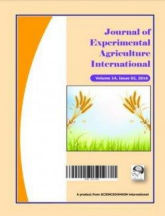

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Micropropagation of Cassava (*Manihot esculenta*) Using Locally Sourced Substitutes in a Routine Medium

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

In spite of global acceptance and wide use of micropropagation as a method for the production of disease free planting material and germplasm conservation, this practice has been slow and non-affordable in Sub-Saharan Africa. This is due to the high cost and non-availability of tissue culture media. Considering the importance of growth factors (micro and macro nutrients) in culture medium, it is inevitable to search for an alternative, cheaper and readily available source of these nutrients. This research therefore provided a natural substitute media formation for Cassava nodal culture. Sugar cane juice was substituted for sucrose (SC) in this research work. The result showed that the explants survived and produced foliage at 20 ml SC and 40 ml SC based media. The forest Top Soil (FTS) modified media produced more foliage (7), at 20 ml/200 ml than conventional media (5). Trona is a soft and porous salty evaporate deposit occurring in association with Neutron, Halite, Thernadite and other salts. Trona is a mixture of Chlorides, Carbonates, and Sulphate salts of Sodium, Calcium, Potassium, and Magnessium thus serving as a good source for these salts. 0.2 g of Trona gave the highest percentage 66% of nodal cutting that developed foliage. In conclusion, there was a positive response observed in the growth of the cassava nodes in the media modified

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with various natural nutrient sources. The use of these natural sources is encouraged because it is less costly and readily available rather than wait for the importation of the costly synthetic culture media.

Keywords: Tissue culture; cassava; micropropagation; nutrients; media; explant.

1. INTRODUCTION

Cassava is a perennial lowland woody shrub with edible roots, which grows in the tropical and subtropical areas of the World. All cultivated forms belong to the species *Manihot esculenta* Crantz; and family Euphorbiacea. It is also called Manioc, Mandioca, Tapioca, Yuca and Sagu in different countries. Cassava has the ability to grow on marginal lands where cereals and other crops do not grow well, it can tolerate drought and can grow in low nutrient soils. The plant grows very tall, at times reaching a height of about 15 feet, with leaves varying in shapes and size. The edible parts are the tuberous roots and the leaves. The tuber is dark brown in colour and grows up to 2 feet long or more depending on the cultivar and the soil conditions [1].

According to the Food and Agricultural Organization (FAO) estimates, about 172 million tonnes of cassava was produced in year 2000 [2]. Africa accounted for 54%, Asia 28% and Latin America and the Caribbean for 18% of the total World production. In 1999 Nigeria produced 33 million tonnes making it the World largest producer.

In Africa, cassava provides a basic daily source of dietary energy. Roots are processed into a wide variety of granules, pastes and flours or consumed freshly boiled or raw. In some of the cassava growing countries in Africa, the leaves are also consumed as a green vegetable, which provides protein and vitamins A and B. In South East Asia and Latin America, cassava is used as a binding agent in the production of paper and textiles, in North America and Europe, cassava is consumed as Tapioca prepared from cassava IITA [3]. Although, cassava is adapted to a wide range of climatic conditions and is tolerant to poor acid soils and drought, several research constraints have been identified in the areas of production processing, and utilization [4]. Pests and diseases, together with poor cultural practices, combine to cause yield losses that may be as high as 50% in Africa [5].

Micropropagation techniques have been developed to provide solutions to some of the cassava production constraints. Micropropagation through tissue culture techniques have been used for disease elimination, pest resistance, germplasm exchange, distribution and germplasm conservation [6]. The medium for micropropagation must contain all components necessary to nourish growth of explants. Though plants do not have the same nutritional requirements, the components of any tissue culture medium must contain the following growth factors: Macro and micro nutrients, carbon source (organics), vitamins, growth regulators, complex organics and inert supports (Gelling agents) [7]. Although micropropagation technique has been developed for cassava, the nutrient medium has utilized both the synthetic and industrially produced components which are beyond the reach and utilization of major stakeholders who are ready to carry out the multiplication of Cassava.

This research work is therefore designed to provide a method of micropropagation which provides all the necessary growth factors from natural sources.

2. MATERIALS AND METHODS

2.1 Extraction and Preparations of Local Materials

(i) **Cane sugar juice extraction:** Fresh sugar cane sticks were purchased from local markets. The stems were cleansed by scrapping. The bark was removed and the cane was shredded with a grater, the shredded cane was then squeezed to release the juice.

(ii) **Preparation of Trona powder:** Impure form of Trona was procured from the local market and ground to powder with pestle and mortar.

(iii) **Forest top soil preparation:** The soil was collected from a forest plot, soaked in excess water and allowed to settle for about 12 hours. The water was decanted into a bottle for use.

(iv) **Lichen and Moss ash preparation:** The Lichens and Mosses were collected from

old Citrus trees (about 10 years and above) by scrapping bark of the trees with a Scapel. The majority of the collection was Lichens. These Lichens were the Crustose type and only a few were foliose Lichens. Mosses collected were of various kinds.

The crypto samples were then placed into three crucibles which were put into an oven. The oven was allowed to operate at a temperature of 600^{o}C for 7 hours. The ash obtained after this procedure (crypto ash) was allowed to cool and stored.

2.2 Media Preparation

Specific aliquots i.e. 20 ml, 40 ml, 60 ml and 80 ml of the sugar cane juice were used to substitute sucrose in the standard Murashige & Skoog (MS) basal medium (Table 1).

0.1 g, 0.2 g, 0.3 g and 0.4 g of powdered Trona were weighed and introduced directly into the medium without MS basal medium.

10 ml, 20 ml, 30 ml and 40 ml of the forest Top soil (FTS) was used as medium to substitute MS basal medium (Table 1). 1.3 g of stored Lichen and Moss ash was weighed and infused into the medium preparation.

2.3 pH Adjustment

The pH of all prepared media was adjusted to 5.7 using 1M NaOH. 0.8 g of agar was added to each medium and made up to 200 ml.

All the media were heated in a microwave to melt the agar. With the aid of an automatic dispenser, the preparations were poured into test tubes and were placed in an autoclave at 121^{o}C at 15psi for 15 minutes, left on the shelf for about 8 hours to cool.

2.4 Culturing of Explants

After taking the necessary precautionary measures of disinfecting the work bench, healthy plants about 4 weeks old were collected. The plantlets were removed from test tubes, the nodes were excised and placed on the medium. The test tube was recapped and sealed with a piece of Parafilm.

The test tubes were placed on the shelf in the culture room under fluorescent light at 27°C room temperature and exposed to 12 hours of light and 12 hours of darkness.

3. RESULTS

The result showed that the media modified with sugar cane juice had the percentage growth of green leaves as high as 87% on 20 ml/200 ml while the lowest percentage was 50% on 60 ml/200 ml which favorably compared with the conventional MS media at 88%.

0.29 g of Trona gave the highest percentage of nodal cuttings that developed green leaves and roots (10%) while 86% and 14% produced only green leaves and roots respectively.

On the average, the percentage foliage production was higher in the media modified with 20 ml/200 ml FTS which was even higher than the foliage production on the conventional media.

Table 1. Routine cassava tissue culture medium

Component	Quantity in 1 litre
MS Basal Medium	4.43 g
Inositol	100 mg
Sugar	30 g
NAA	0.01 mg
BAP	0.05 mg
Agar	4 g

MS – Murashige and Skoog, NAA-Naphthalyacetic acid, BAP – Benzyl Amino Purine

4. DISCUSSION

The cassava explants were observed for 28 days to monitor their survival, green leaves formation and roots formation on each of the modified media.

Generally, all the media prepared from locally sourced materials were effective in sustaining the growth and survival of the cassava explants.

The sugar cane replaced sucrose as a source of energy required for the heterotrophic nutrition of the explants.

It is interesting to note that the percentage of plants that survived or produced foliage especially in the 20 ml SC - based medium can be equated to that of the control. It showed also that the 20 ml and 40 ml SC based media were the best concentration of SC required for the sustainable growth of Cassava.

Table 2. Response of cassava nodes to media modified with SC after 4 weeks

SC Vol. In 200 ml	No growth %	Green leaf with root %	Green leaf (No Root) %	Roots no leaf
20 ml	8± 1.2	13± 3.4	57± 7.5	22± 4.5
40 ml	10± 2.1	5± 2.0	55± 7.1	30± 5.2
60 ml	26± 3.0	0	50± 6.4	24± 4.1
80 ml	11± 2.4	0	72± 8.3	28± 5.5
Control	9± 2.5	33± 6.4	30± 3.0	28± 4.3

SC – Sugar cane juice; Control – Routine MS (2017) Tissue culture medium

Table 3. Response of cassava nodes to media modified with Trona after 4 weeks

Vol. In 200 ml	No growth %	Green Leaf with Roots %	Green leaf %	Root %
0.1 g Trona	34± 3.5	7± 2.1	49± 5.4	10± 2.0
0.2 g Trona	10± 2.3	10± 2.2	66± 6.3	14± 2.4
0.3 g Trona	27± 4.5	4± 1.1	53± 5.2	16± 3.1
0.4 g Trona	14± 2.2	22± 4.6	50± 6.4	14± 2.6
Control	7± 1.7	33± 5.7	50± 5.5	10± 2.2

Control: Routine MS Tissue culture medium

Table 4. Response of Cassava Nodes to media modified with FTS Preparation after 4 weeks

Media (vol. In 200 ml)	No growth %	Green leaf with roots	Green leaf no root	Root no leaves
10 ml FTS	10± 2.4	14± 3.2	66± 4.5	10± 2.4
20 ml FTS	9± 2.4	22± 4.5	47± 3.3	22± 4.6
30 ml FTS	12± 3.1	31± 4.7	37± 4.1	20± 4.2
40 ml FTS	10± 3.2	28± 3.5	40± 4.7	22± 5.5
Control	10± 2.5	42± 5.6	30± 3.5	18± 3.4

Control: Routine MS Tissue culture medium. FTS: Forest Top Soil

Table 5. Response of cassava nodes to media modified with Lichens and Moses after 4 weeks

Media (Vol. In 200 ml)	No growth %	Green leaf with root	Green leaf no root	Roots no leaf
1.3 g Lichen and Moss ash	8± 1.3	10± 2.3	64± 6.7	18± 3.7
Control	10± 2.1	42± 5.7	50± 5.6	8± 2.0

Control: Routine MS Tissue Culture Medium

Fertile top soil (FTS), Trona, Lichen and Moss were used in this study to substitute the MS basal medium containing industrially produced salts. This study has shown that it is possible to use natural and locally available salts in place of the industrially produced salts. This research work agrees with the research works of Santana et al. [8] and Kwarne et al. [9] who used different concentrations of locally available fertilizer to micropropagate Cassava.

Different kinds of fertilizers at different concentrations were also used by Escobar et al. [10] to realize a 24.4% cost reduction for the medium prepared. Trona has been established to be a good source of inorganics for many tropical plants in Africa [11].

It was observed that most of the explants regenerated roots without addition of Auxins, this is in agreement with Yona et al. [12], who reported that cassava explants can naturally develop roots without the addition of Auxins. Alfred and Uchenna [13] also used locally available materials for substrate hardening in the micropropagation of Sweet Potato.

5. CONCLUSION

This research work is an indication that it is possible to formulate nutrient media for sustaining Cassava growth from cheaper, local and safer materials to promote micropropagation of Cassava germplasm through tissue culture.

Future prospects should be to increase input in the development of a natural nutrient medium so as to make micropropagation of not only Cassava but other crops more affordable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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