International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Agricultural Research

ISSN 1816-4897
DOI: 10.3923/ijar.2018.19.25

Research Article
Nutrient Requirements for *in vitro* Propagation of *Ricinus communis* L. Mature Zygotic Embryos

G.C. Louis, C.U. Okafor and C.E.A. Okezie

Department of Plant Science and Biotechnology, Plant Tissue Culture Unit, University of Nigeria, Nsukka, Nigeria

Abstract

Background and Objective: *Ricinus communis* (*R. communis*) is famous for its economic values and of great importance to researchers interested in the raising plantlets from hybrids. This study was carried out on the nutrient requirements for the *in vitro* propagation of *R. communis* employing three basal media using zygotic embryos as explants. **Materials and Methods:** Following the maintenance of asepsis, zygotic embryos were excised from mature seeds and cultured on the three basal media with 3% sucrose and 8 g L^{-1} of agar. Plant growth regulators were not added to the media. This study was done to determine the most suitable basal medium for the growth of *R. communis* zygotic embryo. **Results:** The results obtained showed that the three basal media employed supported *in vitro* regeneration of the embryo explants. The highest mean shoot length (4.450±0.231 cm), the highest mean root length (2.190±0.262 cm), highest mean fresh weight (0.365±0.032 g), highest mean leaf area (1.999±0.189 cm^2), highest mean percent sprouting (91.660±0.000) and highest mean number of roots (4.600±0.163) were observed on Murashige and Skoog medium whereas the highest mean sprout rate (0.330±0.000) was obtained on Murashige and Skoog and Gamborg B5 media. The embryo explants were able to develop into normal plantlets even in the absence of growth regulators. This may determine that endogenous hormones in the zygotic embryos were present at an optimal level to support regeneration. **Conclusion:** Results from this study indicated that Murashige and Skoog basal medium was the best basal medium for the *in vitro* propagation of *R. communis* zygotic embryos and for mass production.

Key words: *Ricinus communis*, Murashige and Skoog, Gamborg B5, Schenk and Hildebrandt, embryo explant

Citation: G.C. Louis, C.U. Okafor and C.E.A. Okezie, 2018. Nutrient requirements for *in vitro* propagation of *Ricinus communis* L. mature zygotic embryos. Int. J. Agric. Res., 13: 19-25.

Corresponding Author: C.U. Okafor, Department of Plant Science and Biotechnology, Plant Tissue Culture Unit, University of Nigeria, Nsukka, Nigeria
Tel: +2347035043578

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Castor plant (*Ricinus communis* L.) is a flowering plant that belongs to the family Euphorbiaceae, mostly indigenous to the tropics[1]. The name *Ricinus* originated from the latin word for tick due to the markings resembling that found in ticks[2]. According to Sujatha *et al.*[3], *Ricinus communis* originated from Abyssinia (Ethiopia) perhaps because of the presence of high diversity[4].

Castor seed is a vital source of vegetable and medicinal oil and has copious benefits to humanity[5]. The oil from castor seeds has many industrial and medicinal applications. The seeds contain 40-60% oil that is rich in triglycerides mainly ricinolein, a toxic alkaloid ricinine and very toxic albumen called ricin[6]. Oil from castor seeds is used in the paint and varnish industry, in the manufacture of a wide range of sophisticated products like nylon fibers, jet engine lubricants, hydraulic fluids, artificial leather and fiber optics[5]. Oil from castor seeds also has great promise in the field of biodiesel production as it is inexpensive, biodegradable, renewable and environment friendly[5]. Many phytochemicals found in the plant tissue and seeds of castor have potential medicinal uses[7]. Castor oil has been used as purgative[8] and has been effective in treating intestinal worm infestation. *In vitro* antiviral activity and hypoglycemic activity were also reported from leaf extracts[9]. Castor plant also provides an option for phytoremediation of soils contaminated with heavy metals since castor plant is tolerant to several heavy metals like lead, nickel, cadmium, zinc and is a non-food plant[10,11].

These benefits provided by this plant has made it necessary that the plant be properly investigated to develop the most reliable method of propagation[12]. Conventional method of cultivation using the seed is limited by problems of seed viability. Propagation through tissue culture techniques employing embryo, as done in this study, is necessary to eliminate the limitations of seed germination. In addition, combustion of fossil fuel contributes to emission of greenhouse gases which lead to atmospheric pollution and global warming[13]. As a result of these, there is the need for substitute for fossil fuel with a clean and renewable fuel such as biodiesel. According to De Oliveira *et al.*[14], oil extracted from castor seeds is a potential feedstock for biodiesel production which will be an alternative for fossil fuel. Nahar[15] also reported that castor plant is a sustainable source of second generation biodiesel feedstock species and the overall supply can be increased with different propagation technologies. Therefore, *in vitro* propagation of castor seed can help make it available all year round.

Embryo culture which involves the removal of the embryo from the seed and subsequent growth *in vitro* until the developing plant can be transplanted to the soil and grown to maturity has made it possible to obtain plants that otherwise would not have been recovered[16]. It is used for production of rare species, rescue of embryos that abort on the mother plant, in hybridization, inter-specific and inter-generic hybrids and production of haploid plants. It is also used to break dormancy in seeds, thereby shortening the breeding cycle by months or even years[17]. Nevertheless, tissue obtained from a plant to be cultured is called an explant and it may include portions of the shoots, leaves, stems, roots, flowers and single undifferentiated cells[18]. These tissues are grown or cultured in basal media with many formulations. These media formulations include white medium, the media of Gamborg *et al.*[19], Murashige and Skoog[20] and Schenk and Hildebrandt[21]. However, these media are high in macronutrients while the other formulations contain less of the macronutrients[16].

Many studies have been documented on the *in vitro* propagation of *Ricinus* from different explants using various media for several purposes. Danso *et al.*[22] reported the *in vitro* regeneration of *R. communis* employing the zygotic embryo. This was achieved using Murashige and Skoog[20] (MS) medium supplemented with different concentration of 6-Benzylaminopurine (BAP), Kinetin and N6-(2-isopentyl)adenine(2ip) (0.0-1.0 mg L^{-1}), sucrose 30 g L^{-1} and myo inositol (100 mg L^{-1}). Ahn *et al.*[23] studied the micropropagation of *R. communis* with hypocotyls tissues from zygotic embryo axis. The hypocotyls tissues produced adventitious shoots when treated with either thidiazuron (TDZ) or BA (N6-Benzyladenine) while indole butyric acid (IBA) induced rooting very effectively and plants established in the soil. In addition, Rahman and Bari[24] reported on the hormonal effects on *in vitro* regeneration of *R. communis*. This was achieved using cotyledonary node and shoot tip. Shoot proliferation was more efficient in cotyledonary node than in the shoot tip with Murashige and Skoog (MS) medium supplemented with 1.5-2.5 mg L^{-1} of N6-Benzyladenine (BA). *In vitro* proliferated shoots were rooted on MS medium. The best rooting (92.7%) was obtained with 0.2 mg L^{-1} of indole-butyric acid (IBA) accompanied with 0.6 mg L^{-1} of silver nitrate ($AgNO_3$). The report showed that the best medium for shoot proliferation of *R. communis* was Murashige and Skoog (MS) medium fortified with 2.0 mg L^{-1} of N6-Benzyladenine (BA) and its recalcitrant property could be minimized to some extent by using silver nitrate ($AgNO_3$) and charcoal in case of root induction. Also Ghorpade *et al.*[25] reported on *in vitro* zygotic embryo germination and

propagation of *Boswellia serrata* Roxb. The zygotic embryos were cultured on B5 (Woody Plant Medium), WPM and Schenk and Hildebrandt (SH) media fortified with different concentrations of sucrose and on Murashige and Skoog medium containing 3% sucrose, polyvinylpyrrolidone (PVP) (0-300 mg L^{-1}), gibberellic acid (GA3), indoleacetic acid (IAA), naphthaleneacetic acid (NAA), IBA or 2,4-Dichlorophenoxyacetic acid (2,4-D) and 6-Benzylaminopurine (BA) or kinetin individually. The highest frequency of embryo germination (96%) and conversion into seedling was obtained on MS medium. Other media were either inferior or induced abnormalities in the seedlings including callus formation from zygotic embryos. Similarly, Okafor and Okezie[26] also reported the supportive role of MS medium on the plantlet regeneration of mature zygotic embryos of *Elaeis guineensis*.

To the best of researchers knowledge, there were no reports in the literature highlighting the nutrient requirements of *R. communis* zygotic embryos on three basal media. This study was designed to determine the most suitable basal medium for *in vitro* culture of *R. communis* embryo and to work out a propagation protocol for *R. communis* employing zygotic embryos.

MATERIALS AND METHODS

Site of the experiment: This study was conducted at the Plant Tissue and Molecular Biology Laboratory of Plant Science and Biotechnology Department, University of Nigeria, Nsukka between October, 2016-February, 2017.

Source of explants: The explants (mature zygotic embryos) employed in this study were excised from matured seeds of *R. communis* obtained from Bio-resources Development and Conservation Programme, along Aku Road, in Nsukka Local Government Area of Enugu State, Nigeria during the season by June, 2016.

Media composition: The basal media used in this experiment were those of MS[20], B5[19] and SH[21]. For MS, the medium was composed of macronutrients (nicotinic acid). On the other hand, B5 was composed of macronutrients ($MgSO_4.7H_2O$, KNO_3, $CaCl_2$, KNO_3, KH_2PO_4, NH_4NO_3, $CaCl_2.2H_2O$ and $MgSO_4.7H_2O$), micronutrients ($MnSO_4.4H_2O$, KI, $ZnSO_4.7H_2O$, H_3O_3, $CoCl_2.6H$ $Na_2MoO_4.2H_2O$ and $CuSO_4.5H_2O$), iron source ($Na_2EDTA.2H_2O$ and $FeSO_4.7H_2O$) and vitamins (myo-inositol, thiamine-HCl, pyridoxine and $2H_2O$, $NaH_2PO_2.H_2O$ and $(NH_4)_2SO_4$), micronutrients (H_3BO_3, $CoCl_2.6H_2O$, $CuSO_4.5H_2O$, KI, $Na_2MoO_4.2H_2O$, $ZnSO_4.7H_2O$ and $MnSO_4.2H_2O$), iron source ($FeSO_4.7H_2O$) and vitamins (myo-inositol, nicotinic acid, pyridoxine-HCl and thiamine-HCl). The SH medium comprised macronutrients (KNO_3, $MgSO_4.7H_2O$ and $NH_4H_2PO_4$), micronutrients (H_3BO_3, $CoCl_2.6H_2O$, $CuSO_4.5H_2O$, KI, $Na_2MoO_4.2H_2O$, $ZnSO_4.7H_2O$ and $MnSO_4.2H_2O$), iron source ($Na_2EDTA.2H_2O$ and $FeSO_4.7H_2O$) and vitamins (myo-inostol, nicotinic acid, pyridoxine-HCl and thiamine-HCl).

Sterilization techniques and embryo transfer: The seeds of *R. communis* were washed in a running tap water to remove visible dirt and then were soaked overnight to imbibe water. This was followed by surface sterilization. The seeds were sterilized by immersion in 70% ethanol (v/v) for 5 sec and subsequently in 10% (v/v) sodium hypochlorite (NaOCl) from commercial bleach (Jik) for 10 min, followed by four rinses in sterile distilled water. Prior to inoculation on the growth media, the seed coats were removed and the embryos were excised from the endosperm using a pair of sterile forceps and scalpels and placed on a 9 cm diameter Whatman No. 1 filter paper in a petri dish. Embryo explants were transferred singly into culturing vessels. 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 by cool white fluorescent tubes. The process of surface-sterilization of seeds and the inoculation of embryo explants were all done in a laminar air-flow hood that was previously sterilized by swabbing absolute ethanol and exposure to ultraviolet light for 30 min.

Experimental design: The experiment was carried out in a Completely Randomized Design (CRD). The experiment comprised twelve replications per treatment. Each replication consisted of one embryo per culture tube.

Regeneration studies: Regeneration of embryo explants of *R. communis in vitro* were monitored daily from the time of inoculation. At the end of 2 weeks, regenerated plantlets under each treatment were scored for the following parameters: Length of shoots and roots produced, leaf area, fresh weight, number of leaves and number of roots produced. Time course in sprouting (%) and sprouting rate were determined from the first day till the eightieth day when sprouting had leveled off in all the treatments. The length of shoots and roots were measured with a string and a meter rule while the number of leaves and roots were determined by counting.

Statistical analysis: Data analysis was carried out using SPSS software. One-way Analysis of variance (ANOVA), followed by Duncan's New Multiple Range Test (DNMRT) was used to test for significance ($p \leq 0.05$) and to compare mean values. Error bars were represented at 5% value.

RESULTS

It was observed that within 3 days of culture of the embryo, the cotyledons which appeared white in colour at the onset gradually turned green on all the basal media. On the fifth day, the green colour of the cotyledons became prominent. There was protrusion of the radicle which emerged from the base of the embryo axis. The embryo elongated both at the root and shoot axes. Result obtained showed that plantlets that emerged from the modified basal media of MS, B5 and SH exhibited healthy growth. On the other hand, regeneration of embryo explant was observed when cultured on 0.7% agar only (no addition of carbon source or mineral salts). It was observed that sprouting occurred at 3 day as seen in the other media and at 6 day, the hypocotyls and the radicles elongated but stunted growth (2.13±0.22 cm) was observed.

Effect of the three basal media (MS, B5 and SH) on percent sprouting and sprout rate of *R. communis* zygotic embryo after 2 weeks of growth: Results from analysis of variance showed that there were no significant differences among the basal media in percent sprouting and sprout rate.

Table 1 shows that MS promoted maximum percent sprouting but did not differ significantly from the other basal media. Control on the other hand, had the least effect on percent sprouting. Also, both MS and B5 had the highest sprout rate but did not differ significantly from the other basal media whereas, SH medium had the least sprout rate.

Effect of basal media (MS, B5 and SH) on shoot length (cm) and root length (cm) of plantlets produced after 2 weeks of growth: Results from analysis of variance showed that there were significant differences among the basal media on shoot length and root length. Table 2 shows that MS had the highest shoot length and differed significantly from the other basal media. On the other hand, control had the least shoot length. Results also showed that MS promoted maximum root length but did not differ significantly from other basal media except from control. Control had the least root length (Table 2).

Effects of basal media (MS, B5 and SH) on fresh weight (g) and leaf area (cm^2) produced after 2 weeks of growth: Results from analysis of mean showed that there were significant differences among the basal media on fresh weight and leaf area.

Results also showed that MS promoted the maximum fresh weight but did not differ significantly from B5 but differed significantly from SH and control. On the other hand, control had the least effect on fresh weight. Results showed that MS had the highest effect on leaf area but did not differ significantly from other basal media except from control (Table 3).

Effects of basal media (MS, B5, SH and control) on number of leaves and roots produced after 2 weeks of growth: Results (Table 4) shows that there were no significant differences among the basal media on number of roots and leaves. However, MS gave the highest effect on number of roots but did not differ significantly from other basal media.

Table 1: Effect of basal media on percent sprouting and sprout rate of *R. communis* after 2 weeks of growth media

Basal media	Percent sprouting (Mean±SE)	Sprout rate (Mean±SE)
MS	91.66±0.00^a	0.33±0.00^a
B5	83.33±0.00^a	0.33±0.00^a
SH	83.33±0.00^a	0.26±0.04^a
Control	75.00±0.00^a	0.29±0.04^a

Mean values with the same alphabet in each column are not significantly different from each other by DNMRT ($p \leq 0.05$)

Table 2: Effects of basal media on shoot and root length of *R. communis* after 2 weeks of growth media

Basal media	Shoot length (cm) (Mean±SE)	Root length (cm) (Mean±SE)
MS	4.45±0.23^a	2.19±0.26^a
B5	3.30±0.22^b	1.70±0.17^{a,b}
SH	3.30±0.20^b	1.95±0.22^a
Control	2.13±0.22^c	1.25±0.16^b

Mean values with the same alphabet in each column are not significantly different from each other by DNMRT ($p \leq 0.05$)

Table 3: Effects of basal media on fresh weight and leaf area of *R. communis* after 2 weeks of growth

Basal media	Fresh weight (g) (Mean±SE)	Leaf area (cm^2) (Mean±SE)
MS	0.37±0.03^a	2.00±0.19^a
B5	0.23±0.01^b	1.59±0.12^a
SH	0.31±0.02^a	1.62±0.15^a
Control	0.19±0.05^b	1.17±0.06^b

Mean values with the same alphabet in each column are not significantly different from each other by DNMRT ($p \leq 0.05$)

Table 4: Effects of basal media on number of leaves and number of roots produced after 2 weeks of growth

Basal media	Number of leaves (Mean±SE)	Number of roots (Mean±SE)
MS	2.00±0.00	4.60±0.16
B5	2.00±0.00	4.44±0.24
SH	2.00±0.00	4.60±0.16
Control	2.00±0.00	4.20±0.29

DISCUSSION

In this study, there is a clear manifestation of a change from semi autotrophy to full autotrophy, a feature of *in vitro* systems. This was observed as the embryo explants of *R. communis* grown *in vitro* opened and changed from white to green colouration about 3 days from the time of inoculation which indicated that photosynthesis had probably started. It was observed that well developed plantlets were regenerated from the zygotic embryos of *R. communis* cultured on hormone-free basal media of MS, SH and B5. This would have been due to the presence of endogenous hormones within the plant, expressed after a lag phase during which a reasonable level of autotrophy had been attained. This is in contrast to findings of Pierik[27], who reported that *in vitro* culture is often impossible without growth hormone. However, Monnier[28] suggested that hormones should not be added to the embryo culture media because they cause structural abnormalities. Supporting this study, Smith and Drew[16] observed that matured embryos can be cultured on simple nutrient medium without growth regulators but culture of immature embryos require the addition of growth regulator. Previous report by Sahin-Demirbag *et al.*[29] achieved no shoot regeneration on MS medium without PGR but high frequency of shoot regeneration was recorded on MS medium containing different concentration of BAP+NAA with a range of 93.33-100%. Cosic *et al.*[30] reported that at different stage (early cotyledonary, cotyledonary and late cotyledonary) of the zygotic embryo of *Brassica oleracea* grown on PGR-free MS medium within 4 weeks developed into 1.5-3.0 cm long plantlets with well-formed roots and elongated hypocotyls.

Embryos cultured on the control (water and agar) had the least growth when compared to other media. This may be due to the absence of basic nutrients in control treatment since the zygotic embryo had been stripped of its food storage tissue. In addition, the growth differences observed in this study among the media may be due to the composition of each medium and the concentration of each component that made up the medium since they were all subjected to the same environmental conditions. In general, tissue growth and the quality of morphogenetic responses are strongly influenced by the type and concentrations of nutrients present in the culture media[31]. However, Murashige and Skoog (MS) medium is known to be a high salt medium when compared to many other formulations, with high levels of nitrogen, potassium and some of the micronutrients, particularly boron and manganese[32]. There was neither callus formation nor proliferation of shoots in this study. This may probably be due to the absence of growth regulators. Studies have shown that plant growth regulators induce callus formation and the growth of callus[33]. Jasrai *et al.*[34] obtained an embryogenic callus from the nodal explant of *Euphorbia pulcherrima* cultured on MS medium supplemented with NAA, 2,4-D, KN and 2ip. Kumari *et al.*[1] reported that the cotyledon explants of *R. communis* cultured on Murashige and Skoogs medium supplemented with B5 vitamins resulted in the formation of callus, multiple shoots and roots.

It was observed that MS medium gave the best response in all the growth parameters evaluated when compared to other basal media. According to Hamed *et al.*[35] evaluated the growth response of shoot tip of Jack fruit (*Artocarpus heterophyllus* L.) in three basal media (MS, B5 and WPM). It was observed that MS medium scored the highest significant number of shoot per explant which was followed by B5 medium. Niratker and Singh[36] also reported on the shoot induction of *Morus indica* in four basal media (MS, B5, SH and WPM). The MS medium was found to be the best medium for shoot induction as the highest shoot length with maximum number of leaves were obtained in MS medium. Three basal media (MS, SH and B5) were compared in this study and all the three basal media supported the *in vitro* regeneration of the *R. communis*, but MS medium was found to be significantly ($p \leq 0.05$) superior to SH and B5 in all growth parameters except in sprout rate where MS and B5 were significantly superior to SH. This may suggest that MS is better than B5 and SH for micropropagation of *R. communis* through embryo culture. Findings of Thangjam and Maibam[37] showed MS to be better in the induction of callus formation from cotyledon explants of *Parkia biglobosa* when compared to B5.

Furthermore, George[38] considered two important factors useful in trying to find media formulations suitable for different plant species and different kinds of cultures. It include: Total concentration of nitrogen in the medium and the ratio of nitrate and ammonium ions. Geroge[38] stated that a high proportion of ammonium nitrogen and the total nitrogen are higher in MS medium than in the most other basal media. Also, ions and the forms in which they are supplied are important in eliciting responses *in vitro*[20]. It is usually observed in most media that one type of ion may be contributed by more than one salt. In Gamborg *et al.*[19] medium, nitrate and potassium ions are contributed by potassium nitrate while sulphate ions are contributed by ammonium sulphate (NH_4SO_4).

Nutritional requirement for optimal growth of a plant tissue *in vitro* may vary with the species and tissues from different parts of a plant may have different requirements for satisfactory growth[20]. McCown and Sellmer[39] reported that some species show analogous response in all media while others show preference for a specific medium for explant establishment and growth. The most widely used culture medium is MS medium, because most plants respond to it favorably[40]. Although it is asserted that no single medium can be suggested as being satisfactory for all types of plant tissues and organs[41]. It has been conjectured by some other researchers that nutritional requirements differ according to species. Woody plant medium (WPM) favoured callus formation better than MS medium[42]. It is attributed that the difference in response to variations in the nutrient and mineral compositions of the two media. In support of this finding, Bhojwani and Razdan[43] reported that the number of adventitious buds differentiated from leaf explants of *Juniperus oxycedrus* was doubled in SH medium when compared to MS medium.

CONCLUSION

The micropropagation protocols for *R. communis* L. described in this study have the potential for the mass production of *R. communis* plantlets and for the multiplication of those endangered species whose propagation through conventional means is difficult. Results obtained in this study indicated that Murashige and Skoog medium (MS) was the most suitable for *in vitro* micropropagation of *R. communis* using embryo explants.

REFERENCES

1. Kumari, K.G., M. Ganesan and N. Jayabalan, 2008. Somatic organogenesis and plant regeneration in *Ricinus communis*. Biol. Plant, 52: 17-25.
2. Weiss, E.A., 1983. Oilseed Crops. Longman Group Limited, London, Pages: 282.
3. Sujatha, M., T.P. Reddy and M.J. Mahas, 2008. Role of biotechnological interventions in the improvement of castor (*Ricinus communis* L.) and *Jatropha curcas* L. Biotechnol. Adv., 26: 424-443.
4. Anjani, K., 2012. Castor genetic resources: A primary gene pool for exploitation. Ind. Crops Prod., 35: 1-14.
5. Ogunniyi, D.S., 2006. Castor oil: A vital industrial raw material. Bioresour. Technol., 97: 1086-1091.
6. Zohary, M., 1987. Flora of Palestine Israel. Acad. Sci. Human., 2: 296-300.
7. Naz, S., F. Tabassum, S. Javad, S. Ilyas, F. Aslam, N. Munir and A. Ali, 2011. Micropropagation and callogenesis of a recalcitrant species *Ricinus communis*. Pak. J. Bot., 43: 2419-2422.
8. Kalaiselvi, P., B. Anuradha and C.S. Parameswari, 2003. Protective effect of *Ricinus communis* L. leaf extract against paracetamol-induced hepatotoxicity. Biomedicine, 23: 97-105.
9. Khafagi, I.K., 2007. Variation of callus induction and active metabolite accumulation in callus cultures of two varieties of *Ricinus communis* L. Biotechnology, 6: 193-201.
10. Romeiro, S., A.M.M.A. Lagoa, P.R. Furlani, C.A. de Abreu, M.F. de Abreu and N.M. Erismann, 2006. Lead uptake and tolerance of *Ricinus communis* L. Braz. J. Plant Physiol., 18: 483-489.
11. Shi, G. and Q. Cai, 2010. Zinc tolerance and accumulation in eight oil crops. J. Plant Nutr., 33: 982-997.
12. Akande, T.O., A.A. Odunsi, O.S. Olabode and T.K. Ojediran, 2012. Physical and nutrient characterisation of raw and processed castor (*Ricinus communis* L.) seeds in Nigeria. World J. Agric. Sci., 8: 89-95.
13. Bankovic-Ilic, I.B., O.S. Stamenkovic and V.B. Veljkovic, 2012. Biodiesel production from non-edible plant oils. Renewable Sustain. Energy Rev., 16: 3621-3647.
14. De Oliveira, D., L.M. Di, C. Faccio, C.D. Rosa, J.P. Bender and N. Lipke, 2004. Optimization of Enzymatic Production of Biodiesel from Castor Oil in Organic Solvent Medium. In: Proceedings of the Twenty-Fifth Symposium on Biotechnology for Fuels and Chemicals Held May 4-7, 2003, in Breckenridge, CO., Finkelstein, M., J.D. McMillan, B.H. Davison and B. Evans (Eds.)., Vol. 31, Springer, Breckenridge, pp: 771-780.
15. Nahar, K., 2015. Castor Bean (*Ricinus communis* L.)-A Biofuel Plant: Morphological and physiological parameters propagated from seeds in Bangladesh. Asian Bus. Rev., 2: 64-66.
16. Smith, M.K. and R.A. Drew, 1990. Current applications of tissue culture in plant propagation and improvement. Funct. Plant Biol., 17: 267-289.
17. Uche, O.C., A.P. Ejiofor and O.C. Eziuche, 2016. Comparative growth rates of *Treculia Africana* Decne: Embryo in varied strengths of murashige and skoog basal medium. World Acad. Sci. Eng. Technol.: Int. J. Agric. Biosyst. Eng., 3: 555-558.
18. Sathyanarayana, B.N. and D.B. Verghese, 2007. Plant Tissue Culture: Practices and New Experimental Protocols. IK International Pvt Ltd., New Delhi, Pages: 176.
19. Gamborg, O.L., R.A. Miller and K. Ojima, 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res., 50: 151-158.
20. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Planta., 15: 473-497.

21. Schenk, R.U. and A.C. Hildebrandt, 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot., 50: 199-204.
22. Danso, K.E., N.T. Afful, C. Annor and H.M. Amoatey, 2011. *In vitro* regeneration of *Ricinus communis* L. and *Jatropha curcas* L. for biofuel production. Biotechnology, 10: 400-407.
23. Ahn, Y.J., L. Vang, T.A. McKeon and G.Q. Chen, 2007. High-frequency plant regeneration through adventitious shoot formation in castor (*Ricinus communis* L.). *In vitro* Cell. Dev. Biol. Plant., 43: 9-15.
24. Rahman, M.A. and M.A. Bari, 2010. Hormonal effects on *in vitro* regeneration of *Ricinus communis* L. cultivar shabje. J. Bio-Sci., 18: 121-127.
25. Ghorpade, R.P., A. Chopra and T.D. Nikam, 2010. *In vitro* zygotic embryo germination and propagation of an endangered *Boswellia serrata* Roxb., a source of boswellic acid. Physiol. Mol. Biol. Plants, 16: 159-165.
26. Okafor, U.C. and C.E.A. Okezie, 2016. Effect of carbohydrate source on the *In vitro* germination of *Elaeis guineensis* Jacq. zygotic embryos on two basal media. Afr. J. Biotechnol., 15: 1531-1540.
27. Pierik, R.L.M., 1997. *In vitro* Culture of Higher Plants. Kluwer Academic Publishers, The Netherlands, Pages: 348.
28. Monnier, M., 1978. Culture of Zygotic Embryos. In: Frontiers of Plant Tissue Culture. International Association of Plant Tissue Culture, Thorpe, T.A. (Ed.)., National Cereal Research Institute, Nigeria, pp: 203-223.
29. Sahin-Demirbag, N., H. Kendir, K.M. Khawar and C.Y. Ciftci, 2008. *In vitro* regeneration of Turkish dwarf chickling (*Lathyrus cicera* L) using immature zygotic embryo explant. Afr. J. Biotechnol., 7: 2030-2033.
30. Cosic, T., B. Vinterhalter, D. Vinterhalter, N. Mitic and A. Cingel *et al*., 2013. *In vitro* plant regeneration from immature zygotic embryos and repetitive somatic embryogenesis in kohlrabi (*Brassica oleracea* var. *gongylodes*). In vitro Cell. Dev. Biol.-Plant, 49: 294-303.
31. Niu, Z.X., L.N. Sun, T.H. Sun, Y.S. Li and H. Wang, 2007. Evaluation of phytoextracting cadmium and lead by sunflower, ricinus, alfalfa and mustard in hydroponic culture. J. Environ. Sci., 19: 961-967.
32. Cohen, D., 1995. The culture medium. Acta Hortic., 393: 15-24.
33. Lee, S.H., B.S. Kim, B.H. Lee, S.H. Won and J.K. Jo *et al*., 2004. Factors affecting callus induction and plant regeneration from mature seed of zoysiagrass (*Zoysia japonica* steud). Korean J. Soc. Grassland Sci., 24: 29-36.
34. Jasrai, Y.T., K.N. Thaker and M.C. D'souza, 2003. *In vitro* propagation of *Euphorbia pulcherrima* Willd. through somatic embryogenesis. Plant Tiss. Cult., 13: 31-36.
35. Hamed, A.M., E.A.M. Ali and E.S. Boshra, 2007. *In vitro* propagation of jackfruit (*Artocarpus heterophyllus* L.). J. Applied Sci. Res., 3: 218-226.
36. Niratker, P.C. and M. Singh, 2015. Effect of different type of media on *in vitro* regeneration of mulberry (*Morus indica*): An economically important tree. Ann. Biol. Res., 6: 22-26.
37. Thangjam, R. and R.S. Maibam, 2006. Induction of callus and somatic embryogensis of cotyledonary explants of *Parkia timoriana* (DC.) Merr. A multipurpose tree legume. J. Food Agric. Environ., 4: 335-339.
38. George, E.F., 2008. Plant Tissue Culture Produce. In: Plant Propagation by Tissue Culture, George, E.F., M.A. Hall and G.D. Klerk (Eds.)., Springer, The Netherlands, pp: 500-504.
39. McCown, B.H. and J.C. Sellmer, 1987. General Media and Vessels Suitable for Woody Plant Microculture. In: Cell and Tissue Culture in Forestry, Bonga, J.M., D.J. Durzan and M. Nijhoff (Eds.)., Vol. 1, Springer, The Netherlands, pp: 1-16.
40. Thirunavoukkarasu, M., P.K. Panda, P. Nayak, P.R. Behera and G.B. Satpathy, 2010. Effect of media type and explant source on micropropagation of *Dalbergia sissoo* Roxb.-An important multipurpose forest tree. Int. J. Plant. Sci., 1: 155-162.
41. Smith, R.H., 2000. Plant Tissue Culture Techniques and Experiments. Academic Press, USA., Pages: 228.
42. Orkpe, U. and A.I. Adesoye, 2008. *In vitro* Regeneration Studies on *Parkia biglobosa* (Jacq.) R. Br Ex Don, A Semi-Domesticated Multipurpose Tree. In: Proceedings of the 21st Annual Conference of the Biotechnology Society of Nigeria (BSN), Edema, M. and S. Uzochukwu (Eds.)., University of Agriculture, Abeokuta, Nigeria, pp: 71-78.
43. Bhojwani, S.S. and M.K. Razdan, 1996. Plant Tissue Culture: Theory and Practice. Elsevier Science, The Netherlands, Pages: 779.