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Application of *in-vitro* micropropagation technique for sustainable production of four local taro cultivars [*Colocasia esculenta* (L.) Schott] in Cameroon

Manju B. Evelyn[1], Mbong A. Grace[1,2], Fokunang N. Charles[2,3]*, Tembe-Fokunang A. Estella[3] and Rachid Hanna[4]

[1]Laboratory of Applied Botany, Faculty of Science, University of Dschang, Box 67 Dschang, Cameroon.
[2]Department of Biological Sciences, Faculty of Sciences, University of Bamenda, Cameroon.
[3]Department of Pharmacotoxicology and Pharmacokinetics, the University of Yaoundé 1, Cameroon.
[4]Department of Plant Health, International Institute of Tropical Agricultural (IITA), Cameroon.

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Taro leaf blight disease has recently been reported in Cameroon to cause between 50 and 100% yields loss of taro in most of the agro-ecological crop growing regions. This has led to a significant reduction in disease-free planting materials, edible crop and increased. The Meristem culture technique has been used to produce crop plants free of viruses and fungi especially in vegetative propagated colocassia plants. This aimed at applying *in-vitro* micro-propagation technique for sustainable production of four local taro cultivars in Cameroon. This study was conducted at the Root and Tuber Tissue Culture Laboratory, of the Institute of Agricultural Research for Development (IRAD), Bambui from April 2015 to November 2016. Micro-plants from four local taro cultivars were produced *in vitro* from apical meristem tips. The tip meristems were excised from corms of the four local taro cultivars. The excised explants were surface sterilized with alcohol and sodium hypochlorite in sequence steps at different concentrations. Meristems were cultured at establishment stage on Murashige and Skoog (MS) medium with 30 g of sugar, 1.1 ml of 6-benzylaminopurine and 7 g of agar. Shoots proliferation was induced in MS with 2.2 ml of 6-benzylaminopurine (BAP). Result shows a significant difference at p≤0.5 in number of shoots, petiole length, open leaf and corm diameter among the cultivars and no significant variation in mean number of senescence leaf with respect to all the cultivars, at 60 days of shoot tip culture. At rooting stage, taro shoots were cultured on MS media supplemented with 10 ml of 0.1 mg/ml naphthalene acetic-acid (NAA). Roots were produced on all the cultivars with excellent mean growth rate of 14.7 ± 0.69 recorded in cultivar with dark green petiole and small leaves.

Key words: Taro cultivars, *in-vitro* micro-propagation, benzylaminopurine, naphthalene acetic-acid, Murashige and Skoog.

INTRODUCTION

Taro [*Colocasia esculenta* (L.) Schott] is a major staple food and remains an important crop to many cultural and agricultural traditions worldwide (Ooka and Brennan, 2000). It is consumed as a staple crop in West Africa, particularly in Ghana, Nigeria and Cameroon (Joshua, 2010). All parts of the plant including corm, cormels,

rhizome, stalk, leaves and flowers are edible and contain abundant starch (Bose et al., 2003). Its leaves contain higher levels of protein and are also excellent source of carotene, potassium, calcium, phosphorous, iron, riboflavin, thiamine, niacin, vitamin A, vitamin C and dietary fibres (Bradbury and Holloway, 1998). It is the fourteenth most consumed vegetable worldwide (Rao et al., 2010). Besides its nutritional value, taro is used as a medicinal plant and provides bioactive compounds used as an anti- cancer drugs (Kundu et al., 2012).

Worldwide, the top producers of taro are Nigeria, China, Ghana and Cameroon (FAOSTAT, 2013). The world production of taro corms was estimated at 12 million tons, with Africa producing 7.1 million tons. Out of this total production in Africa, Cameroon produces 1.5 million tons as compared to Nigeria whose total production was estimated at 3.2 million tons (FAO, 2012).

In Cameroon, taro is mostly propagated using vegetative material, grower's planting material, consisting of; (1) side corm, produced as a result of lateral proliferation of the main plant in the previous crop; (2) small corms resulting from the main plant in the previous crop; (3) Huli, the apical part 1 to 2 cm of the corm with the basal 15 to 20 cm of the petioles attached; and (4) corm pieces; resulting when large corms are cut into smaller pieces (Onwueme, 1999). These methods of propagation are not always suitable due to large quantities of seeds, high percentage of seed rotting and susceptibility to pathological agent. Taro is affected by viral diseases which decrease the yield (Ooka, 1994). Zettler et al. (1989) summaries several pathogen of quarantine importance including the viruses alomae (meaning the death of taro) bobone (meaning curled or folded leaf) and a disease caused by a small bacilliform virus, the insect taro beetle (*Papuana* ssp.), taro hopper (*Tarophagus prosperina*) and nematode (*Hirschimaniella miticausa*) and the fungi taro leaf blight caused by *Phytophthora colocasiae.* In Cameroon, Mbong et al. (2013) reported that the constraints to taro production are diseases and pests. The crop is susceptible to fungal, bacterial, viral and nematode infections (Gadre and Joshi, 2003). Among these various diseases, taro leaf blight disease is one of the major important economic diseases of taro because it reduces corm yield of up to 50% (Singh et al., 2006) and leaf yield of up to 95% in susceptible genotypes and favourable environmental factors (Nelson et al., 2011). *P. colocasiae* causes corms to rot both in the field and in storage, and this has led to heavy storage lost (Brunt et al., 2001). In 2010, taro leaf blight disease was reported in Cameroon and it caused between 50 and 100% yields lost of taro in most of the crop growing regions. This has led to a reduction in planting materials, food, house hold income, increase poverty and some farmers have abandoned their farms and are now growing other crops (Guarion, 2010; Fontem and Mbong, 2011).

Despite reduction in planting materials and also disease planting material, *in-vitro* multiplication techniques of taro have given modest multiplication rates (Malamug et al., 1992). Meristem culture technique is used to produce plants free of viruses and fungi especially in vegetative propagated plants (Abo El-nil and Zettler, 1976). Plant tissue culture techniques have become a powerful tool for propagation of taro to overcome many problems facing traditional methods of propagation. Different explants were used to produce disease free planting materials (Hartman, 1973; Chung and Goh, 1994; Behera and Sahoo, 2008). The method for the production of four Cameroonian local taro cultivars micro-plants, described in this research is the first report to increase productivity of crop through the acquisition of pathogen- free cultivars in Cameroon, therefore the main aim of this study was to produce taro cultivars through *in-vitro* micro- propagation using shoot tip culture.

MATERIALS AND METHODS

The study was conducted in the field and Laboratory of Tissue Culture at Institute of Agricultural research (IRAD), Bambui, North West Region, Cameroon. The position of the experimental site was recorded using GPS mark Garmin etrex 20. IRAD; Bambui is situated at 32, 0627' N latitude, 0659' E longitude and altitude 1262 m above sea level.

Media preparation

Preparation of stock solution

Stock solutions were prepared by dissolving the amount of ingredients in various volume of sterilized distilled water (Table 1) using a magnetic stirrer. The stocks were kept in the refrigerator at 4°C.

Preparation of Murashige and Skoog medium

The medium was prepared by putting 600 ml of sterilized distil water in a beaker and 30 g of sugar was to it while stirring on a magnetic stirrer until all the sugar was dissolved. Fifty millilitre of macro and 5 ml of micro elements were added to the solution, stir until dissolved. Five millilitre vitamins stock, 5 ml Fe-EDTA complex and 5 ml of ascorbic acid were measured, added to the solution and stirred. 1.1 ml of 6-benzylaminopurine was also added and stirred. The volume of the solution was made up to 1000 ml of distilled water. The pH of the medium was adjusted to 5.7 and 7 g of agar was added to the mixture and heated until the solution was clear. The clear medium was distributed (2 ml per tube) by means of sterile pipette into cylindrical test tubes of 13 mm in diameter and 100 mm in height. The tubes were sealed with aluminium foil or corks and medium was sterilized in an autoclave for 121° C, 103.4

*Corresponding author: E-mail: charlesfokunang@yahoo.co.uk. Tel: +237670902446.

Table 1. Composition of stock solution (Murashige and Skoog, 1962).

Types of stock	Quantity of stock (ml)	Ingredient
Amounts	**Sterilize distilled water**	
MS group 1	1000	
19 g		KNO_3
Macro elements (g)		
16.5		NH_4NO_3
4.4		$CaCl_2.2H_2O$
3.7		$MgSO_4.7H_2O$
1.7		KH_2PO_4
MS group II (mg)	100	
278 (Fc-EDTA complex)		$FeSO_4.7H_2O$
373		$Na_2EDTA.2H_2O$
MS group III (mg)	100	
446		$MnSO_4.4H_2O$
Micro elements		
124		H_2BO_3
172		$ZnSO_4$
16.6		KI
5		$Na_2MoO_4.2H_2O$
0.5		$CuSO4.5H_2O$
0.5		$CoCI_2.6H_2O$
MS group IV	100	
40 mg		Glycine
Vitamins (mg)		
8		Thiamine-HCL
10		Pyridoxine-HCL
10		Nicotinic acid
Ascorbic acid	100	
400 mg		Ascorbic acid
BAP 0.1 mg/ml	10	
1 ml		BAP 1 mg/ml stock
NAA 0.1 mg/ml	50	
5 mg		NAA

KPa for 15 min. The forceps, blades, cotton and paper napkins were wrapped in aluminium foil and sterilized alongside the medium. The medium was allowed to cool and solidify overnight. Only one type of medium was used for all the incubation stages of *in-vitro* culture (Murashige and Skoog, 1962).

Collection and surface sterilization of taro corms

Four local taro cultivars of interest for preliminary yield trials screening (dark green petiole with small leaves, red petiole with small leaves, green petiole with large leaves and green petiole with small leaves) with young healthy off shoots of taro plants bearing rhizomes of 10 cm long were collected from IRAD Bambui experimental farms in the month of September 2015. These cultivars were 5 months old and carried to the tissue culture laboratory for surface sterilization. Cultivars were washed in running tap water. Roots and leaves were removed. Plants were trimmed into smaller pieces of plant material (explants). Explants were trimmed into 10 mm height; 3 mm at the base, with some corms measuring 3 mm in thickness attached using a knife. Corms were sterilized in 20% sodium hypochloride under an alcohol-swapped laminar air- flow chamber; the plants were immersed in 10% sodium hypochloride containing 2 drops of tween 80 per 100 ml in a closed vessel and were shaken for 45 min. The bleach was decanted and rinsed in sterile distilled water to remove all bleach. Explants were immersed in 70% Etoh alcohol for 2 to 3 min. Etoh alcohol was decant from explants and rinsed with sterile distilled water. Explants were immersed in 5% sodium hypochloride for 5 min, decant and rinse in sterile distilled water 3 times. Third and second leaf sheath were trimmed and explants were placed directly on a solidified cool Murashige and Skoog medium in sterile test tubes. Tubes were labelled with cultivars' names and placed in a growth room under light intensity (white fluorescent lamps) at 18°C. This was the establishment stage. The surfaced sterilized taro corms and sterilized taro corms in Murashige and Skoog medium were used for the study as indicated in Figure 1.

Shoot proliferation stage

Adventitious shoots or buds were produced 4 weeks after

(a) **(b)**

Figure 1. Sterilized taro cultivar in MS media. **(a)** Surface sterilized taro corms. **(b)** Sterilized taro corms in MS media.

incubation in Murashige and Skoog medium and were sub-cultured twice in the same media to obtain explants. The concentration of 6-benzylaminopurine (BAP) was double to 2.2 ml during the third subculture in order to initiate bud formation for 8 weeks. These cultivars were removed from each of the test tubes and placed on a sterilized typing sheet in an alcohol-swapped laminar air-flow chamber in order to avoid contamination from bacterial and fungal growth. The number of contaminated cultivars was counted during each sub culture and number of shoots and leaves were also counted during the third subculture. Petiole length and corm diameter was measured with a ruler during third subculture. Data on number of shoot, number of open leaves, and length of longest petiole, senescence leaf and diameter of corms were recorded.

Root initiation stage

Explants were removed from the test tube and subculture on a rooting media to stimulate rhizogenesis. This media consist of the same media as above with 2.2 ml of 6-benzylaminopurine being replaced by 10 ml of 0.1 mg/ml naphthalene acetic-acid (NAA) in Murashige and Skoog medium. The numbers of shoot, roots, in each test were counted and petiole lengths, root length, diameter of corms were measured using a ruler. Weight of explants was taken by weighing each cultivar from the test tube with an electronic balance. Data on number of shoot, petiole length, number of leaf, senescence leaf, diameter of corms, number of roots, root length and weight of explants were recorded after 60 days of shoot tip culture (Hartmann and Davies, 1990).

Statistical analysis

All data collected from number of shoot, number of leaves, senescence leaf, diameter of corms, number of roots, root length and weight of explants were subjected to analysis of variance (ANOVA) using statistical software (J M P 8). Mean variability amongst the cultivars were determined. Their treatment means were separated using Student's t test (STT) and the Least Significant Difference (LSD) at statistical significance of 95% confidence interval.

RESULTS

Effect of benzylaminopurine on four local cultivars of taro shoots proliferation.

The response of taro shoots cultured on MS medium with 2.2 ml of 6-benzylaminopurine on the number of shoots, petiole length, open leaf, senescence leaf and corm diameter after 60 days of shoot tip culture is shown in Table 2. Corms, shoots and leaves were produced on all the four cultivars in shoot tip culture with the longest number of petiole length and maximum number of open leaves, and corm diameter recorded on green petiole large leaves with mean values of 4.9 ± 1.21, 3.75 ± 0.20 and 0.59 ± 0.16, respectively. Cultivar red petiole small leaf recorded maximum number of shoot of 5.75 ± 0.59. The minimum mean number of shoots, petiole length, open leaf and corm diameter were also recorded with cultivars, dark green petiole small leaf, green petiole small leaf and red petiole small leaf with mean values of 1.7 ± 0.28, 1.8 ± 0.5, 2.35 ± 0.25 and 2.35 ± 0.25 respectively. There was no significant variation in mean number of senescence leaves with respect to all the cultivars at 60 days of shoot tip culture.

The different taro cultivars corm shoots in proliferation under M and S media is indicated in Figure 2.

Table 2. Effect of Murashige and Skoog medium containing 2.2 ml of 6-benzylaminopurine on the number of shoots, petiole length, open leaf, senescence leaf and corm diameter after 60 days of shoot tip culture.

Cultivars	Number of shoots	Petiole length (cm)	Open leaf	Senescence leaf	Corm diameter (cm)
Dark green petiole small leaf	1.7 ± 0.28^{c}	2.3 ± 0.96^{b}	2.6 ± 0.24^{b}	0.55 ± 0.12^{a}	0.25 ± 0.05^{b}
Red petiole small leaf	5.75 ± 0.59^{a}	1.97 ± 1.03^{b}	2.6 ± 0.16^{b}	0.3 ± 0.09^{a}	0.21± 0.06 ^{b}
Green petiole large leaf	3.1 ± 0.41^{bc}	4.9 ± 1.21^{a}	3.75 ± 0.20^{a}	0.65 ± 0.12^{a}	0.59 ± 0.16^{a}
Green petiole small leaf	4.3 ± 0.53^{ab}	1.8 ± 0.5^{b}	2.35 ± 0.25^{b}	0.3 ± 0.15^{a}	0.24 ± 0.05^{b}

Means followed by the same letters in the same column are not significantly different at p = 0.05 (STT). Values are mean number of shoots, petiole length, open leaf, senescence leaf and corm diameter followed by standard error.

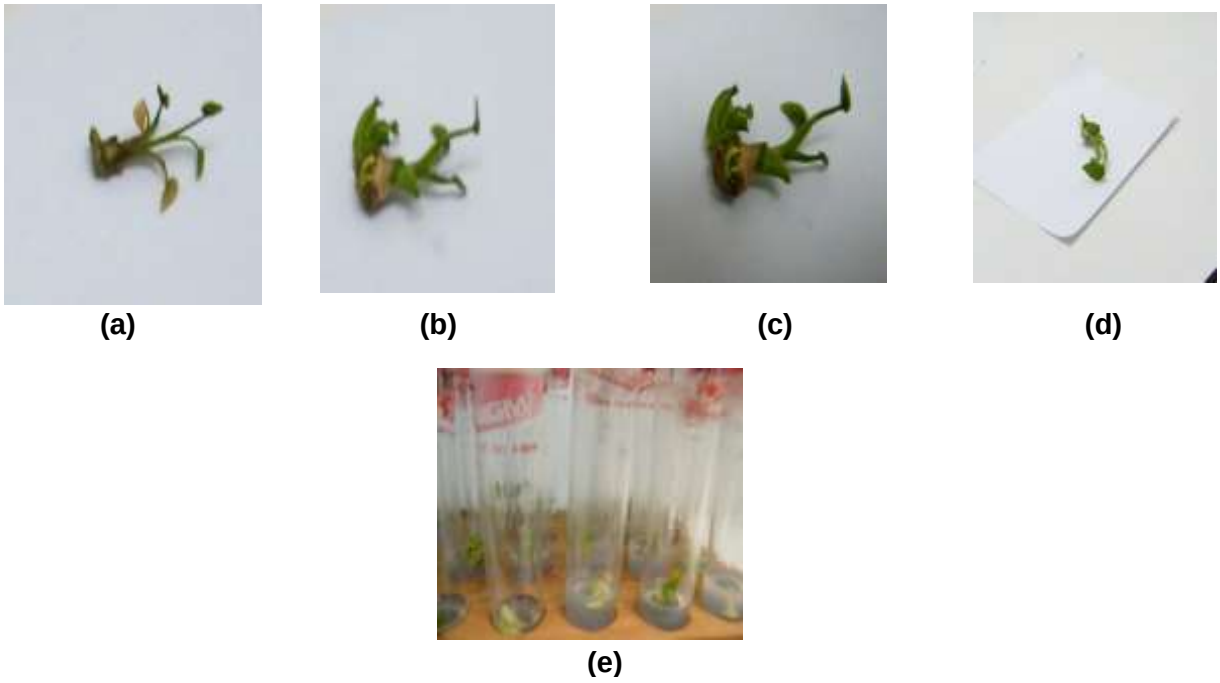

Figure 2. Taro shoots after shoot proliferation. **(a)** Dark green petiole small leaves. **(b)** Red petiole small leaves. **(c)** Green petiole large leaves. **(d)** Green petiole small leaves. **(e)** Taro shoots in proliferation media.

Effect of NAA on rooting of four local cultivars of taro shoot

Roots were produced on all the cultivars with maximum number of roots and the longest length of roots produced by cultivar dark green petiole small leaf with mean value of 14.7 ± 0.69 and green petiole small leaf with mean value of 3.67 ± 0.17, respectively. The minimum number of roots and the shortest length of roots were recorded in cultivars red petiole small leaf with mean values of 7.65 ± 0.69 and 1.93 ± 0.1 (Table 3). The highest mean value weight of explants of 1.87 ± 0.15 was recorded in cultivar dark green petiole small leaf and the least weight of 1.03 ± 0.11 in cultivar red petiole small leaf. Cultivar red petiole small leaf recorded low performance in Murashige and Skoog medium supplemented with 10 ml of 0.1 mg/ml NAA. There was a significant variation amongst the cultivars on number of roots, length of roots and weight of explants.

There were differences in petiole length and senescence leaf amongst the cultivars. There were no significant variations in number of shoots, open leaf and corm diameter. Cultivar dark green petiole small leaf performed best in this medium (Table 4). Cultivar dark green petiole recorded the highest mean number of shoots of 0.45± 0.13 and least mean shoot number of 0.3 ± 0.15 was recorded in cultivar green petiole small leaf. A mean maximum petiole length score of 10.62 ± 0.58 was recorded in cultivar dark green petiole small leaf and a minimum petiole length of 2.80± 0.37 in cultivar red petiole small leaf. The highest mean number of open leaves of 3.5 ± 0.22 were observed in cultivar dark green petiole small leaf and the least lowest number of open leaves of 2.75 ± 0.27 in cultivar red petiole small leaf.

Table 3. Effect of Murashige and Skoog medium supplemented with 10 ml of 0.1 mg/ml NAA on the number of roots, root length, and weight of explants after 60 days of shoot tip culture.

Cultivar	Number of roots	Length of roots (cm)	Weight of explants (g)
Dark green petiole small leaf	14.7 ± 0.69[a]	2.66 ± 0.11[b]	1.87 ± 0.15[a]
Red petiole small leaf	7.65 ± 0.69[c]	1.93 ± 0.10[c]	1.03 ± 0.11[b]
Green petiole large leaf	8.85 ± 0.79[c]	2.99 ± 0.12[b]	2.01 ± 0.13[a]
Green petiole small leaf	11.9 ± 0.8[b]	3.67 ± 0.17[a]	2.18 ± 0.19[a]

Means followed by the same letters in the same column are not significantly different at p = 0.05 (STT). Values are means number of roots, length of roots and weight of explants followed by standard error.

Table 4. Effect of Murashige and Skoog medium supplemented with 10 ml of 0.1 mg/ml NAA on the number of shoots, petiole length, open leaf, senescence leaf and corm diameter after 60 days of shoot tip culture.

Cultivar	Number of shoots	Petiole length (cm)	Open leaf	Senescence leaf	Corm diameter (cm)
Dark green petiole small leaf	0.45± 0.13[a]	10.62 ± 0.58[a]	3.5 ± 0.22[a]	0.5± 0.12[a]	0.57 ± 0.03[a]
Red petiole small leaf	0.4 ± 0.19[a]	2.80± 0.37[c]	2.75 ± 0.27[a]	0.3± 0.11[ab]	0.48 ± 0.03[a]
green petiole large leaf	0.35 ± 0.14[a]	10.28± 0.69[a]	3.45 ± 0.28 [a]	0.4± 0.10[ab]	0.51± 0.03[a]
green petiole small leaf	0.3 ± 0.15[a]	5.32 ± 0.74[b]	3.00± 0.23[a]	0.1 ± 0.08[b]	0.52 ± 0.02[a]

Means followed by the same letters in the same column are not significantly different at p = 0.05 (STT). Values are means number of shoots, petiole length, open leaf, senescence leaf and corm diameter followed by standard error.

Figure 3. Taro shoots after rooting. (a) Dark green petiole small leaves. **(b)** Red petiole small leaves. **(c)** Green petiole large leaves. **(d)** Green petiole small leaves.

After rooting the taro shoots were presented as shown in Figure 3.

DISCUSSION

Results obtained from shoot proliferation media indicated that there was a significant difference (p = 0.5) observed in number of shoots, petiole length, open leaf and corm diameter among the cultivars. This could be due to the genotypes of the cultivars. Reports from Toledo et al. (1998) states that different potatoes varieties respond differently to shooting due to genetic variability. The phenotypes of some the cultivars revealed that some have large leaves, long petiole and fast growing rates while others have small leaves, small petiole and slower growing rates. Shoots, leaves and corms were produced on all the cultivars when cultured on MS medium with 2.2 ml of 6-benzylaminopurine. This can be supported by report of Chung and Goh (1994) who reported that the addition of BAP into a culture medium enhanced growth and development of shoot auxiliary buds of *Colocasia esculenta* var *esculenta.* Seetohul et al. (2008) also reported that the highest multiplication rates of *in vitro* shoot tip of taro was recorded in MS medium supplemented with either BAP at 2 mg/l or thidiazuron (TDZ) at 0.9 mg/l. Reports from El-sayed et al. (2016) stated that higher concentration of BAP (kinetin, 6 mg/l)

produced low number of shoots in comparison with lower concentration of BAP at 2 mg/l. This may be due to the toxicity of the high concentration of cytokines which caused a delay in shoot formation. 6-benzylaminopurine is a cytokines which plays an important role in plant at growth and development. It also regulates flowering and fruits formation by stimulating cell division (Kianamiri and Hassani, 2008). It is known that the multiplication stage requires cytokines during micro propagation of various plants. This requirement appears to differ depends on the type of crop plants, explants type, phase of development, the concentration of growth regulators, the interaction between growth regulators and environmental conditions (Yokoya and Handro, 1996).

Roots were observed on all the cultivars when placed in MS media supplemented with 10 ml of 0.1 mg/ml NAA. Roots play a role in the mineral and water uptake of the plant. This can be supported by reports from Schiefelbein et al. (1997) which state that roots have an essential role in the functions of plants and development, supplying water and nutrients to the plants. Reports from Behera and Sahoo (2008) stated that the rooting of taro shoots was better in 0.5 MS medium + 1.5 or 2.0 mg NAA/1 than 0.5 MS medium + 1.5 or 2.0 mg IBA/1 after 15 days of culture. NAA is a synthetic auxin which when added to a culture stimulates root development from stem cuttings and cell elongation (Chambers, 2003). The significant variation amongst the cultivars on weight of explants could be as a result in growth rates in plants, where those with faster growth rate recorded higher weight and those with slower growth rates this could be due to genetic variability.

No significant variation was detected among cultivars on number of shoots, open leaf and corm diameter when cultured in rooting media. This result may be attributed to the role of auxin for enhancement of rooting and not shoot, where the development of lateral buds is inhibited by auxin produced at the apical meristem and transported down the stem (Yokoya and Handro, 1996; Bhuiyan et al., 2011).

Conclusion

All the four local Cameroonian cultivars multiplied when culture in MS medium supplemented with BAP and NAA during this experiment. Cultivar green petiole large leaves and cultivar dark green petiole small leaves performed best in both media respectively. Cultivar red petiole small leaves performed least when growth parameters such as number of shoot, leaves weight of plant and roots were taken in to consideration.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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