 4 respectively. All the cultivars stem cuttings assayed showed regeneration activity and growth on M9 medium. Significant differences between global averages of plant height were observed for both media ($p < 0.05$), due to cultivars IDEA52, IDEA98 and MBRA383. However the interaction media-cultivar was found not significant ($p > 0.05$) which means that the cultivars height was independent from media, so similar height was observed in the same cultivar in both media. 3 groups of cultivars were identified according to their height, the first group represented by IDEA87 with low growth rate, a second group represented by CM6740-7 with high growth rate and the third group of intermediate growth rate represented by the rest of the cultivars assayed. The results of number of nodes, which represent the multiplication rate, ranged 3 - 7 at 60 days in the different cultivars assayed. Statistical analysis showed no significant differences ($p < 0.05$) and similar number of nodes between media. However in this case the differences are due to 75% of the cultivars and no interaction cultivar-media was observed which means that the number of nodes does not depend on the media. The cassava cultivars with lower number of nodes produced was IDEA87 but in general M9 produced an acceptable multiplication rates for all the cultivars assayed (60% of the cultivars with > 4 nodes/60 days).

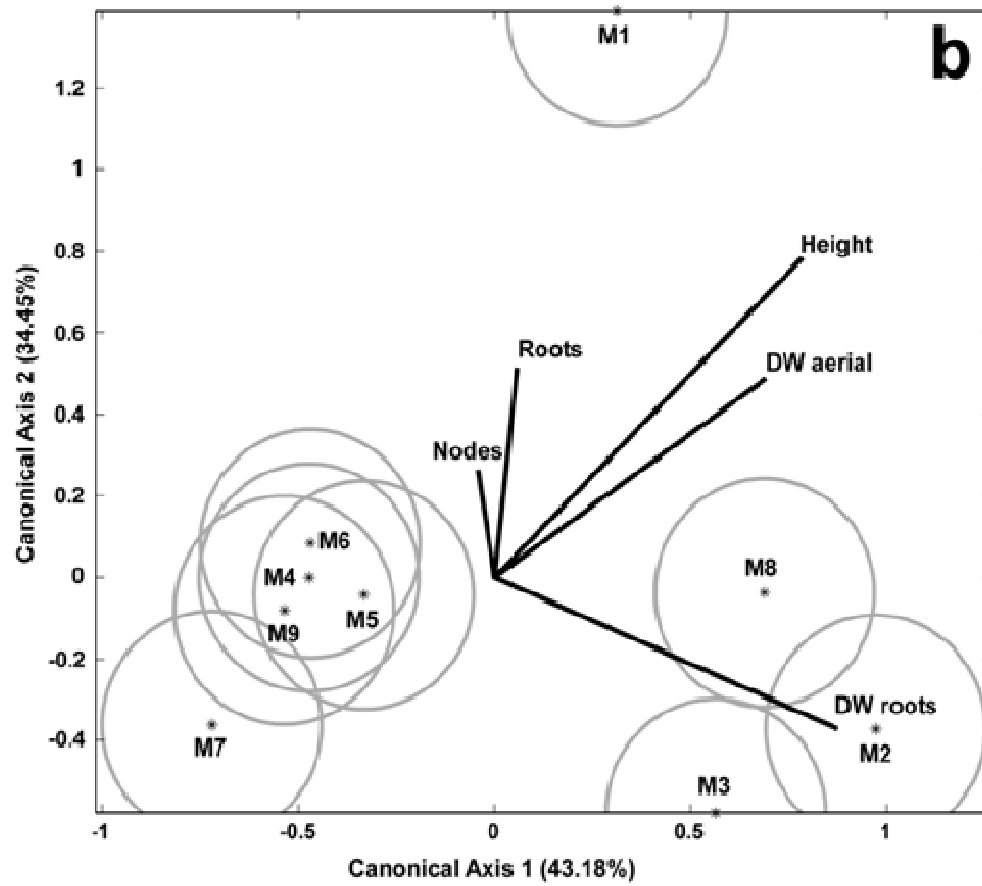
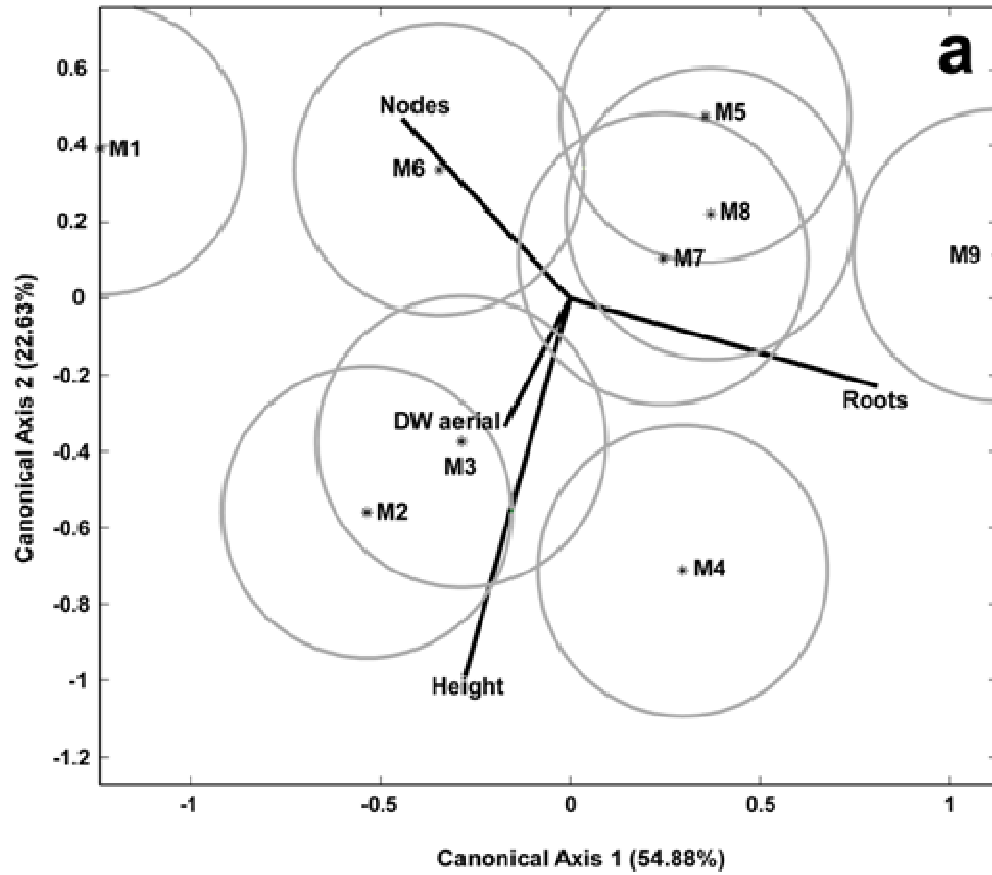
DISCUSSION

Because of its big potential to produce healthy planting material, through out the years, tissue culture-based plant propagation has emerged as one of the most important agro-technologies. However, the lack of information about its existence and the benefits of these technologies and the cost of *in vitro* plant production are obstacles for its access to growers and farmers in developing countries. Thus, efforts to develop low-cost technologies to the people are necessary (Thro et al., 1999).

Plants grown under *in vitro* conditions need especially designed medium containing the nutrients required by the plant to maintain adequate development. A conventional plant culture medium usually contains a basal solution with major and minor mineral elements, a source of carbon (that is, sucrose), vitamins, growth regulators, a gelling agent for semi-solid media and water (Trigiano and Gray, 2000).

Roca (1980, 1984) established the conditions for cassava propagation using conventional tissue-culture techniques. In his work, 2 media were used, medium 4E, used for propagation, which includes MS salts (Murashige and Skoog, 1962), 0.04 mg l^{-1} 6-benzylaminopurine (BA), 0.05 mg l^{-1} GA_3 , 0.02 mg l^{-1} NAA, 1 mg l^{-1} thiamine, 100 mg l^{-1} m-inositol, 2% sucrose and agar, at pH = 5.7-5.8 and medium 17 N, used for rooting, which included MS/3, 25 mg l^{-1} of the fertilizer Plantex R, 0.1 mg l^{-1} NAA, 0.1 mg l^{-1} GA_3 , 1 mg l^{-1} thiamine, 100 mg l^{-1} m-inositol, agar and 2% sucrose. However, a frequent modification of the cassava nodal micropropagation *in vitro* culture media involves altered macronutrient and micronutrient concentration.

In a conventional medium, if agar is used as a gelling agent, it can represent up to 70% of the total cost, followed by the minerals, water, sucrose and other minor media components (Prakash et al., 2004). As a substitute for agar, cheaper sources of gelling agent such as starches and plant gums have been used. In a previous work Romay et al. (2006) used a modified cassava starch as a



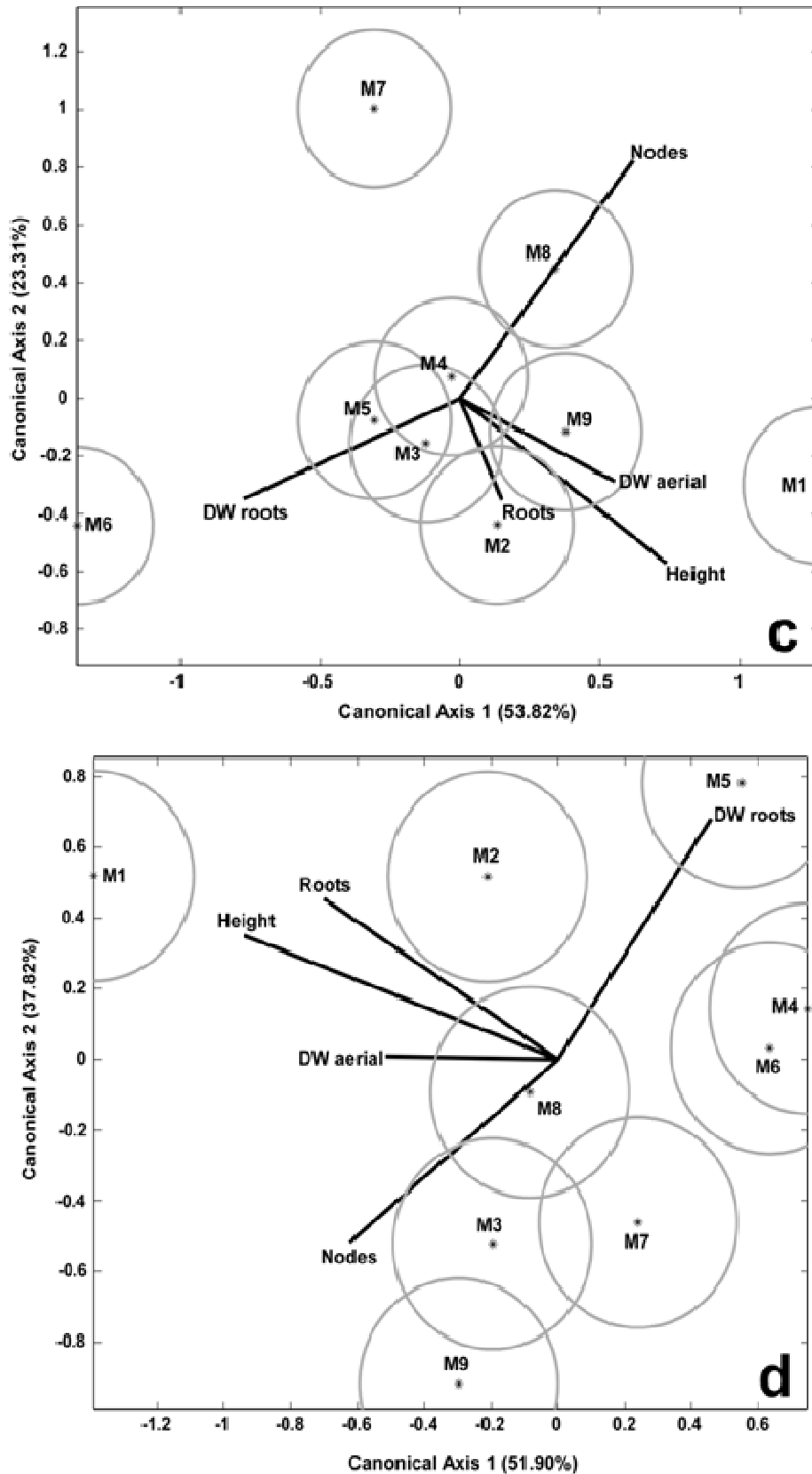
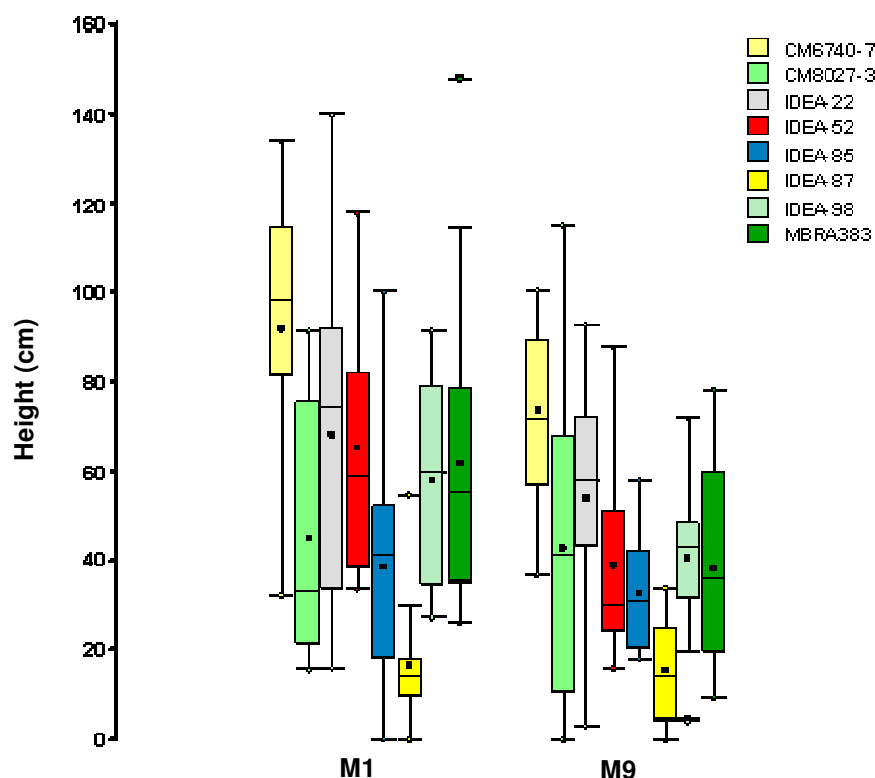


Figure 2. Canonical biplot representation of the first analysis of growth parameters observed for *in vitro* cassava plants: (A) 15 days-old; (B) 30 days-old; (C) 45 days-old and (D) 60 days-old.

Table 3. Cumulative goodness-of-fit of the variables to explain the treatment averages after adjusting the Canonical biplot.

Growth parameter	15 days	30 days	45 days	60 days
Dry Weight (aerial)	26.45	71.75	84.20	60.31
Dry Weight (roots)	nm	80.43	78.86	77.03
Height	81.31	88.62	82.98	88.46
Nodes	49.97	09.06	82.94	78.62
Roots	85.03	34.16	13.98	95.16

nm: Non measurable.

**Figure 3.** Graph showing the results of the height of different cultivars of *in vitro* cassava plants growing under M1 and M9 medium 60 days after sub culturing. Black squares represent the average value (n = 15).**Table 4.** Comparative costs of medium with various substituted components.

Medium	Cost of 1 l of media (US\$)	Decrease (%) in cost compared to M1
Sigma Premix	5.25	NA
M1	2.75	0
M2	1.91	30.5
M3	2.75	0
M4-M5-M6	2.08	24.4
M7-M8	2.60	5.5
M9	0.19	93.1

substitute of gelling agent. The modified cassava starch resulted as well as Phytigel™, showing no differences at the number of nodes and height of cassava *in vitro* plants. Similar results have been shown in this paper (M1 vs. M2) with a calculate cost reduction of 30% (Table 4). In spite of the constrains in the media preparation and detection of contaminants, the low cost and nationwide availability of this modified cassava starch make it ideal for simplifying the cassava *in vitro* propagation media. Additionally, compared to Phytigel™, cassava modified starch did not liquefied during the plant culturing. The use of liquid media (absence of gelling agent) for cassava propagation has also been assayed previously but slight-

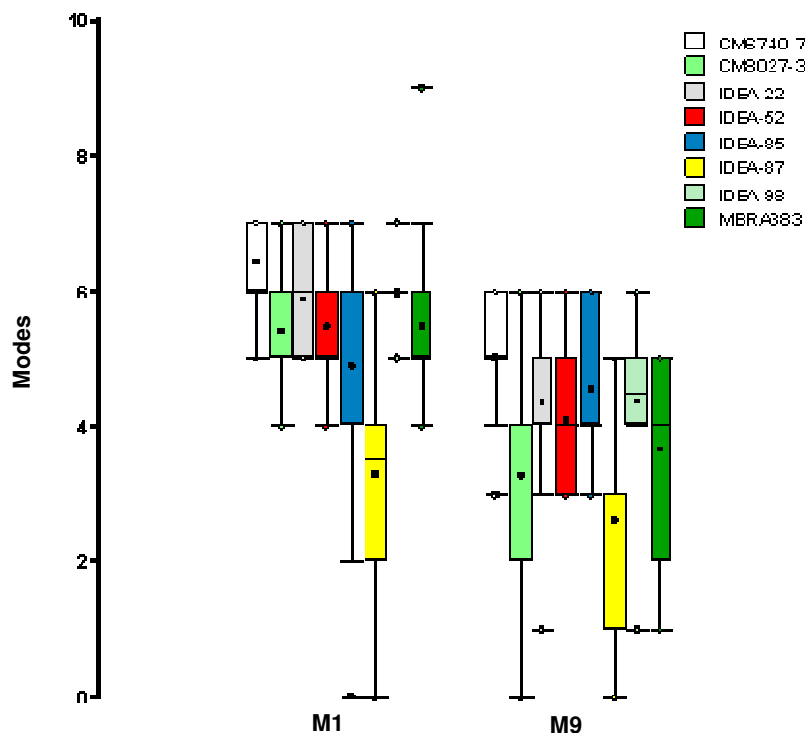


Figure 4. Graph showing the results of number of nodes of different cultivars of *in vitro* cassava plants growing under M1 and M9 medium 60 days after sub culturing. Black squares represent the average value ($n = 15$).

ly vitrified plants have been obtained.

Based on media 4E, devoid of BA to prevent callus formation at the base of the explants, we assayed different strategies to reduce cost and laborious procedures. To replace the basal mineral nutrients solution, we used different concentrations (1, 2 and 2.5 g/l) of a local available fertilizer. Higher concentrations of fertilizer were very toxic to the tissues, showing chlorosis at the beginning and necrosis after several days of exposure (data not shown). However, the results presented here showed that when fertilizer concentration was adjusted, healthy plants with similar growth rates than those observed for MS macro and micro-nutrients were obtained. The intermediate fertilizer concentration of 2 g/l seemed to be optimal for the cassava propagation. Escobar et al. (2005) tried also different kind of fertilizers at different concentrations. However, these authors did not report any fertilizer combination that could match 4E media in maintaining the growth of cassava *in vitro* plants. The calculated media cost reduction using fertilizers was 24.4% (Table 4); but apart from this, it is important to mention that fertilizers with similar composition at the ones used here are produced in Venezuela and available worldwide at low-cost to farmers.

Many laboratories have reported the use of table sugar (sucrose) on *in vitro* plant propagation medium (Ganapathi et al., 1995; Kodym and Zapata-Arias, 2001; Prakash et al., 2004; Kaur et al., 2005; Tyagi et al.,

2007). The results obtained in our work also confirmed that table sugar and molasses are suitable and cheap alternative to pure carbohydrates. Molasses is not only a source of carbohydrates but vitamins as well, especially inositol, niacin, pyridoxine, pantothenic acid, thiamin, riboflavin, biotin, choline and minerals (Curtin, 1973; Baker, 1979; Curtin, 1983, United States Sugar Corporation: <http://www.suga-lik.com/molasses/composition.html>). Our results showed that cassava plants grown on molasses containing media seemed to be stronger, greener and vigorous. However, preliminary assays showed that high molasses concentration could inhibit plant growth. The 2 different molasses concentration used for the experiments presented showed similar growth ability (number of nodes) indicating that either of the concentration can be used.

Plant growth regulators are key media components. The use of agriculture products containing plant growth regulator in plant tissue culture media has been reported previously (Escobar et al., 2005). The concentrations of NAA and GA₃ in the media used by Escobar et al. (2005) and this study were the same (0.02 mg l⁻¹ NAA and 0.05 mg l⁻¹ GA₃) as in modified 4E media. Furthermore, many root promoters commercially available, including Radixone 3AS, contains IAA, IBA and NAA as an active ingredient. The addition of IAA and IBA to modified 4E medium did not affect the numbers of roots produced (M2 vs M3). Results obtained in our work confirmed that

cheap and freely available plant growth regulators are suitable as substitute to very expensive and pure plant hormones.

Water is one of the major medium components. Distilled water obtained by electrical distillation is expensive and required a sophisticated distillation apparatus. However, rain water or tap water can be used as a substitute as it was shown previously by Ganapathi et al. (1995), Sharma and Singh (1995) and Kaur et al. (2005) in banana, ginger and strawberry respectively. Good growth was also obtained for cassava growing on M9 indicating that there is not need to include sophisticated equipment to field laboratories in order to produce water suitable for the cassava seed production. However, previous to the use of any source of water it is advisable to order a water analysis by a commercial laboratory just to make sure that the source of water is appropriate for its use on tissue culture media.

An ideally medium must allows to maintain a high number of cultivars of a specie with a homogenous growth rate. However, due to the high diversity of cassava varieties, small differences in the performance of them are expected. After analyzing the different cultivars growing on M1 and M9, all cultivars showed a good adaptation to the M9 media. Differences in plant height and number of nodes were observed between cultivars due to the genotype and no due to the media. The multiplication rate obtained (3-7/2 months cycle), it is acceptable for all the varieties assayed. The production of cassava seed includes cycles of 2 months and a good *in vitro* multiplication rate for the production of seed is considered as 1:4 in a cycle. A small adjustment of the media may be required in order to increase the multiplication rate of some promissory varieties or long term maintenance of germoplasm bank with homogenous growth rates.

In short, the fully substituted designed media presented in this work is simple, easy to prepare and allows a low-cost strategy to obtain a high quality *in vitro* micropropagated cassava plants with multiplication rates similar to traditional media. The results will have a high impact on the production and availability of cassava seed once introduced to cassava seed producers.

Finally, it is important to mention that the canonical biplot analysis (CBA) represents an improvement over traditional methods for discrimination among groups using multiple observed variables since it is allowing the study of treatment-variable relations more appropriately and facilitates the interpretation of the results.

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