Original Research Article

Response of Some Cameroonian Cocoyam *Xanthosoma sagittifolium* (L.) Schott. Cultivars/Landraces to Tissue Culture Techniques

ABSTRACT

Aims: This study was aimed at exploring tissue culture technique as a tool for mass propagation of some Cameroonian cocoyam (*Xanthosoma sagittifolium*) cultivars/landraces (red, yellow and white skin colour).
Study design: The experiment was laid out in a completely randomized design with three treatments in four replications.
Place and Duration of Study: The study was conducted in the tissue culture laboratory of the Institute of Agricultural Research for Development, Bambui, Cameroon, in the first half of 2018.
Methodology: Explants were gotten from three Cameroonian cocoyam landraces (red, white and yellow skin colour). Shoot tips were excised and cultured on Murashige and Skoog (MS) medium supplemented with 30g of sucrose, 5ml of ascorbic acid, 4ml of 6-benzylaminopurine (BAP 1mg/l), 1ml indole-3- acetic acid (IAA 1mg/l) and 6g of agar at pH of 5.8±0.1 for shoot initiation and proliferation. Data was collected after 4 weeks (number dead, number rooted, number of roots, number of buds) and 12 weeks (number of leaves, shoot length, number contaminated) of initiation.
Results: All the landraces responded positively to the growth media since none died. The number of explant rooted did not vary significantly ($p > .05$). the highest number of roots and buds were from the white cultivar, followed by the red cultivar. Analysis of variance revealed significant differences ($p = .05$) in most of the parameters measured except for number rooted. High numbers of leaves and shoot length were recorded from the red cultivar, followed by the yellow and white cultivars. However, the white cultivar (4.2) was more susceptible to pathogen than the yellow (3.5) and red (2.67) cultivar ($F = 19.13$, $df = 2, 8$, $p < .001$)
Conclusion: It is recommended that the three cocoyam cultivars be followed from growth media to the field and evaluate their growth and yield parameters. The implications of this finding vis-à-vis rapid and mass propagation of Cameroonian cocoyam cultivars is explained.

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Keywords: cocoyam, cultivar, explant, tissue culture, IRAD Bambui, Xanthosoma sagittifolium

1. INTRODUCTION

Cocoyam *Xanthosoma sagittifolium* (L.) Schott belongs to the family Araceae, grown in tropical and subtropical regions in moist and shady habitats [1]. It is believed that cocoyam originated from tropical America and was introduced in tropical Africa in 1840 [2]. Cocoyam

is grown mostly for its edible corm, cormels and leaves [3]. The corms, cormels and leaves are important sources of carbohydrate and other minerals for human and animals. Cocoyam has high carbohydrate content (70 – 80%) in the form of digestible starch [4]. According to [5], this high digestible starch content provides energy and increases satiety in consumers. Cocoyam also contains a reasonable amount of good quality protein, vitamin C, thiamine, riboflavin, niacin and some essential amino acids [6][7]. In West Africa, cocoyam is an important root crop [8]. In Cameroon in particular, cocoyam, which is the third most cultivated food after cassava and plantain [9] plays both a nutritional and a cultural role, with a specialized method of preparation from different parts of country with very strong traditional and historical links. The leaves, corm and cormels are consume in different forms as highlighted in Table1. With over 10 million tons of cocoyam produced in 2012, cocoyam has the potential to alleviate poverty and hunger, and can greatly contribute to food security [9]. Cocoyam like many other aroids is neglected and underutilized, receiving very little research attention [10]. Research conducted so far has been focusing on food qualities: chemical, pasting and functional properties of starches and flour by traditional methods [11-15]. Despite the health and economic benefits of cocoyam, production has been or remained stagnant for many years [16] as a result of low productivity and unavailability of planting material [17], fungal and viral disease infection [18] and high susceptibility to physical damage during harvesting leading to high postharvest losses. Hence, methods that are more sensitive are needed to improve production of cocoyam by making planting material affordable and readily available. In Cameroon [19] and [20] identified Cameroon cocoyam as belonging to the species *X. sagittifolium* and categorized them into white, red and yellow cultivars depending on the colour of the tuber skin. Mass. This study was conducted to determine the response of three common indigenous Cameroonian cocoyam cultivars on growth media as first step for micropropagation of cocoyam with the ultimate goal of mass propagation of high quality planting material for the Cameroonian cocoyam industry. Njualem et al. [21] has reviewed the importance of tissue culture techniques and micropropagation in commercial agriculture.

Comment [O.P2]: This objectives isnot specific Are you determining growth response on growth media or carrying cocyam micropropagation or both. The conclusion did not state otherwise.

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Table 1. Some Cameroonian delicacies from cocoyam

Common name	Photo	Description
Porridge cocoyam		Peeled cocoyams, boiled with some vegetable (bitter leaf), dry fish and other traditional condiments. Best eaten with the hands.
Achu		Boiled cocoyam, peeled and pounded in a mortar, then moulded in a circular fashion. Soup made of palm oil and some fish/meat is added. Best eaten with the hand.

Cocoyam fufu		Peeled and boiled cocoyam is pounded into a soft malleable elongated/oval/spherical shape fufu. It is eaten with soup or vegetable. Best eaten with the hands.
Ekwang		Grated cocoyam is folded in cocoyam leaves. Then cooked with palm oil, dried fish and other condiments. Best eaten with the hands.
Kwacoco and banga soup		Grated cocoyam is folded in plantain leaves and boil. It is eaten with palm oil (banga) soup. Best eaten with the hands.
kwacoco		Grated cocoyam, mixed with palm oil with dried fish and other condiments as additions, then folded in plantain leaves and boiled. Sometimes eaten with tomato sauce.
Cocoyam and vegetable		Peeled and boiled cocoyam. Eaten with vegetable. Best eaten with the hands.
Cocoyam and palm oil		Peeled and boiled cocoyam. Eaten with palm oil.
Roasted cocoyam		Roasted cocoyam. Eaten with roasted plum. Very common on roadsides.

2. EXPERIMENTAL DETAILS

2.1. Study location and source of planting material

The study was carried out at the tissue culture laboratory of Bambui Regional Center of the Institute of Agricultural Research for Development (IRAD-BRC), located at the Western highlands in the NorthWest Region. The Bambui Regional Center is located at latitudes 630^0 N, longitude 10 15^0-E on an altitude of 1600 m above sea level. The study was carried out from March 14th to August 14th 2018. Three cocoyam cultivars (red, white and yellow skin colour) were used for the study (Figure 1). The red and white skin were collected from Nkongsamba (Littoral Region) and the yellow skin from Bambili (North West Region). More than 80.0% of the inhabitants are farmers. Here, plantain, cocoyam, rice, maize, and beans play a huge role in their every diet and energy sources.

Figure 1. Some cocoyam cultivars/landraces used in the study; (A) red skin cocoyam, (B) white skin cocoyam, and (C) white skin cocoyam.

2.2. Sterilization of equipment and materials

Prior to medium and stocks preparation, all equipment including distilled water, baby food jars and the materials used for culture initiation such as; forceps, cotton, A_4 papers, and scalpels were sterilized in a pressure pot, at a temperature of 121^{0}C and at a pressure of 103.4Kpa for 20minutes.

2.3. Stock and medium preparation

The stocks were prepared according to the international potato center´s (CIP) protocol [22] with slight modifications [21]. The culture medium was prepared according to [23] and supplemented with 1mg/l 6- benzylaminopurine (BAP),1mg/l indole-3-acetic acid (IAA), 0.1g/l myo-inositol, 30g/l sugar and 5ml ascorbic acid stock. The procedure used is outline in [21].

2.4. Preparation and sterilization of explant

The explants were prepared from plants harvested directly from the farm. The roots were cleaned, cormels and leaf sheaths reduced and cut off until only the shoot tip of the plants was left. Shoot tips of each cultivar were submerged in a well labeled beaker filled with tap water to prevent dehydration of the explants. The explants were washed thoroughly under running tap water using a brush to remove all soil and debris. Two solutions were used to sterilize the explants inside the laminar flow hood: (1) 96% alcohol for 2minutes followed by (2) 30% sodium hypochlorite with a few drops of tween 80 for 15mintues which was prepared by putting 300ml of sodium hypochlorite (la Croix) in a 1000ml measuring cylinder then sterile distilled water was added to make it up to 1000ml and 20 drops of tween 80 added. After sterilization of the explants, they were thoroughly rinsed 3-4 times with sterile distilled water.

2.5. Shoot tip excision

This was done under a laminar flow hood previously swapped with 70% alcohol. Using a blade mounted on a blade holder and forceps, the shoot tip of 10 x 2 x 6mm with 3-4 leaf primordial was obtained. The shoot tips were placed on the medium in an upright position. The jars were labelled and incubated in the growth room at a temperature of 25 ^{o}C ± 2 with a photoperiod of 16 hrs/day and allowed to grow for 90 days during which data was collected. The cultures were transferred to a fresh medium four weeks after initiation.

2.6. Data collection and analyses

Data was collected on (1) number of dead explant, (2) number of rooted explant four weeks after initiation, (3) number of roots four weeks after initiation, (4) number of leaves of each explant four weeks after initiation, (5) shoot length using a graduated ruler at the end of the study, and (6) number of shoots contaminated by visual inspection. The experiment was laid in a completely randomized designed (CRD) with three replicates. The data was analyzed using, the Statistical Package for Social Science (SPSS) (vers. 23.0) One-way analysis of variance (ANOVA) was used to compare if there were significantly different means. Means significantly different were separated using the Duncan's Multiple Range Test (DMRT) at probability level 0.05. Excel 2016 was used to plot the graphs.

3. RESULTS

3.1. Number of dead explant, number rooted, number of roots and number of buds on explants of three cocoyam cultivars/landraces on growth media

The number of dead explant was zero for all cocoyam cultivars. Consequently, no statistics was conducted (Table 2). The number of rooted explant did not vary significantly ($F = 0.530$, $df = 2, 8$, $p = .593$). The highest number of rooted plant was 1.083 from the yellow cocoyam cultivar (Table 2). The number of roots per explant was significantly different ($F = 3.252$, $df = 2, 8$, $p = .046$). white cocoyam produced the highest number of roots (7.3), followed by yellow cocoyam (4.0) and red cocoyam (3.3) (Table 2). The number of buds was also significantly different ($F = 1.981$, $df = 2, 8$, $p = .05$). The number of buds was highest (4.4) for white cocoyams, followed by red cocoyam (3.86) and yellow cocoyam (1.75).

Table 2. Mean (± s.e) Number of dead explant, number rooted, number of roots and number of buds on explants of three cocoyam cultivars/landraces on growth media

Cocoyam cultivar/landrace	Number dead	Number rooted	Number of roots	Number of buds
White cocoyam	0.0	0.80 ± 0.13 a	7.30 ± 1.54 a	4.40 ± 1.34 a
Red cocoyam	0.0	0.867 ± 0.16 a	3.267 ± 0.81 b	3.867 ± 0.97 a
Yellow cocoyam	0.0	1.083 ± 0.26 a	4.0 ± 1.21 ab	1.75 ± 0.52 b
F	-	0.530	3.252	1.981
P	-	.593	.05	.05

Means with the same letter(s) in the same column are not statistically different (DMRT, $p < .05$)

3.2. Numbers of leaves

The number of leaves on the explant after 12 weeks of culturing is shown in figure 2. The mean number of leaves varied with cocoyam cultivar/landrace ($F = 1.505$, $df = 2, 8$, $p = .023$). The number of leaves ranged from 2.7 (white cocoyam) to 3.75 (yellow cocoyam) and 4.4 for red cocoyam.

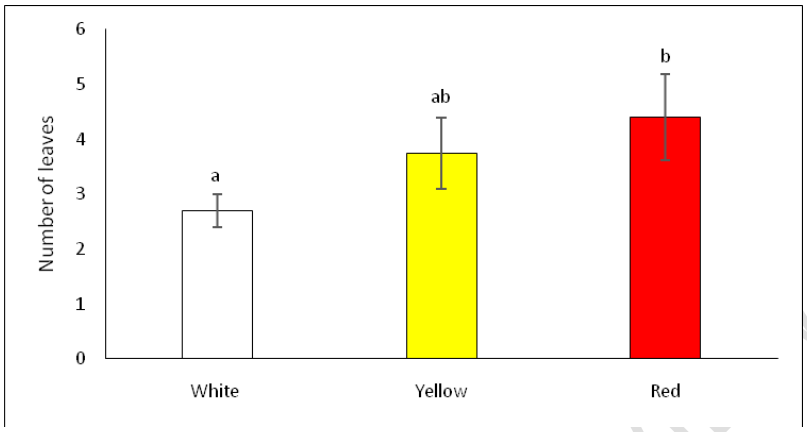

Figure 2. Number of leaves on some Cameroonian cocoyam cultivars/landraces on growth media.
Mean bars with the same letter(s) are not significantly different (DMRT, p < .05)

3.3. Shoot length (cm)

The short length of some Cameroonian cocoyam cultivars/landraces after 12 weeks of culturing is presented in figure 3. The analysis revealed that shoot length varied with cultivar/landrace (F = 3.845, df = 2, 8, *p* = .031). Red cocoyam cultivar/landrace produced the longest shoot length (7.1 cm). The shoot length for the white and yellow cocoyam cultivar/landraces were 4.68 and 5.25, respectively.

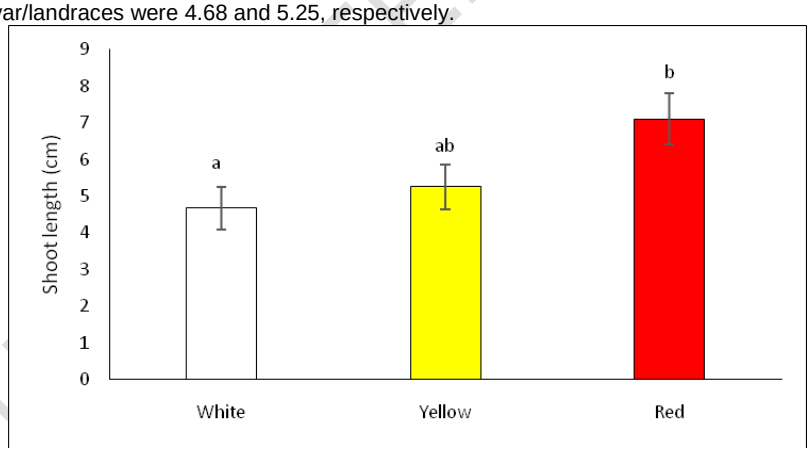

Figure 3. Mean number of shoot length of some Cameroonian cocoyam cultivars/landraces on growth media.
Mean bars with the same letter(s) are not significantly different (DMRT, p < .05)

3.4. Number of shoots contaminated

The number of shoots contaminated after 12 weeks of culturing varied with cocoyam cultivar (figure 4). The variation in the mean number of shoots contaminated ranged from 4.2 in white landrace to 2.67 in red landrace. The ANOVA indicated significant differences (F = 19.13, df = 2, 8, *p* < .001) in the number of shoots contaminated per cocoyam landrace.

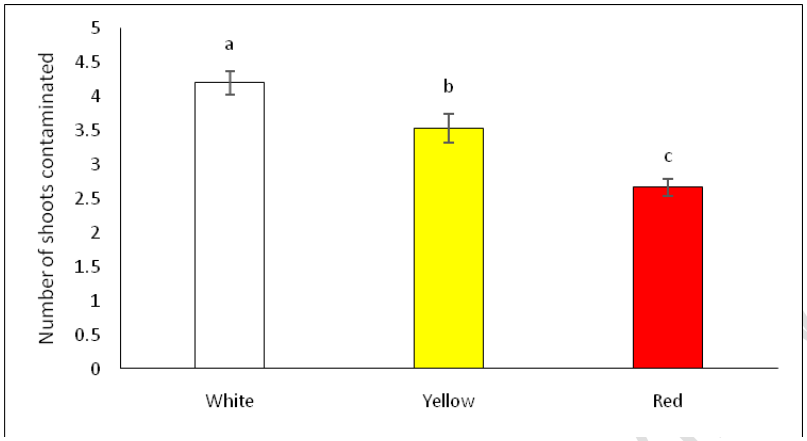

Figure 4. Mean number of contaminated shoots of some Cameroonian cocoyam cultivars/landraces on growth media.
Mean bars with the same letter(s) are not significantly different (DMRT, $p < .05$)

4. DISCUSSION

The role of cocoyam as a source of carbohydrate and some vital minerals cannot be over emphasized [24] especially in Cameroon and Nigeria were it serves as a major staple [9][25] Mass production of these cocoyam cultivars/landraces to meet domestic and international markets require novel techniques. One such technique is tissue culture. Tissue culture is an easy way to rapid and mass propagate plantlets of tuberous crops and a simple method of preserving the valuable germplasm [21]. However, the use of growth media is delicate and expensive compared to other conventional methods [26]. In the current study, no explant died on the growth media. This implies that mass propagation of Cameroonian cocoyam landraces is almost guaranteed on a growth media. This has huge cost implications. All cultivars performed the same as the parameter number of rooted explant is concerned. However, the number of roots from white cocoyam cultivar was very high. In fact, the number of roots on white cocoyam cultivar is almost twice that of yellow cocoyam cultivar. This particular trend was observed on the parameter number of buds. This finding suggest that not only are these cultivars different morphologically [19][20] but are different in their response to growth media. Comparing the three landraces, white cocoyam cultivar can be considered to have a high regenerative capacity which can be attributed to genotypes of the cultivars. Hence the rate of bud proliferation is cultivar dependent [27]. Mbouobda et al. [16] suggest that red and white cultivars are closer to each other than to the yellow cultivar as evident in the current study.
The number of leaves was significantly different with red cocoyam cultivar having the highest number of leaves. Since the leaves are the photosynthetic organs of the plant, it can be inferred that red cocoyam cultivar will produce larger corms than the others. For the number of leaves, red cocoyam and yellow cocoyam cultivars had similar performances compared to the white cultivar. This finding suggest that red and white cultivars are not always closer to each other more than to the yellow cocoyam cultivar as purported by [16]. Shoot length (cm) parameter followed a similar trend as the number of leaves. The shoot length for the red cocoyam cultivar was highest and closer to that of the yellow cultivar compared to the white cultivar. This discrepancy can be explained based on the different genotypes [28]. In

addition, [29] argues that such discrepancies can also be explained by concentration and combination of cytokinin and auxin in the culture media.
The number of shoots contaminated was significantly different for the different cultivars. The findings suggest that white cocoyam cultivar was most susceptible to pathogen. This high degree of susceptibility of the white cocoyam cultivar to pathogen could explain why the white cocoyam cultivar had the lowest shoot length and number of leaves. A diseased plant cannot function very well as relatively healthier plants [30]. Interestingly, the number of roots and the number of buds (which were measure four weeks after initiation) were higher in the white cultivar than in the red and yellow cocoyam cultivar. It is clear from the current study that the white cocoyam cultivar does better on growth media for early measured parameters but eventually get diseased (because of high susceptibility to pathogens) and performs poorly for late measure parameters (number of leaves, length of shoots, number contaminated).

5. CONCLUSION

This study concludes that the different cultivars/landraces of Cameroonian cocoyam respond differently to growth media. It would be fascinating to follow these cultivars from growth media to the field and evaluate growth and yield parameters in order to improve and increase a holistic understanding of the role of tissue culture in the Cameroonian cocoyam food chain.

Comment [O.P4]: The conclusion drawn here isa general statement. The author should make vivid or vital conclusion which other scientst can rely on. He should give specific objectives achieved during this study.

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