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ANTIOXIDANT CAPABILITIES OF THREE VARIETIES OF *ELAEIS GUINEENSIS* JACQ. ON DIFFERENT BASAL MEDIA

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Abstract

The effects of three media, namely; Murashige and Skoog (MS), Gamborg *et al.,* (B5), and Eeuwens (Y3) were assessed for mass propagation of oil palm (*Elaeis guineensis* Jacq. var. *Dura, Pisifera* and *Tenera*) zygotic embryos *In vitro*. The experimental design was a 3 x 4 factorial in a completely randomized design with each treatment consisting of ten replicates. While maintaining asepsis, the embryos were cultured on each of the three basal media as well as control which were devoid of salts of the basal media. The growth of oil palm embryos was influenced by the type of media and the variety (*Dura, Pisifera* and *Tenera*) involved as all the basal media used, regenerated plantlets directly from mature zygotic embryos of oil palm. For *Dura* variety, Y3 medium gave the best result in fresh weight, shoot length, leaf area, and number of leaves; B5 medium gave the highest sprout rate and per cent sprouting while MS medium had the best result for root length**.** For *pisifera* variety, Y3 medium was significantly superior at $p \leq 0.05$ to MS and B5 in fresh weight, shoot length**,** root length, leaf area, number of leaves**,** and per cent sprouting while for *Tenera* variety, B5 medium produced the best results for all the parameters. Dura cultured *In vitro* on Y3 media was significantly higher in terms of total phenolic content and the scavenging antioxidant activity when compared to other varieties in different basal media. Similarly, *In vitro* leaf extracts were found to have better antioxidant activity when compared to the extracts from wild leaf. The protocol stated in this work can enable a large amount of true-to-type oil palm plants which can produce better antioxidant when compared to the conventionally grown plants.

Key words: *Elaeis guineensis* embryo explant, Murashige and skoog medium, Gamborg *et al.*, medium, Eeuwens medium, Antioxidant activity.

Introduction

E. guineensis Jacq, (Arecaceae) is a perennial, monocotyledonous, monoecious tropical crop mainly propagated for its vegetable oil producing capabilities (Muniran *et al.,* 2008; Mgbeze & Iserhienrhien, 2014; Verheye, 2014). Oil palm has roots in west and central Africa (Obahiagbon, 2012). Oil palm is an extended stout single stem, about 20-30 m high, terminating in a crown of between 20–100 leaves (Dransfield *et al*., 2005). *E. guineensis* has three varieties, categorized on the basis of whether or not a shell is present in the fruit. They are: *Dura, Pisifera* and *Tenera* which is a hybrid of *Dura* and *Pisifera* (Thuzar *et al*., 2012) *Dura* produces fruits with a thick shell (2-8 mm) comprising 25-55% weight and 35-55% medium mesocarp weight. It is an important source of germplasm. Generally, this plant propagates exclusively by seeds which are genetically variable in nature (Verheye, 2014).

The oil from Palm is currently considered the most promising source of biodiesel fuel and hence, a likely alternative to fossil fuels, which have promoted global carbon dioxide emission (Nellemann *et al*., 2007; UNEP, 2011). However, oil palm production has been limited by several agronomic factors. Firstly, the biological characteristics of oil palm do not allow its vegetative propagation by conventional horticultural means such as cutting, budding and grafting because it has a solitary growing point (Mgbeze & Iserhienrhien, 2014) and high genetic variability is observed among hybrids, as well as low planting density (Duval *et al*., 1994). Secondly, about ten years or more are needed to assess the yield of its produce and also the genetic improvement of oil palm through conventional breeding is extremely slow and costly, as the breeding cycle can take up to 10 years (Duval *et al.,* 1993; Muniran *et al.,* 2008). In addition, the low seed- germination rate associated with seedling establishment makes it difficult to use seeds as explant source. *In vitro* propagation through tissue culture of embryos bridges these gaps and hastens germination which offers solutions to such aforementioned seed propagation problems. This is because embryo culture technique has been used in overcoming embryo non-viability and the constraint to seed germination (seed dormancy) caused by the seed coat and endosperm; reduction of germination time; production of interspecific and intergeneric hybrids and also to provide a long term storage of germplasm (Okezie & Okonkwo, 1992).

Plant tissue culture, therefore involves the science of developing plant cells, tissues or organs isolated from 'mother' plant under controlled environmental and nutritional conditions on standard artificial growth medium (Thorpe, 2007; Mgbeze & Iserhienrhien, 2014). Numerous studies on oil palm tissue culture have been conducted since 1970 using different types of explants and media formulations (Hardon *et al.*, 1987). The use of tissue culture was projected to improve oil production and this was later confirmed by significant increases in yield (up to 30%) compared to commercial *Dura* × *Pisifera* (D × P) seedlings in large-scale field trials (Eng-Ti *et al*., 2008). Muniran *et al*., (2008) established the micro-propagation protocol for oil palm cultured in full strength media of modified Chu (N6), Murashige and Skoog (MS) and Eeuwens (Y3) medium using immature zygotic embryo. The result revealed that the modified Eeuwens (Y3) medium was the most effective on induction of callus, somatic embryogenesis and rooting, as well as for direct regeneration. Fernando *et al*., (2004) also reported

micropropagation of coconut (*Cocos nucifera* L.) through embryonic shoot culture. Callus was successfully initiated from plumule explants when cultured in Murashige and Skoog and Eeuwens medium with the inclusion of between 24 – 400 uM 2,4-D and activated charcoal. In the same vein, Suranthran *et al*., (2011) has also worked on the influence of plant growth regulatory substance and activated charcoal on *In vitro* growth and regeneration of oil palm (*Elaeis guineensis* Jacq. var. *Dura)* zygotic embryo. The total phenolics and antioxidant evaluation of field grown *Elaeis guineensis* leaves have been carried out by Han & May (2010); Vijayaranthra & Sasidharan (2012); Yusof *et al*., (2016). But to the best of our knowledge, no study has compared the three varieties of oil palm *In vitro* on MS, B5 and Y3 basal medium and accessed their antioxidant activities in comparison to the wild species. In other words, this study was done to develop a protocol for the regeneration of oil palms embryo *In vitro* using three different culture media and to determine the differential sprouting response of embryos from three varieties of oil palm and to ascertain differences in their antioxidant activities of the leaves as compared to the field grown leaves.

Materials and Methods

Source of explant: The explants (mature zygotic embryos) used in this study were excised from mature fruits of *Elaeis guineensis* Jacq. (*Dura, Pisifera and Tenera*) obtained from Ada Palm in Ohaji Egbema Oguta Local Government Area of Imo State, Nigeria. The fruits were identified and confirmed in the Herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Culture media composition: The culture media used for this work were MS (Murashige & Skoog, 1962), B5 (Gamborg *et al.*, 1968) and Y3 (Eeuwens, 1976). MS medium was composed of macro salts (KNO_3, KH_2PO_4, NH_4NO_3, $CaCl_2.2H_20$ and $MgSO_4.7H_2O$), micro nutrients ($MnSO_4.4H_2O$, KI, $ZnSO_4.7H_2O$, H_3BO_3, $COCl_2.6H_20$, $Na_2MoO_4.2H_2O$ and $CuSO_4.5H_2O$), iron source ($Na_2EDTA.2H_2O$ and $FeSO_4.7H_2O$) vitamins (Thiamine HCL, Glysine and Nicotinic acid) and myo-inositol. Likewise, B5 medium was composed of macro salts ((KNO_3, $NaH_2PO_2.2H_2O$, $(NH_4)_2SO_4$, $CaCl_2.2H_20$ and $MgSO_4.7H_2O$), micro salts ($MnSO_4.4H_2O$, KI, $ZnSO_4.7H_2O$, H_3BO_3, $CoCl_2.6H_20$, $Na_2MoO_4.2H_2O$ and $CuSO_4.5H_2O$), iron source ($FeSO_4.7H_2O$) vitamins (Thiamine HCl, and Nicotinic acid) and myo-inositol. Eeuwens medium was composed of macro salts ((KNO_3, KCl, $NaH_2PO_2.2H_2O$, NH_4Cl, $CaCl_2.2H_20$ and $MgSO_4.7H_2O$), micro nutrients ($MnSO_4.4H_2O$, KI, $ZnSO_4.7H_2O$, H_3BO_3, $CoCl_2.6H_20$, $Na_2MoO_4.2H_2O$, $NiCl_2.6H_2O$ and $CuSO_4.5H_2O$), iron source ($Na_2EDTA.2H_2O$ and $FeSO_4.7H_2O$) vitamins (Thiamine HCl, Biotin and Nicotinic acid) and myo-inositol.

Aseptic techniques: After soaking the seeds for 21days to imbibe water, they were immersed in 70% (v/v) ethanol (70%) and stirred for one minute. This was followed by immersion in 1.0 % (v/v) sodium hypochlorite containing two drops of Tween 20 for 20 minutes and thorough rinsing in four changes of sterile distilled water. The seeds were then withdrawn and placed on sterile blotting paper to dry. The seed coats were removed to free the seeds (Fig. 1a-c) from which the zygotic embryos (Fig. 2) were excised. The embryos were aseptically excised by means of sterile forceps and scalpels, on a 9 cm-diameter Whatman No.1 filter paper in a Petri dish. Embryos were moved singly into culture vessels. The process of surface sterilization of seeds and embryo isolation were carried out in a laminar air-flow hood previously made sterile by wiping with absolute ethanol and exposed to ultraviolent light for 50 minutes. The temperature, light cycle and intensity of the growth room were 25 ±2°C, 16-h light/8-h dark cycles and 2500 lux respectively.

Design of experiment and statistics: In this study, experiment was carried out in a 3x4 factorial in a Completely Randomized Design (CRD). Experimental design consisted of twelve treatments with ten replications per treatment which was repeated three independent times. Data acquired from growth parameters namely; fresh weight, shoot length, root length, leaf area, number of leaves and number of roots were subjected to analysis of variance (ANOVA) by the selection of ten plantlets. Treatment means were tested for significance at $p \leq 0.05$ using Duncan's New Multiple Range Test (DNMRT).

Fig. 1a b c. Seeds of *E. guineensis* Jacq. var. *Dura, Pisifera and Tenera* respectively.

Fig. 2. Oil Palm zygotic embryos.

Regeneration studies: The growth and regeneration of *E. guineensis* embryo explants *In vitro* were observed daily from the first day of inoculation. Rate of emergent and percentage spouting were obtained from the first to the fourteenth day when all the treatments had reached the maximum sprouting rate. At the end of the experimental time (eight weeks), regenerated plantlets from each treatment were removed from the culture tubes, washed off the basal medium in a running water, and each of the plantlets were randomly selected and recorded for the following parameters: fresh weight of sprouts, length of shoot and roots, leaf area, number of leaves and roots produced in culture.

Antioxidant activities of leaf extracts: The wild fresh leaves and freshly produced plantlets' leaves from the three varieties cultured in each basal media were dried at 70^{o}C and the methanolic extracts were tested for their antioxidant activities using the DPPH (1,1-diphenyl 2, picrylhydrazyl) method according to Brand-Williams *et al.,* (1995) with some modifications. Triplicate experiments were performed at three independent times. The samples were covered with aluminum foil, shaken gently and kept in the dark for 20 min, after which the absorbance was read at the absorbance wavelength of 517 nm. The percentage scavenging activity was determined as difference in absorbance of blank and extract, divided by absorbance of blank multiplied by 100.

Total phenolics: The aforementioned extracts in aliquots of 2.5 mg/ml were assayed for their total phenolic contents using Folin Ciocalteu reagent according to the methods of Ghasemi *et al*., (2009) and expressed in mg/g GAE using Gallic acid as standard (r^2=0.98). Absorbance was read at 725 nm.

Statistical analysis: Data were reported as means±SEM, where appropriate. One-way analysis of variance (ANOVA) and correlation analysis were used to analyze the experimental data and Duncan multiple test range was used to compare the group means obtained after each treatment with control measurements. Differences were considered significant when $p \leq 0.05$.

Results

In this study, it was observed that from the first to the third day of embryo growth, there was no visible physiological or morphological change. Enlargement and expansion of the embryos were observed from the fourth day when about 30 % of the embryos had doubled their size. This was followed by curving by the 7th day in culture. By the 10th day, the embryos which appeared white in colour from the onset had enlarged and begun to turn greenish yellow in all the treatments. Between the 12th and the 14th day, the green colour had become more prominent leading to the emergence of embryonic root then followed by the embryonic shoot from the plumular end. By the 21st day, the elongation of shoot and root had become more prominent in *Dura* variety cultured on B5 medium, on *Pisifera* variety cultured on Y3 medium and *Tenera* variety cultured on B5 medium**.** On the other hand, embryo growth was equally observed on the control medium (only agar and sucrose, no basal media or PGR). Sprouting of the embryos commenced from the 4th day of culture, and continued expansion till the 14th day.

For *Dura* variety, Table 1 showed that there was a significant difference among the three basal media in terms of sprout rate and percent sprouting since $p \leq 0.05$. The highest sprout rate of 0.11 ± 0.01 and per cent sprouting of 86.67 ± 6.67 were obtained in B5 medium followed by Y3 medium which had 0.08 ± 0.00 and 80.00 ± 0.00 respectively, while MS medium had the least sprout rate of 0.00 ± 0.00 and percent sprouting of 40.00 ± 0.00. For *Pisifera* variety, Y3 medium had the highest sprout rate of 0.08 ± 0.00 (which did not differ significantly from B5 and control), and percent sprouting of 100.00 ± 0.00 (which differed significantly from MS, B5 and control). For *Tenera* variety, B5 differed significantly from MS and Y3 as well as control in terms of sprout rate and percent sprouting. The highest sprout rate of 0.13± 0.01 and percent sprouting of 93 ± 6.67 were obtained in B5 medium. Table 1 also showed that there was significant difference among the three basal media in terms of sprout rate and percent sprouting since $p \leq 0.05$.

Analysis of variance indicated that there were significant differences among the three basal media on fresh weight and shoot length of plantlets produced from *Dura* variety since $p \leq 0.05$. Table 2 showed that Y3 medium had the highest effect on fresh weight of 0.15 ± 0.01 which did not differ significantly from B5 while differing from MS and control. The same Y3 medium (Plate 4a) had the highest shoot length of 0.77 ± 0.13 cm which differed significantly from MS, B5 and control. For *Pisifera* variety, Y3 medium had the highest effect on fresh weight of 0.15 ± 0.02 (g) which did not differ significantly from B5 while differing from MS and control. The same Y3 medium had the highest shoot length (Fig. 5a) of 0.83 ± 0.08 cm which differed significantly from MS, B5 and control meaning that proliferation of shoot of *Pisifera* variety *In vitro* was best on Y3medium. For *Tenera* variety, B5 medium had the highest effect on fresh weight (0.21±0.04 g) and shoot length (0.42±0.06 cm) which differed significantly from MS and Y3 medium (Table 2).

Table 1. Sprout rate and per cent sprouting of *Elaeis guineensis* var. *Dura, Pisifera* and *Tenera* embryo explants as affected by the three basal media (MS, B5 and Y3) after two weeks of growth.

Variety	Basal media	Sprout rate	Per cent sprouting
Dura	MS	0.00 ± 0.00^{c}	40.00 ± 0.00^{c}
	B5	0.11 ± 0.10^{a}	86.67 ± 6.67^{a}
	Y3	0.08 ± 0.00^{b}	80.00 ± 0.00^{ab}
	Control	0.06 ± 0.00^{b}	66.67 ± 6.67^{b}
Pisifera	MS	0.00 ± 0.00^{b}	40.00 ± 0.00^{c}
	B5	0.06 ± 0.00^{a}	60.00 ± 0.00^{b}
	Y3	0.08 ± 0.00^{a}	100.00 ± 000^{a}
	Control	0.05 ± 0.00^{a}	60.00 ± 0.00^{b}
Tenera	MS	0.07 ± 0.00^{b}	80.00 ± 0.00^{b}
	B5	0.13 ± 0.01^{a}	93.33 ± 6.67^{a}
	Y3	0.05 ± 0.00^{b}	60.00 ± 0.00^{c}
	Control	0.07 ± 0.00^{b}	66.67 ± 6.67^{ab}

Values represent mean ± SE. Means values within a column followed by different letters are significantly different from each other by DNMRT ($p \leq 0.05$)

Table 2. Fresh weight and shoot length of the three varieties of *Elaeis guineensis* as affected by the three basal media (MS, B5 and Y3).

Variety	Basal media	Mean fresh weight (g)	Mean shoot length (cm)
Dura	MS	0.11 ± 0.02^{b}	0.40 ± 0.03^{b}
	B5	0.14 ± 0.01^{a}	0.35 ± 0.05^{b}
	Y3	0.15 ± 0.01^{a}	0.77 ± 0.13^{a}
	Control	0.05 ± 0.00^{c}	0.22 ± 0.04^{b}
Pisifera	MS	0.08 ± 0.01^{b}	0.55 ± 0.04^{b}
	B5	0.12 ± 0.02^{a}	0.28 ± 0.05^{c}
	Y3	0.15 ± 0.02^{a}	0.83 ± 0.08^{a}
	Control	0.03 ± 0.00^{c}	0.31 ± 0.03^{c}
Tenera	MS	0.07 ± 0.02^{bc}	0.22 ± 0.04^{b}
	B5	0.21 ± 0.04^{a}	0.42 ± 0.06^{a}
	Y3	0.10 ± 0.01^{b}	0.29 ± 0.04^{ab}
	Control	0.03 ± 0.00^{c}	0.24 ± 0.05^{b}

Table 3. Leaf area (cm^2) and number of leaves of the three varieties of *Elaeis guineensis* as affected by the three basal media (MS, B5 and Y3).

Variety	Basal media	Mean leaf area (cm^2)	Mean number of leaves
Dura	MS	0.13 ± 0.01^{bc}	1.20 ± 0.13^{a}
	B5	0.28 ± 0.05^{b}	1.20 ± 0.13^{a}
	Y3	0.49 ± 0.10^{a}	1.50 ± 0.22^{a}
	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Pisifera	MS	0.50 ± 0.02^{a}	1.30 ± 0.21^{a}
	B5	0.23 ± 0.03^{b}	1.22 ± 0.15^{a}
	Y3	0.52 ± 0.06^{a}	1.40 ± 0.16^{a}
	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
Tenera	MS	0.09 ± 0.02^{bc}	1.00 ± 0.00^{b}
	B5	0.71 ± 0.12^{a}	1.40 ± 0.16^{a}
	Y3	0.28 ± 0.05^{b}	1.00 ± 0.00^{b}
	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

Table 4. Root length (cm) and number of roots of the three varieties of *Elaeis guineensis* as affected by the three basal media (MS, B5 and Y3).

Variety	Basal media	Mean root length (cm)	Mean number of roots
Dura	MS	2.31 ± 0.25^{a}	1.00 ± 0.00^{a}
	B5	1.44 ± 0.27^{a}	1.00 ± 0.00^{a}
	Y3	1.65 ± 0.55^{a}	1.00 ± 0.00^{a}
	Control	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Pisifera	MS	0.20 ± 0.07^{bc}	0.50 ± 0.17^{b}
	B5	0.73 ± 0.15^{b}	0.90 ± 0.10^{a}
	Y3	1.90 ± 0.39^{a}	1.00 ± 0.00^{a}
	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Tenera	MS	0.13 ± 0.04^{b}	0.50 ± 0.17^{b}
	B5	2.43 ± 0.85^{a}	2.80 ± 0.92^{a}
	Y3	2.35 ± 1.07^{a}	1.00 ± 0.00^{b}
	Control	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Analysis of variance indicated that there were significant differences among the different basal media on leaf area and number of leaves of plantlets produced from *Dura* variety since $p \leq 0.05$. Y3 medium (Fig. 4a) promoted maximum leaf area of 0.49 ± 0.01 cm^2 which differed significantly from MS and B5 medium (Table 3.). Y3 also had the highest number of leaves (1.50 ± 0.22) but did not have significant difference on MS and B5. The control did not produce leaves at the end of two months' study. For *Pisifera* variety, Y3 medium promoted maximum leaf area of 0.52 ± 0.06 cm^2 which did not differ significantly from MS while differing from B5 and control. Y3 also had the highest number of leaves (1.40 ± 0.16) but there was no significant difference among the three basal media in terms of number of leaves. The control produced no leaves at the end of two months' study. For *Tenera* variety, B5 had the highest leaf area (0.71 ± 0.12 cm^2) and number of leaves (1.40±0.16) which differed significantly from MS and Y3 while the control produced no leaf at the end of the study.

For *Dura* variety, Table 4 showed that MS medium promoted maximum root length of 2.31 ±0.25 cm which did not differ significantly from B5 and Y3 medium except on control, while there was no significant difference among the three basal media in terms of number of roots, thus, 1.00 ± 0.00 was recorded for all. The control did not produce roots at the end of two months' study thus, 0.00 ± 0.00 was recorded (Fig. 4d). For *Pisifera* variety, Y3 medium had the highest root length of 1.90 ± 0.39 cm which differed significantly from B5, MS and control. Y3 and B5 had the best result (1.00 ± 0.00 and 0.90± 0.10 respectively) which differed significantly from MS in terms of number of roots, while control produced no roots on the different basal media at the end of two months' study. For *Tenera* variety, B5 medium had the highest root length of 2.43 ± 0.85 cm which did not differ significantly from Y3 while differing from MS and control. B5 also had the best result (2.80 ± 0.92) on number of roots which differed significantly from Y3 and MS, while control produced no root on the different basal media at the end of two months' study (Table 4).

The content of total phenols in extracts was measured by Folin-Ciocalteau's reagent at a wavelength of 750 nm, quantified with reference to gallic acid per g of extract. The total phenol content of methanolic extracts of *In vitro* leaves *E. guineensis* were significantly higher than that of the wild species except for the leaf extracts of Tenera and Pisifera in MS medium that were lower than the wild leaf extract.

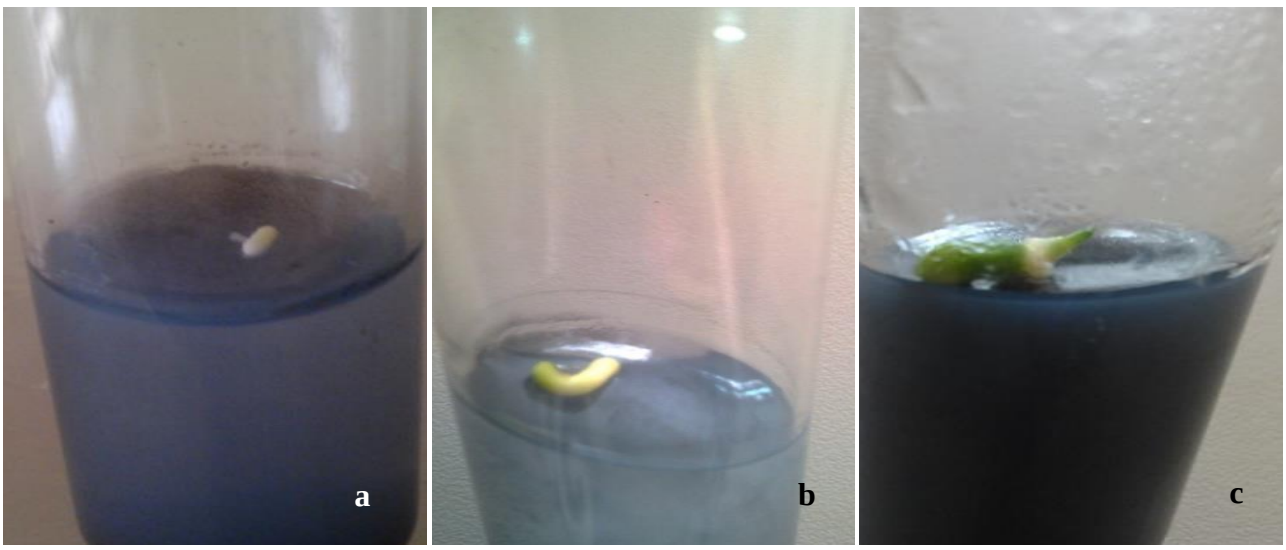


Fig. 3. (a)1-day (b) 10-day (c) 14-day old Plantlets from zygotic embryo of *E. guineensis* varieties during the first 14 days in culture.

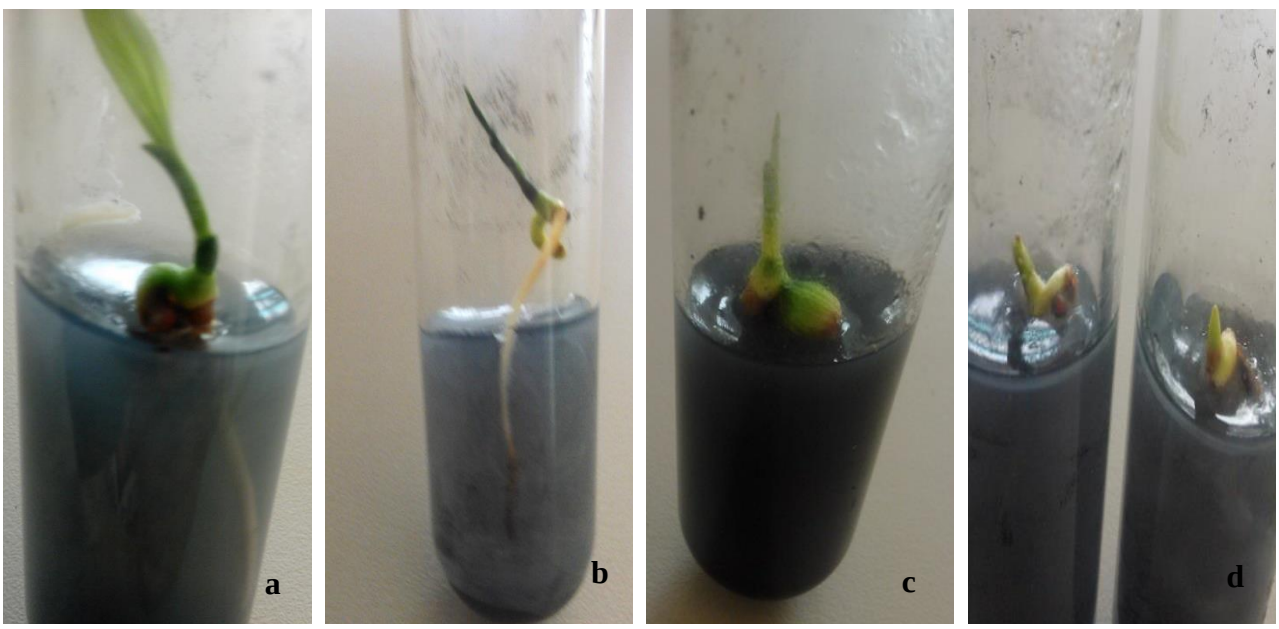


Fig. 4. *Dura* variety on (a)Y3 (b) MS (c) B5 (d) Control medium after two months in culture.

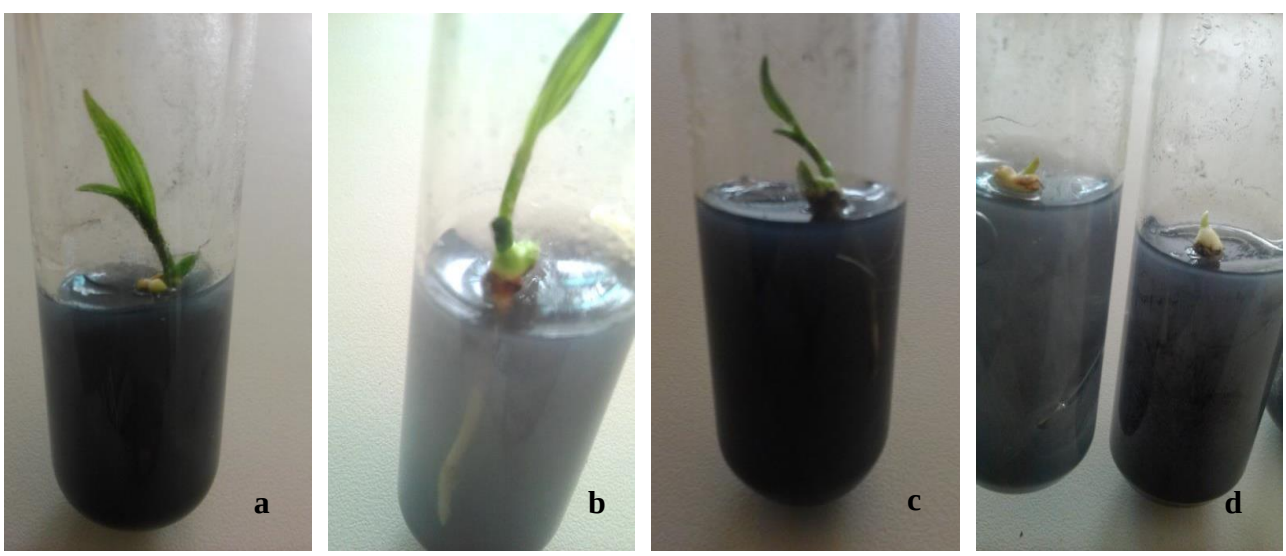
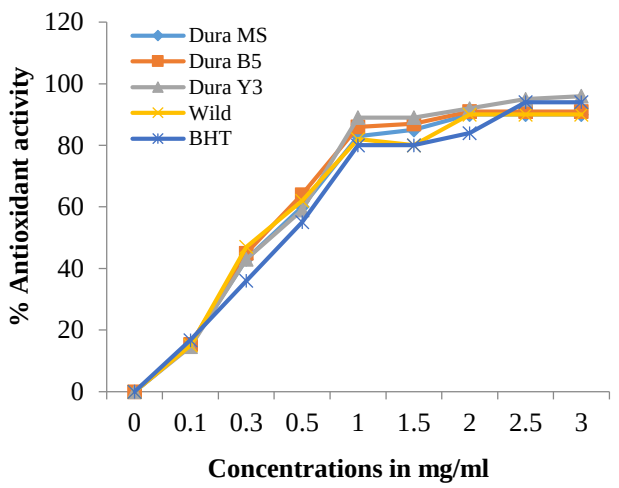


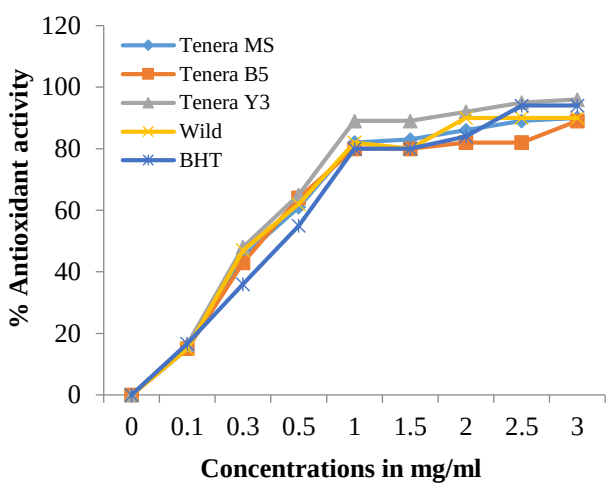
Fig. 5. *Pisifera* variety on (a)Y3 (b) MS (c) B5 (d) Control medium after two months in culture.

Fig. 6. *Tenera* variety on (a)Y3 (b) MS (c) B5 (d) Control medium after two months in culture.



*AA (%) = Radical scavenging activity of extract expressed as percentage inhibition of the free radical, DPPH.

Fig. 7. Antioxidant activities of methanolic leaf extracts of *Elaeis guineensis* var. Dura cultured on three different nutrient media.



*AA (%) = Radical scavenging activity of extract expressed as percentage inhibition of the free radical, DPPH.

Fig. 8. Antioxidant activities of methanolic leaf extracts of *Elaeis guineensis* var. Tenera cultured on three different nutrient media.

Table 5. Total phenolic contents and antioxidant activities of *In vitro* and wild leaf methanolic extracts.

Methanolic extract of leaf	Basal media	Total phenolic content (TPC)	Antioxidant activity (IC_{50}) (DPPH)
Dura	MS	8.22 ± 0.05^{e}	0.403 ± 0.002^{b}
	B5	8.25 ± 0.19^{e}	0.304 ± 0.001^{e}
	Y3	9.04 ± 0.51^{a}	0.300 ± 0.001^{f}
Tenera	MS	8.02 ± 0.20^{g}	0.304 ± 0.002^{e}
	B5	8.45 ± 0.73^{d}	0.310 ± 0.001^{d}
	Y3	8.96 ± 0.04^{b}	0.304 ± 0.005^{e}
Pisifera	MS	8.01 ± 0.33^{g}	0.310 ± 0.001^{d}
	B5	8.26 ± 0.06^{e}	0.309 ± 0.002^{d}
	Y3	8.92 ± 0.96^{c}	0.304 ± 0.002^{e}
Wild	-	8.11 ± 1.03^{f}	0.317 ± 0.003^{c}
BHT	-	-	0.476 ± 0.001^{a}

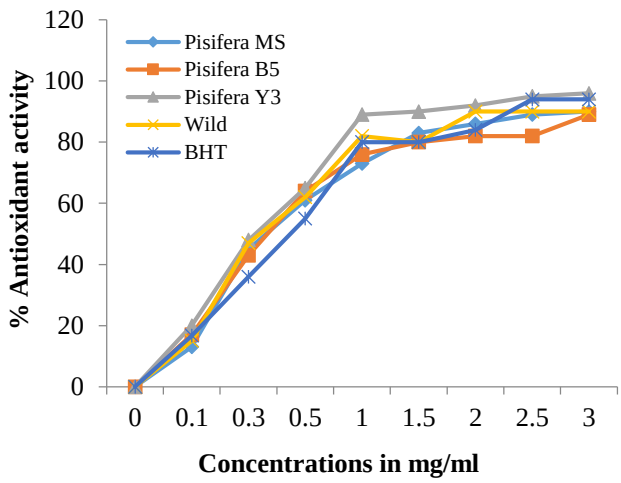
The DPPH scavenging activity of all samples, *Dura* (Fig. 7), *Tenera* (Fig. 8), *Pisifera* (Fig. 9) and BHT (Fig. 10) followed a dose-dependent pattern. IC_{50} values represent the inhibition concentration for 50% scavenging of total DPPH radicals. The IC_{50} values for the *In vitro* leaf extract, wild leaf extract as well as the reference antioxidant BHT are presented in Table 5. The *In vitro* and wild leaf extracts exhibited higher ($p<0.05$) scavenging potential than BHT. On the other hand, *In vitro* leaf extracts exhibited higher ($p<0.05$) scavenging potential than the wild leaf extract except for leaf extract from Dura in Y3 medium showed the weakest scavenging activity.

Discussion

This study compares the growth rates of three varieties of El*aeis guineensis* Jacq. on three different basal media. Mature zygotic embryos of three varieties of *Elaeis guineensis* Jacq. (*Dura*, *Pisifera* and *Tenera)* were cultured on three different basal media viz: Murashige and Skoog (MS), Gamborg *et al.*, (B5), and Eeuwens (Y3) without plant growth regulatory substance (PGRs). Embryos increased in size and

germination was observed in all the varieties cultured on the three basal media. Within four days of zygotic embryo culture, swelling and expansion were observed which led to the embryonic axis turning green indicating that photosynthesis had taken place. After that, the emergence of an out-growth close to the root called haustorium by the 10th to the 14th day was observed (Plates 3b and 3c). The haustorium became elongated and embryonic shoot emerged leading to shoot formation from the shoot apex. Similar morphological growth is in line with the works of Suranthran *et al.*, (2011) and Okafor and Okezie (2016) who observed enlargement, extension and greening of zygotic embryos within five days of culture, as well as plumule development which eventually led to the formation of shoot within fourteen days of culture. Thawaro & Techato (2009; 2010) also observed similar form of growth which included enlargement and enlogation of zygotic embryos between 10th to 12th days of culture followed by initiation of shoots at the 14th day. Complete plantlet was established approximately one month after culture in *E. guineensis* var. *Tenera.* Embryos cultured on control devoid of basic nutrients sprouted but produced neither leaves nor roots. This may be due to the absence of basic nutrients necessary for growth as the zygotic embryos has been stripped of its food storage tissue indicating that basal media has a vital role in plant morphogenesis. In this study, plantlets were successfully rejuvenated directly from mature zygotic embryos of *E. guineensis* varieties on MS, B5 and Y3 basal media. The outcome from the experiment conducted showed that the three basal media sustained the growth of oil palm zygotic embryos *In vitro* and it is consistent with the work of Muniran, *et al.*, (2008) who compared the growth of oil palm on three different media and Thurzar *et al.*, (2012) who worked on recloning of rejuvenated plantlets from elite oil palm cv. *Tenera.* The emergence and development of the mature zygotic embryos of *Elaeis guineensis* varieties into plantlets on each of the basal medium without plant growth regulators may be a clear indication that a high level of endogenous hormone are found in the cultured explants. This may further suggest that matured embryos are hormone independent and entirely autotrophic (George, 2008). Smith & Drew (1990) observed that matured zygotic embryos can be cultured on simple nutrient medium without growth regulators but the culture of immature embryos requires the addition of growth regulators. Thawaro & Te-Chato (2010) also stated that the culturing of zygotic embryos requires only basic nutrients for germination.

The inclusion of activated charcoal (AC) to the entire basal medium inhibited the action of phenolic compound from the oil palm embryo, thus promoting the development of the embryo *In vitro.* Hence, all culture media supplemented with activated charcoal (AC) showed almost 100 % viability irrespective of the absence of plant growth regulators. This is in line with the report of Suranthran *et al.*, (2011) who worked on the influence of plant growth regulatory substances and AC on *In vitro* development and enlargement of oil palm (*E. guineensis* Jacq. var *Dura*) zygotic embryo. The latter recorded that all culture media treated with AC regardless of presence or absence of synthetic hormone had almost 100 % viability while those without the inclusion of AC showed poor viability. Fridborg, *et al.*, (1978) also showed that AC adsorbs a number of compounds and culture metabolites which often inhibits specific developmental stages of somatic embryos. Diro & Van Staden (2004) also observed that the inclusion of activated charcoal into the media reduced oxidative browning in *Esente ventrocosum*, promoting germination of embryos and improve the growth of seedlings.



*AA (%) = Radical scavenging activity of extract expressed as percentage inhibition of the free radical, DPPH.

Fig. 9. Antioxidant activities of methanolic leaf extracts of *Elaeis guineensis* var. Pisifera cultured on three different nutrient media

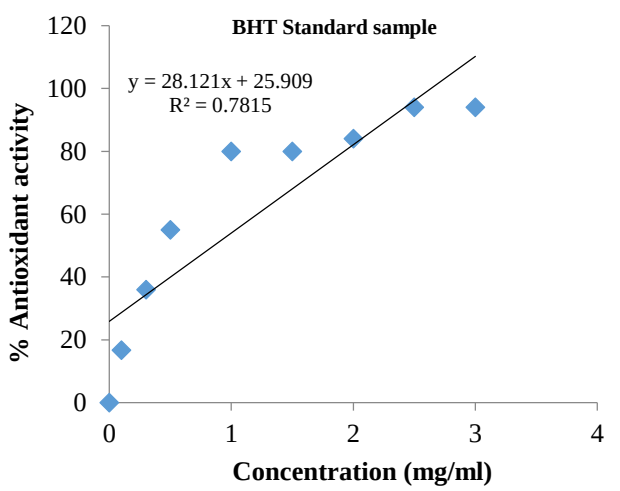


Fig. 10. BHT standard curve.

*The total phenolic and antioxidant activity of the leaf extracts of the three varieties of Elaeis guineensis in three basal media.
*Data in mean ± SE (n=3) replicated three independent times (Total =9)
*AA (%) = Radical Scavenging Activity of Extract expressed as percentage inhibition of the free radical, DPPH.
*TPC = Total Phenolic Content expressed as Gallic Acid Equivalents (GAE) in milligrams per g plant material
*Values with dissimilar alphabets along the same column are significantly different at $p \leq 0.05$

This study has revealed that the growth and the subsequent development of oil palm varieties on the three standard basal media *In vitro* was strongly dependent on the interaction between the different varieties and the basal media since each variety showed different growth rates on different basal media. The growth differences observed among the varieties may probably be due to the composition and concentration of each component that make up the medium. Also the composition, size and vigour of the embryo from each variety might be a contributing factor, since they were all subjected to the same environmental conditions. George (2008) considers two important factors that are useful in finding the media formulation appropriate for different plant species which includes; the fraction of nitrate and ammonium ions and the over-all concentration of nitrogen in the medium. Also Bhojwani & Razdan, (1996) noted that the main change in the composition of a range of commonly used tissue culture media was based on the quantity of various salts and ions, hence the active factor in the medium was the ion of different type rather than the compound. According to them, a high quantity of ammonium nitrogen and total nitrogen are much higher in MS medium than in the majority of media. Ions and the forms in which they are supplied are important in eliciting particular *In vitro* responses (Murashige & Skoog, 1962). Bhojwani and Razdan, (1996); Pierik, (1997); and Smith, (2000) asserted that no single medium can be suggested as being entirely satisfactory for all types of plant species and organs. Though Murashige and Skoog (1962) basal medium is very common because most plants respond to it favorably. However, in this work, plantlets regenerated on Y3 and B5 medium showed marked difference in most of the growth parameters assessed when compared to the ones in MS medium. For *Dura* and *Pisifera* variety, Y3 basal medium was found to be the best when compared to others as it recorded highest responses in all the growth parameters tested. Y3 medium regenerated plantlets with long rooting. This is in line with the works of Okafor & Okezie (2016); Muniran, *et al.,* (2008) who compared the effect of two (MS and Y3) and three basal media (N6, MS and Y3) respectively on *E. guineensis: Dura* and the result showed that Y3 medium was the most appropriate for direct regeneration of somatic embryogenesis of *Dura* variety. Muniran *et al.,* (2008) also observed that plantlets regenerated on modified Y3 medium both in presence and absence of IBA showed high percentage and prolific rooting. This could be ascribed to the content of the medium which has higher level of KCl (Potassium Chloride) and NH_4Cl which provides more Cl- ions that acts like innate auxin in the root induction and their growth. In addition, the composition of salts (major and minor) in Y3 basal media has been reported to be more appropriate for palm species (Eeuwens, 1976). Y3 medium also has the richest source of amino acids and high content of KI which might have played a crucial role in the progression and expansion of the species *In vitro*. Okafor & Okezie (2016) also confirmed the superiority of Y3 over MS in root development and in general growth of oil palm zygotic embryos. For *Tenera* variety, the plantlets regenerated on B5 medium showed marked increase in yield as it produced profuse rooting. It was also possible that B5 medium performed better than MS and Y3 medium in *Tenera* variety due to high content of potassium ion, as well as low quantity of ammonium and nitrogen in B5 media and the composition of the variety.

The zygotic embryos of *E. guineensis* Jacq. varieties used in this study showed significant responses on the three basal media. Hence, the three basal media (MS, Y3 and B5) compared supported the *In vitro* regeneration of embryo explants of *E. guineensis* varieties, but Y3 medium was found to be significantly ($p \leq 0.05$) superior to MS and B5 in the growth parameters studied for *Dura* and *Pisifera* while B5 medium was found to be significantly superior to MS and Y3 in all the parameters studied for *Tenera* variety. The findings of this study may suggest that Y3 and B5 medium are better than MS medium for micropropagation of the embryo explants of oil palm varieties.

On the other hand, *In vitro* regenerated plantlets' leaf, in this study contained higher amounts of phenolics and antioxidants than the wild species. Although, the phenolics and antioxidant contents reported may have been different when compared to the works of Han & May (2010); Vijayaranthra & Sasidharan (2012); Yin *et al*., (2013) and Yusof *et al*., (2016) who reported the antioxidant capabilities of field grown leaves of *Elaeis guineensis*. The differences may have resulted due to difference in media conditions and constituents, addition of activated charcoal (that may have reduced toxic substances) and difference in climatic conditions (for the field grown plant). The study also showed that basal media may have a role to play in increasing the antioxidant quantities in plants. The three varieties in Y3 medium in this study had significantly higher antioxidant activities than ones in B5 and MS media. The current work is in line with the work of Nagesh & Shanthamma (2011) who compared the total phenolic and antioxidant activity of the leaves of field grown and micropropagated plants of *Mollugo nudicaulis*; Devendra *et al*., (2012) who compared the phytochemicals from the leaves of field grown and micropropagated plants of *Crotalaria*, Mohanty *et al*., (2015) on *Curcuma aromatica*; Prasad *et al*., (2016) on leaf extract of *Aerides odorata*; Behera *et al*., (2018) on *Paederia foetida*;. The authors confirmed that *In vitro* produced leaf extracts had higher antioxidants or phenolics when compared to the extracts field grown leaves. Also, the result obtained contrasted to the work of Nikolova *et al*., (2013) on *Arnica montana* leaf extracts who reported that *ex vitro* and field grown leaf extracts had significantly higher radical scavenging activity than the extracts of *In vitro* samples.

Conclusion

The results obtained in this work, through comparison of three basal media on three varieties of *Elaeis guineensis* Jacq. provides a foundation for further refinement of the *In vitro* regeneration protocol for micropropagation of oil palm for its commercial cultivation, mass production and its antioxidant capabilities. This study has also provided a solution to the shortening of long germination period of oil palm for its mass production especially for *Pisifera* variety which has high abortion rate.

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