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Effect of carbohydrate source on the *in vitro* germination of *Elaeis guineensis* Jacq. zygotic embryos on two basal media

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In this study, seven carbohydrates, namely, glucose, fructose, galactose, sucrose, maltose, raffinose and starch were screened for the most suitable carbon source for the *in vitro* culture of oil palm (*Elaeis guineensis* Jacq. var. tenera) zygotic embryos on the basal media of Murashige and Skoog (MS) (1962) and Eeuwen (Y3) (1976). The embryos were excised aseptically and cultured on both media containing the different carbohydrates. Results obtained showed that while both media supported *in vitro* plantlet regeneration from the embryo explants, Y3 medium was significantly superior to MS medium (P=0.05) in length of root and shoot of plantlets produced. Sucrose was also significantly (P=0.05) superior to glucose and maltose while starch, raffinose, fructose and galactose had the least value in all growth parameters studied. In addition, more uniform plantlets were produced in Y3 media in which sucrose served as carbon source relative to the other six carbon sources tested. The protocol reported here has potential for speeding up germination process within a short period of time for oil palm.

Key words: *Elaeis guineensis,* Eeuwen (Y3) medium, Murashige and Skoog (MS) (1962) medium, embryo explants, carbohydrates.

INTRODUCTION

Oil palm, a member of the family Arecaceae, genus *Elaeis*, has two known species namely, *Elaeis guineensis* (African oil palm) and *Elaeis oleifera* (American oil palm). Oil palm is an arborescent, monocotyledon and an oleaginous tropical perennial crop, used as vegetable oil as well as a substitute for biofuel (a renewable energy source of energy) (Marlucia et al., 2014). There are three varieties of oil palm classified based on the presence or absence of a shell in the fruit: Dura which produces fruits with a thick shell; Pisifera which is without a shell in its rare fruits; Tenera, a hybrid of these two varieties and produces fruits with an intermediate shell (Kamnoon and Preamrudee, 1999).

Oil palm breeding by conventional methods has several limitations which are, (i) the perennial nature of the plant (which may require about five to ten years to assess the value of a progeny); (ii) the allogamous nature and, (iii) the use of seeds as the sole means of propagation

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Figure 1a. Seeds of *Elaeis guineensis* Jacq. (Mag. 1x).

material (Corley, 1982). For these reasons, *in vitro* propagation bridges the gap and offers solutions to such possible problems.

Plant tissue culture techniques and in particular embryo culture ensures rapid *in vitro* regeneration of plantlets as well as shortening of germination time (Pullaiah and Subba-Rao, 2009; Marlucia et al., 2014). Previous reports by Wan Nur Syuhada et al. (2016) (successfully used immature embryos for plantlet regeneration of oil palm through indirect organogenesis); Kantamaht et al. (2010), Alves et al. (2011), Suranthran et al. (2011) and Thuzar et al. (2012) also achieved maximum results on *E. guinensis* embryo-derived callus, hybrids, Dura and Tenera varieties, respectively when the carbon sources used were at three percent w/v. This appears to justify the concentration of carbon source used in this work even though MS was the only basal medium used by the authors cited. Muniran et al. (2008) also achieved good results when carbon sources used were at three percent even though they compared the effect of three basal media on *E. guineensis* var. Dura. Subsequently, the carbon sources also used did not include a trisaccharide and a polysaccharide. The present work, however, compared two basal media (MS and Y3), used seven carbon sources which comprises monosaccharides (glucose, galactose and fructose), disaccharides (sucrose and maltose), a trisaccharide (raffinose) and a polysaccharide (starch).

Carbohydrates are required *in vitro* due to the heterotrophic nature of cultured cells, to replace the carbon, which plants normally fix from the atmosphere by photosynthesis *in vivo* for growth, development and other physiological processes (Mehwish et al., 2013) Carbohydrates primarily contain only carbon, hydrogen and oxygen (Noggle and Fritz, 2006). In plants, they serve as the principal vehicle in which the energy of sunlight is captured and they make up their structural scaffold (Datta, 2007). Many explants in culture are generally not autotrophic and so require a carbon source that plays an important role as an energy source, as well as an osmotic agent (Smith, 2013). Thus, they are necessary as a source of energy and a carbon substrate for biosynthesis and its continuous supply to plants cultured *in vitro* is essential, since photosynthetic activity of *in vitro* grown tissues is usually reduced. For all these reasons, sugars have a great potential effect on the physiology, growth and differentiation of cells (Gibson, 2000). Carbohydrates may be divided into large groups namely; monosaccharides (glucose, fructose, galactose, etc.), oligosaccharides which comprised of disaccharides (sucrose, maltose, etc.) and trisaccharides (raffinose, etc.) and polysaccharides (starch, etc.) (Datta, 2007). Hilae and Te-chato (2005) investigated the effect of different carbon sources and strengths of MS medium on oil palm somatic embryo induction of shoot and root from haustorium-stage embryos. A reduced strength of MS and high concentration of alcohol sugar or sucrose was found to enhance root formation. Boonsanong and Kamnoon (1996) reported the superiority of Y3 or ½ MS when compared with MS in the formation of developed growth of root from zygotic embryos. In addition, Muniran et al. (2008) compared the efficacy of three basal media: N_6, MS and Y3 in the micropropagation of oil palm. The superiority of Y3 in callus induction, somatic embryogenesis and rooting was observed as compared to MS (Marlucia et al., 2014). Also, a combination of both MS and Y3 as reported by Wan Nur Syuhada et al. (2016) showed highest number of friable callus when immature embryo of oil palm is used as the explant. This study was therefore carried out to employ two types of modified basal media, namely, Murashige and Skoog (1962) and Eeuwen (1976), and to use a wide range of carbon sources (glucose, fructose, galactose, sucrose, maltose, raffinose and starch) as energy sources for purposes of determining the most suitable for studying the growth and development of oil palm embryos.

MATERIALS AND METHODS

Site of experiment

This study was conducted at the Tissue Culture and Molecular Biology Laboratory of the National Biotechnology Development Agency (NABDA) located at University of Nigeria, Nsukka.

Source of explants

The mature zygotic embryos employed in this study were excised from mature seeds of *E. guineensis* Jacq. 'tenera hybrid' obtained from the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Edo State, Nigeria. The seeds were identified and confirmed at the herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Oil palm seeds (Figure 1a) were cracked with a hammer to extract the embryo after removing the pericarp from the fruit. Prior to extracting the embryo (Figure 1b), the seeds were immersed in a mild solution of water

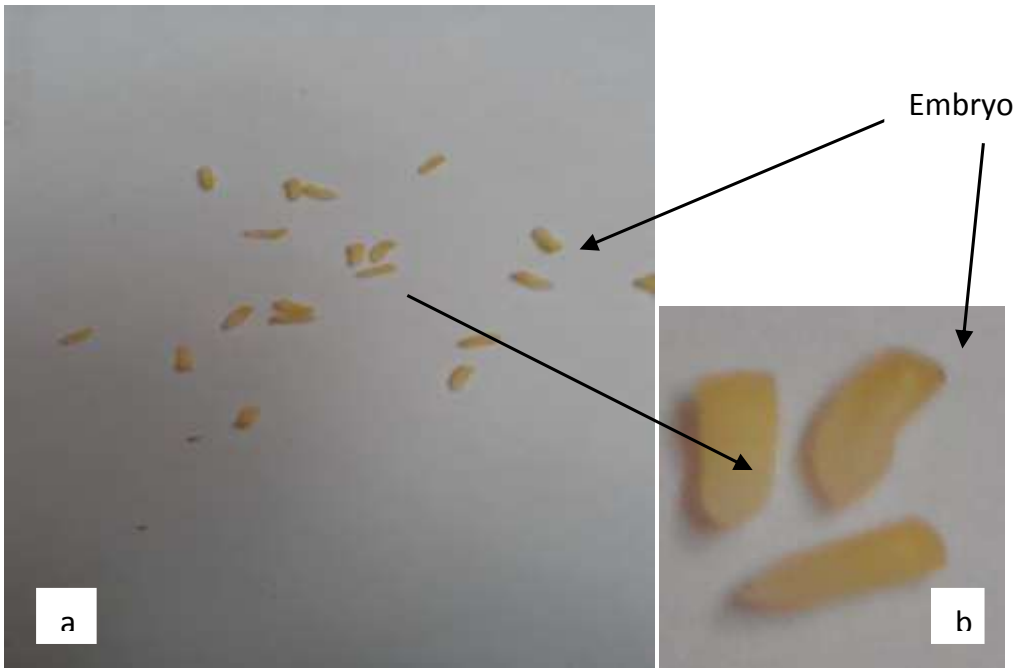

Figure 1b. i: Oil palm zygotic embryos (light yellow in colour) measuring between 0.9-1.2 cm in length (Mag 1x) ii: enlarged oil palm zygotic embryo (Mag. 3x).

and Tween-20 and were swirled for one minute according to the method of Suranthran et al. (2011).

Culture media

The basal culture media employed in this study were those of Murashige and Skoog (1962) and Eeuwen (1976) (Table 1). Each was supplemented with 3% (w/v) of the carbohydrates, namely, glucose, fructose, galactose, sucrose, maltose, raffinose and starch. Cultures with no carbohydrate source were employed as controls.

Sterilization techniques and embryo transfer

The seeds (Figure 1a) were washed in running tap water to remove visible dirt and other adhering particles and then surface-sterilized by immersion in seventy percent ethanol (v/v) for five seconds and subsequently in one percent (v/v) sodium hypochlorite (NaOCl) from commercial bleach (Clorox) for ten minutes, followed by four rinses in sterile double distilled water. Prior to inoculation on the growth media, the seeds were freed of the seed coats and endosperm exposing the embryo (Figure 1b), using a pair of sterile forceps and scalpels, on a 9 cm-diameter Whatman No. 1 filter paper in a Petri dish. The seed testa and endosperm were discarded after every manipulation to minimize contamination. Embryos were transferred singly into culture vessels (one per vessel). In the course of embryo transfer, the pair of forceps was dipped in 70% ethanol, flamed over a spirit lamp, and held to cool within the sterile hood before picking up the embryos into the culture media. Similarly, the mouth of the inoculated culture vessel was flamed over a spirit lamp before closing. These were done to minimize contamination. The cultured explants were maintained in the growth room at 25 ±2°C under 16-h light/8-h dark cycles at a light intensity of 2500 lux provided by cool white fluorescent tubes (Quickstart, B.G. Tropical Daylight 6,500k, England). The process of surface-sterilization of seeds and the inoculation of embryos were all carried out in a laminar air-flow hood, previously made sterile by swabbing with absolute ethanol and exposed to ultraviolet light for 30 min.

Experimental design and statistical analysis

The experiment was set up as a completely randomized two-factor factorial design with the main factors being basal media salt formulation at two levels (MS and Y3 media) and carbon sources at seven levels (glucose, fructose, galactose, sucrose, maltose, raffinose and starch) plus control, making it a total of eight levels. A total of 480 embryos (240 for each of the basal medium) were used for this experiment, that is, ten replicates (each replication consisted of one embryo per culture tube) for eight treatments, done three independent times, and used in order to obtain a mean ± standard error for all the growth parameters studied. Data pertaining to growth parameters which included: Leaf area, fresh weight, length of roots and length of shoots was also subjected to analysis of variance (ANOVA) by the selection of ten plantlets. Treatment means were tested for significance ($P \leq 0.05$) using Duncan mutiple range test (DMRT) error bars were represented at 5% value.

Plant regeneration studies

The growth and development of embryos were monitored on daily basis from the day of inoculation. Time course in sprouting (%) and sprouting rate were determined from the first day till the fourteenth day when sprouting had leveled off in all the treatments. At the end of eighth week, regenerated plantlets under each treatment were withdrawn from the culture media and scored for the following growth parameters: length of shoots and roots produced, fresh weight of sprouts produced (weighed on a Sartorius sensitive

Table 1. Compositions of Murashige and Skoog (1962) and Eeuwens (1976) basal media.

Component	Y3	MS
Macroelements (mg/l)		
KNO_3	2020	1900
KCl	1492	-
KH_2PO_4	-	170
NH_4Cl	535	-
NH_4NO_3	-	1650
$NaH_2PO_4.2H_2O$	312	-
$CaCl_2.2H_2O$	294	440
$MgSO_4.7H_2O$	247	370
Iron salts (mg/l)		
$Na_2EDTA.2H_2O$	37.3	37.3
$FeSO_4.7H_2O$	27.8	27.8
Microelements (mg/l)		
$MnSO_4.4H_2O$	11.2	22.3
KI	8.3	0.83
$ZnSO_4.7H_2O$	7.2	8.6
H_3BO_3	3.1	6.2
$CoCl_2.6H_2O$	0.24	-
$Na_2MoO_4.2H_2O$	0.24	0.25
$CuSO_4.5H_2O$	0.16	0.025
$NiCl_2.6H_2O$	0.024	-
Supplements (mg/l)		
Meso inositol	100	100
Thiamine-HCl	0.5	0.1
Pyridoxine-HC	0.05	0.5
Calcium pentothenate	0.05	-
Nicotinic acid	0.05	0.5
Biotin (B-complex)	0.05	-
Gibberelic acid	0.038	-
Glycine	-	2
Sucrose	45000	30000
Coconut water	5%	-
Activated charcoal	1000	-
Agar (g/l)	7.5	7.5
pH	5.5	5.8

weighing balance Sartorius BS 323S, USA) and plantlet leaf area. The experiment was repeated three independent times for reproducibility.

RESULTS

The pattern of oil palm zygotic embryo development and morphogenesis during the first ten days of culture is shown in Figure 1c (i-iv). Swelling and expansion of embryos was observed within the first 3 days (Figure 1c-iii) and it was followed by the curving of plantlets approximately 3 to 5 days of culture (Figure 1c-iv). The embryo (Figure 1b) which was light yellow in colour at the time of inoculation enlarged and began to turn green leading to the emergence of radicle from the radicular end and plumule from the plumular end and a haustorium within 5 days in culture. The radicle and plumule finally gave rise to the root and new shoot, respectively, while the haustorium served an absorptive function (Figure 1c-vi).

Sprouting commenced on the third day in culture. Sprouting in both MS and Y3 basal media supplemented with sucrose had 50% sprouting on the 5^{th} day and maximum sprouting percentage of 81.2±2.58 and 68.00±2.99 on the 14^{th} day for Y3 and MS basal media, respectively. For glucose, 50% sprouting in both MS and Y3 basal media was achieved on the 6^{th} and 5^{th} day, with a maximum of 60.40±3.84 and 58.40±2.01 on the 14^{th} day, respectively. Maltose, however, had 50% sprouting on the 12^{th} and 7^{th} day and maximum percent sprouting of 50.00±1.84 and 51.40±3.72 on the 14^{th} day for MS and Y3 basal media, respectively. There was no sprouting in those embryo explants supplemented with fructose, galactose, raffinose, starch and control within 14 days in culture (Table 2).

Sprout rate of the plantlets (determined as the reciprocal of the number of days to 50% sprouting) was dependent on both the type of carbon source employed and basal media used. The highest rate of sprouting (0.20±0.01) was recorded for Y3+sucrose, Y3+glucose and MS+sucrose each since 50% sprouting was achieved on the 5^{th} day, while MS+glucose had 0.16±0.05 because 50% sprouting was achieved on the 6^{th} day while maltose in both Y3 and MS had sprout rates of 0.14±0.00 and 0.08±0.00, respectively, because 50% sprouting was achieved on the 7^{th} and 12^{th} day, respectively. Embryo explants in untreated (control), fructose, galactose, raffinose and starch in both media did not sprout and a value of 0.00±0.00 was recorded for them (Table 3).

One-way analysis of variance (ANOVA) showed that Y3 and MS media were significant at 5% level in all growth parameters studied except for fresh weight and leaf area. The two media brought about *in vitro* plantlet regeneration from embryo of *E. guineensis*. The media had significant effects on the length of root and shoot of the plantlets. The lengths of roots (Figure 2) and shoots (Figure 3) produced by the plantlets, achieved on Y3 medium were 4.18 ± 1.29 and 1.08 ± 0.54 cm; and 3.84 ± 0.18; 2.98± 0.73 cm for sucrose and glucose, respectively, while the same parameters achieved on MS medium recorded 2.08 ± 0.77, 0.68 ± 0.25; and 3.34 ± 0.86; 2.00±0.84 cm for sucrose and glucose, respectively. This may suggest in this study that, Y3 medium was found superior to MS medium for *in vitro* regeneration of *E. guineensis* through embryo culture in

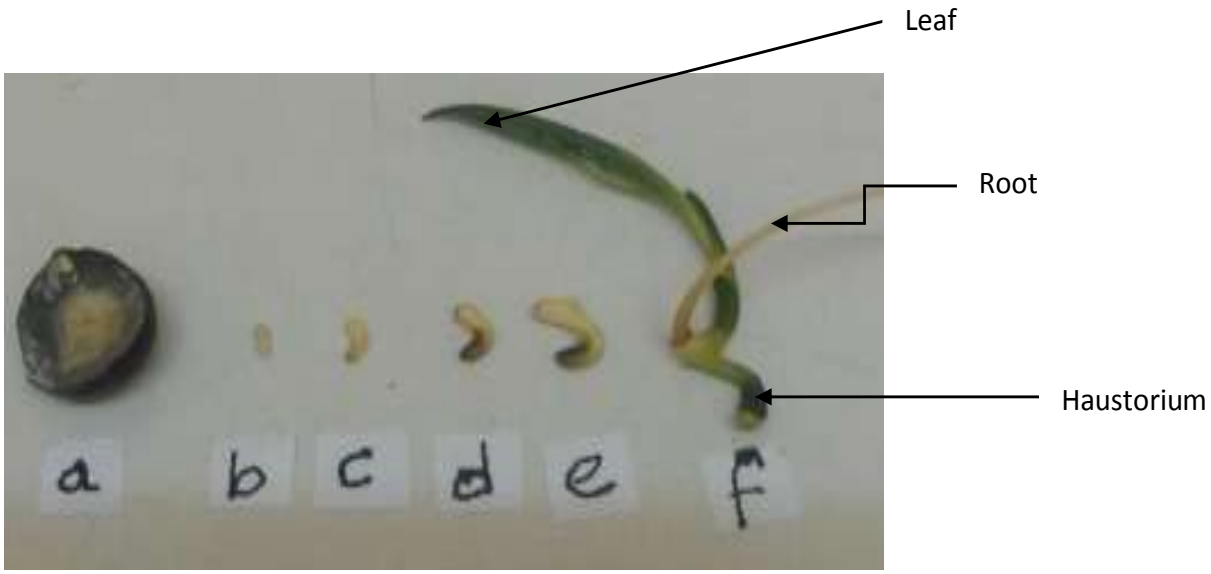

Figure 1c. Pattern of development of the plantlets from zygotic embryo of oil palm supplemented with sucrose on Y3 medium. i: Longitudinal section of oil palm seed showing the embryo, endosperm and testa (Mag. 1x); ii: Excised zygotic embryo (Mag. 0.5x); iii: Curved zygotic embryo after three days in culture (Mag. 0.5x); iv: Curved zygotic embryo between four and ten days in culture (Mag. 0.25x); v: Swollen and curved zygotic embryo after fourteen days in culture (Mag. 0.5x); vi: Plantlet after eight weeks in culture showing leaf, root and haustorium (Mag. 1x).

Table 2. Sprouting percentage of *Elaeis guineensis* embryo explants after two weeks on Y3 and MS basal media containing different carbon sources at three % (w/v) concentration.

Carbon sources	Media	
	MS	Y3
Sucrose	68.00±2.99^{a1}	81.20±2.58^{a2}
Glucose	60.40±3.84^{b1}	58.4±2.01^{b1}
Maltose	50.00±1.84^{c1}	51.4±3.72^{c1}
Fructose	0.00±0.00^{d1}	0.00±0.00^{d1}
Galactose	0.00±0.00^{d1}	0.00±0.00^{d1}
Raffinose	0.00±0.00^{d1}	0.00±0.00^{d1}
Starch	0.00±0.00^{d1}	0.00±0.00^{d1}
Control	0.00±0.00^{d1}	0.00±0.00^{d1}

Means followed by the same letters do not differ, in the column, at 5% level of probability using Duncan's multiple range test and means followed by the same number do not differ, in the row at 5% level of probability using DMRT.

Table 3. Rate of sprouting as affected by the various carbon sources at three percent (w/v) and the basal media.

Carbon sources	Media	
	MS	Y3
Sucrose	0.20±0.01^{a1}	0.20±0.01^{a1}
Glucose	0.16±0.05^{a1}	0.20±0.01^{a1}
Maltose	0.08±0.00^{b1}	0.14±0.00^{b2}
Fructose	0.00±0.00^{c1}	0.00±0.00^{c1}
Galactose	0.00±0.00^{c1}	0.00±0.00^{c1}
Raffinose	0.00±0.00^{c1}	0.00±0.00^{c1}
Starch	0.00±0.00^{c1}	0.00±0.00^{c1}
Control	0.00±0.00^{c1}	0.00±0.00^{c1}

Means followed by the same letters do not differ, in the column, at 5% level of probability using Duncan's multiple range test and means followed by the same numbers do not differ, in the row at 5% level of probability using DMRT.

the length of root and shoot. Explants in Y3 medium supplemented with sucrose had the highest fresh weight of 1.77 ± 0.48 g while the least root length of 0.04 ± 0.02 g was recorded for the explants in galactose+Y3 (Figure 4). ANOVA showed that there was significant difference in carbon sources used, but significant difference did not exist between MS and Y3 under each carbon source. This means that the use carbon source irrespective of basal media used had a significant effect on the fresh weight of sprouts. In Y3 medium, explants supplemented with sucrose had the highest leaf area of 6.70 ± 0.91 cm^2 as compared to glucose while there was no leaf for the untreated explants (control), fructose, galactose, maltose, raffinose and those supplemented with starch, thus, 0.00 ± 0.00 cm^2 was recorded for them (Figure 5). ANOVA showed that there was no significant difference in basal media but sucrose was a better carbon source than glucose. This means that only the application of carbon source had a significant effect on the leaf area of

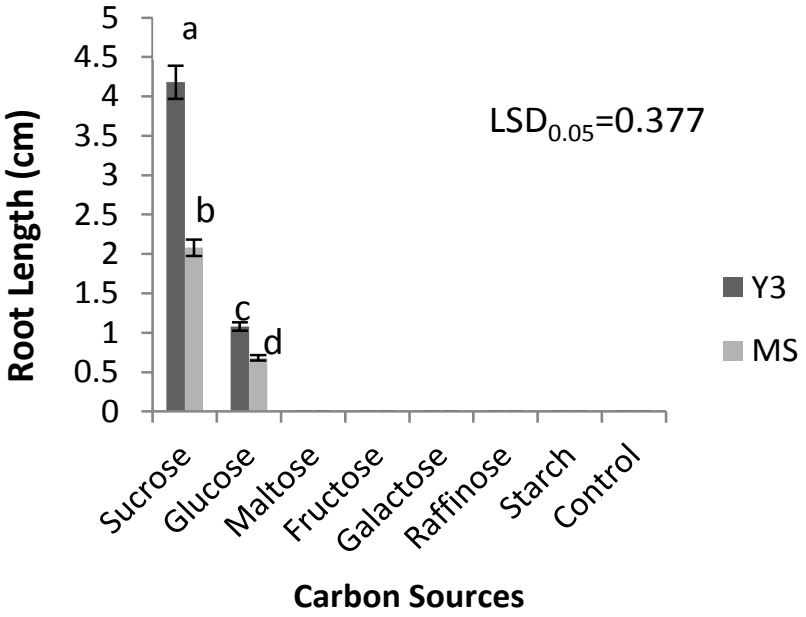

Figure 2. The effects of different carbon sources and basal media on the root length of plantlets of *E. guineensis* (Jacq.) produced in MS and Y3 media. Each point represents a mean of ten replicate treatments. Vertical bars represent ± standard error of the mean. Means followed by the same letters from one column to another do not differ at 5% level of probability using Duncan's multiple range test.

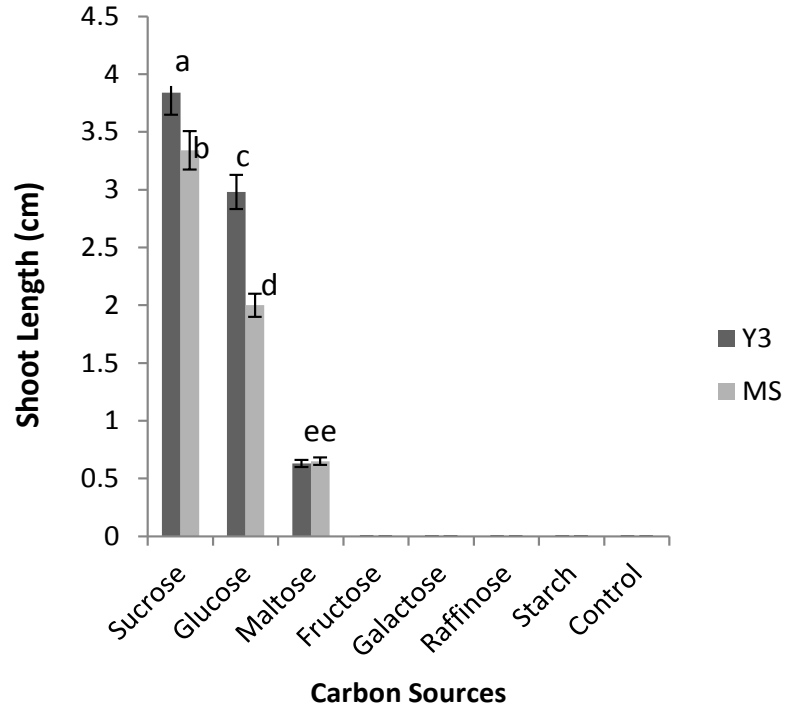

Figure 3. The effects of different carbon sources and basal media on the shoot length of plantlets of *Elaeis guineensis* (Jacq.) produced in MS and Y3 media. Each point represents a mean of ten replicate treatments. Vertical bars represent ± standard error of the mean. Means followed by the same letters from one column to another do not differ at 5% level of probability using Duncan's multiple range test.

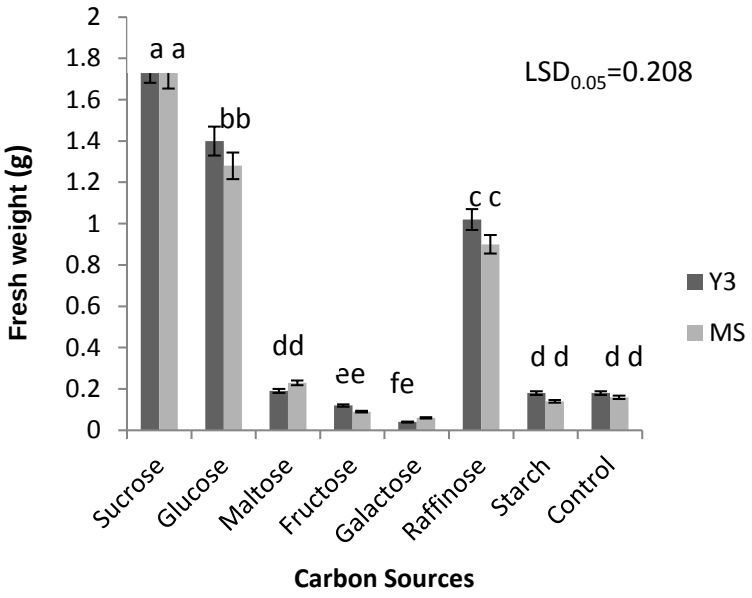

Figure 4. The effects of different carbon sources and basal media on the fresh weight of plantlets of *Elaeis guineensis* (Jacq.) produced in MS and Y3 media. Each point represents a mean of ten replicate treatments. Vertical bars represent ± standard error of the mean. Means followed by the same letters from one column to another do not differ at 5% level of probability using Duncan's multiple range test.

plantlets.

DISCUSSION

The addition of carbon sources to *in vitro* cultures is necessary prior to autotrophy as seen in this study. The embryonic axis that turned from yellow to green about three to five days from the time of inoculation indicate that the explants had become autotrophic. Similar result was obtained by Suranthran et al. (2011) who observed swelling, expansion and greening of embryos within five days of culture. The authors observed plumule emergence which eventually led to the emergence of shoot from the shoot apex within fourteen days of culture. The control (devoid of carbon source) did not show any marked differentiation into plumule and radical, an indication that sugars are mandatory for morphogenesis.

Sucrose was superior to other carbon sources employed in all growth parameters studied, producing healthy and uniform plantlet (Figure 1d). The reason may be that it is the most common carbohydrate translocated in the phloem sap of many plants (George, 2008). In addition, sucrose, a non-reducing sugar with no free carbonyl group has all its aldehydes (the reactive part of the sugars) hidden, preventing them from undergoing glycosylations that could kill the plant. This, however, makes sucrose very stable and convenient to transport sugars (David and Michael, 2008). The presence of the hydrolytic enzyme 'invertase' in plant cell wall and vacuole that breaks down sucrose into glucose and fructose for plant metabolism may be responsible for the superiority of sucrose over other carbon sources (Arnd and Guo-Qing, 1999). The superiority of sucrose over the other sugars employed as carbon source in this study is consistent with earlier reports for other plants such as *Phoenix dactylifera* (Veramendi and Navarro, 1996; Othmani et al., 2009) used as a sole carbon source and *Dianthus caryophyllus* (Karami et al., 2006). Baskaran and Jayabalan (2005) suggested that among the three carbon sources, sucrose proved to be better than fructose or glucose for shoot regeneration of *Eclipta alba*. Glucose, on the other hand supported *in vitro* plantlet regeneration of zygotic embryos of oil palm, although, produced lesser root, shoot, leaf area and fresh weight as compared to sucrose. This may be as a result of a series of chemical reactions (glycosylation) that could inhibit growth since glucose is a reducing sugar (with a free carbonyl group). In contrast to the results obtained, Amiri and Kazemitabar (2011) suggested that glucose was better for inducing shoot proliferation than other carbon sources in *Datura stramonium***.**.

Maltose, a reducing sugar favored the formation of plumule that resulted in the production of shoot with neither leaves nor root when Y3 was used (Figure 1e). This may have resulted to the slow hydrolysis of maltose by *in vitro* plantlet as reported by George (2008). The result obtained in this study is consistent with that of

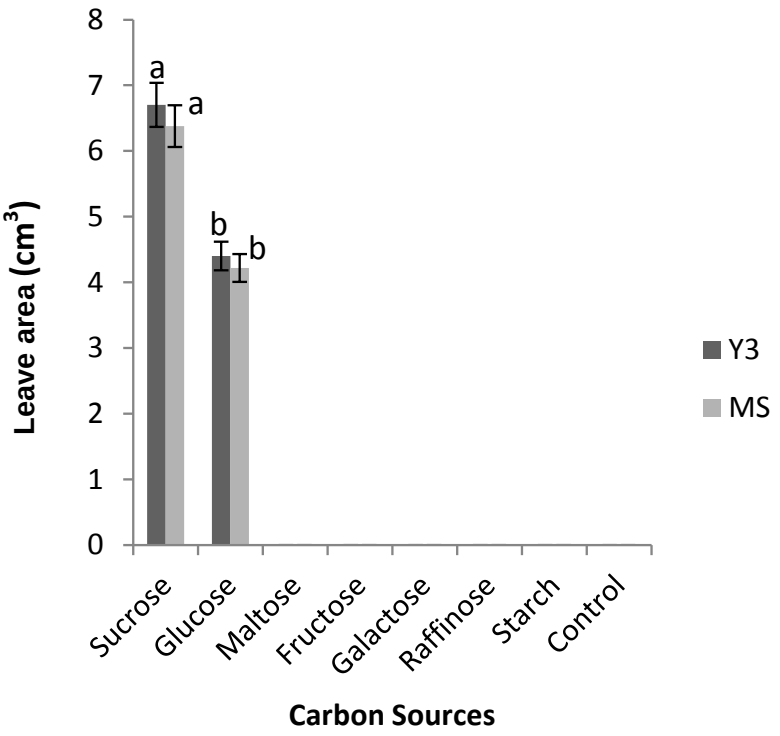

Figure 5. The effects of different carbon sources and basal media on the leaf area of plantlets of *Elaeis guineensis* (Jacq.) produced in MS and Y3 media. Each point represents a mean of ten replicate treatments. Vertical bars represent ± standard error of the mean. Means followed by the same letters from one column to another do not differ at 5% level of probability using Duncan's multiple range test.

Hossain et al. (2005). The authors reported that maltose is inferior to sucrose and glucose in the production of root in *Centella asiatica*. This could be attributed to the fact that plants may not have the necessary enzyme 'maltase' to hydrolyse maltose into glucose. These, however, may have also resulted to low rate of sprouting seen in the results obtained.

The inability of the embryos to utilize the more complex carbohydrate (starch) may have been as a result of the absence of the full complements of enzymes needed for their degradation or conversion into simpler utilizable carbohydrates such as sucrose and glucose. Raffinose, on the other hand could not support embryo growth in both media possibly due to the presence of galactose (a product in the hydrolysis of raffinose). The embryo explants elongated but could not be differentiated into plumule and radicle (Figure 1f). This may be as a result of the lack of galactose kinase which reduces the toxicity of galactose by converting it to galactose-1-phosphate as found by George (2008) who reported the toxicity of galactose in plant tissues of orchids. This, however, may be the same reason the embryo explants of *E. guineensis* could not sprout when raffinose was employed as the carbon source. In addition, the elongation resulted because sucrose, a product of the hydrolysis of raffinose may have been taken up by the embryo explants but could not differentiate because galactose may have inhibited the growth resulting in increase in fresh weight especially on Y3 medium. Mehwish et al. (2013) reported absence of roots when xylose, lactose, turanose, raffinose, cellulose, starch or mannitol were employed as carbon source for the propagation of *Chrysanthemum* both in light and dark conditions.

Fructose, on the other hand did not support the zygotic embryo regeneration of *E. guineensis* possibly due to the production of furfural derivatives by fructose during autoclaving as reported by George (2008). This may be toxic to the explants and may have caused the failure of embryos to sprout when fructose or galacose was used. The superiority of Y3 to MS could be related to the quantity of ions in the basal medium. Bhojwani and Razdan (1996) showed that the main difference in the composition of a range of commonly used tissue culture media is based on the quantity of various salts and ions. Plantlets in Y3 medium irrespective of the carbon source employed showed marked difference in their root growth as compared to the ones in MS medium. This observation may have been attributed to the high nitrogen content of MS that may have affected root organogenesis of *E. guineensis* plantlets. Wan and

Schuyler (2004) supported this by reporting that reducing the nitrogen content in the MS medium by halving could alleviate the problem of toxicity, and thus resulting in a high frequency of shoot organogenesis in *Pinus pinea* L.

In addition, the compositions of macro and micro elements in Y3 media has been reported to be more suitable for palm species as compared to White medium, or Murashige and Skoog (1962) and Eeuwens (1976). The plantlet regenerated on Y3 medium showed profuse longer roots when compared with MS, this could be attributed to the content of the medium. Eeuwens medium contains higher concentration of KCl and NH_4Cl, which provides more Cl^- ions.

The Cl^- ions are known to act like natural auxins in the somatic embryogenesis, rooting and callus induction of oil palm immature embryos (Muniran et al., 2008, Masani et al., 2013). In addition, Y3 medium is richer in micro salt content (KI), which might play a vital role in the root induction. Boonsanong and Kamnoon (1996) also confirmed the superiority of Y3 over MS in root development and in general growth of oil palm zygotic embryos. Marlucia et al. (2014) also reported that Y3 medium generates better oil palm plantlets for hardening when a 90-day old embryo was used as explant. The zygotic embryos of *E. guineensis* (Jacq.) used in this study showed different responses to carbon sources and basal medium. Eeuwen medium supplemented with sucrose (3%) was noted to favour the growth of the embryos *in vitro* by increasing the growth of plantlets especially in their fresh weights and long profuse roots as compared to other carbon sources in both media.

Conclusion

The micropropagation protocols for *E. guineensis* Jacq. as described in this study have the potential for shortening the germination time required to obtain *E. guineensis* plantlets when compared with normal soil germination. The plantlets, after hardening, would be raised *ex vitro* to ensure a steady supply of vegetable oil from the seeds. Somaclonal variants arising from this propagation method (Bhojwani and Razdan, 1996) would include those with desirable attributes that would enhance steady availability of elite cultivars of this plant for purposes of providing a cleaner and more environmentally friendly substitute for fossil fuel and a source of commercial vegetable oil.

Conflict of Interests

The authors have not declared any conflict of interests.

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