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Germination response of coconut (*Cocos nucifera* L.) zygotic embryo

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ABSTRACT: The study investigated the effects of liquid and solid media in the propagation of coconut (*Cocos nucifera*) zygotic embryos at initiation stage. Eeuwen's medium supplemented with growth hormones naphthalene acetic acid (NAA) and indole butyric acid (IBA) at different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5mg/l) were used for this experiment in both liquid and solid states. Results showed that liquid state medium was better compared to solid state, with a successful shoot emergence of 46.7% of inoculants, compared to 13.3% in the solid state. Within 2 – 4 weeks, 77.8% of germinated zygotic embryo developed shoots in the liquid medium compared to 50% rate in the solid medium. Comparing zygotic embryo development in NAA and IBA, results showed that root yields were better in NAA, with inoculants in 1.5 mg/l NAA showing profuse rooting compared to 0.5 – 2.0 mg/l IBA with no root development.
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***Keywords*:** Coconut, Eeuwen's medium, hormones, root yield, zygotic embryo

The coconut palms (*Cocos nucifera* L.), usually termed as a "tree of life", is grown throughout the tropic regions (Waaijenberg, 1994). Virtually all parts of the coconut tree are utilized; from the nuts, husks, inflorescences, stems, and roots. The leaves are used for roofing and brooms, trunks are used for construction, and the copra can be processed into oil mainly for the soap industry, cosmetics, and candle wax (Campbell *et al.*, 2000). Coconut cultivars are generally classified into the Tall and Dwarf types. Tall coconuts grow to a height of about 20-30 m and are allogamous, late flowering, and their nuts are medium to large in size; they are hardy and thrive in a wide range of environmental conditions (Child, 1974). Dwarf coconuts grow to a height of about 10 - 15 m and are autogamous, early flowering, and generally produce a large number of small nuts with distinctive colour forms (Child, 1974).

The coconut palm is suffering from drastic production constraints, including pests and diseases and susceptibility to natural disasters. In addition, a number of aging coconut plantations are now being uprooted in order to make way for the planting of more portable crops (Muhammed, 2013). Therefore, there is an urgent need to implement efficient coconut germplasm via *in vitro* technique of tissue culture that allows germination and conversion into plantlets in a controlled environment.

Embryo culture micropropagation has several advantages over conventional propagation methods; for instance, the production of disease free, high quality planting materials and the rapid production of many uniform plantlets in a limited space area are achievable by this technology. In the present study, West African tall and Malayan orange dwarf zygotic embryos were used as the explants. Thus, the aim of this study was to understudy the germination responses of coconut (*Cocos nucifera* L.) zygotic embryo via liquid and solid nutrient media.

MATERIALS AND METHODS

This study was carried out using the facilities of Tissue Culture Laboratory of the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria.

Source of explants: Embryos were obtained from10 to 12 month old nuts, from Nigerian institute for oil palm research (NIFOR) substation Badagry in Lagos state. Harvested from mother plant of the West African Tall (WAF) and Malayan orange Dwarf (MOD), coconut palms which had been identified to be highly productive by the plant breeders.

Preparation of culture medium: The basal mediums used for this experiment were Eeuwens (1976) medium. The basal mediums were supplemented with growth hormones (auxins) naphthalene acetic acid (NAA) and indole butyric acid (IBA) at different concentrations 0.5, 1.0, 1.5, 2.0 and 2.5mg/l. The medium contained 30g/l sucrose plus 2.5 g/l activated charcoal, pH was adjusted to 5.7, medium was prepared without or with agar autoclaved at a temperature of 121°C for 20 min before use.
Embryo Extraction: The collected nuts (10 to 12 months old) were split transversely using a machete and endosperm surrounding embryo was excised from the split nuts using a clean knife, with a method adapted by (Molla *et al.*, 2004).

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Surface sterilization of endosperm extracts: The endosperm extracts were washed using detergents and rinsed under a running tap for 10 minutes, they were further rinsed in the laminar flow chamber with distilled water and sterilized for 3 minutes in 0.1% mercuric chloride. The endosperm were thoroughly rinsed with sterile distilled water, the embryos were then excised from endosperm using sterile scalpel. A method adapted by (cutter *et al* 1954) Cutter. Thereafter, embryos were then sterilized in 0.2% sodium hypochlorite for 3minutes, rinsed thrice in sterile distilled water and inoculated in a liquid medium and solid medium.

Culture conditions: After two weeks of culture, the liquid medium was decanted out and the embryos were transfer into a fresh medium and incubated in darkness for additional two weeks before transferring to light. All subsequent *in vitro* culture was carried out under dark and light photoperiod, 12-14 and 8-10 hours respectively. After six weeks, embryos that had started forming shoot were transferred onto semi-solid medium supplemented with the following plant growth regulators, naphthalene acetic acid (NAA) (0.5 – 2.5 mg/l) and indole butyric acid (IBA) (0.5 – 2.5 mg/l). Plantlets were transferred onto fresh medium every four weeks until they developed leaves and secondary roots.

RESULTS AND DISCUSSION

The culture of zygotic embryos of coconut as described in this report hold great potential for use in propagation work. In numerous other difficult-to-culture crops, using immature embryos and growth hormones lead to success in the propagation of the former.

Results showed that the better media state for inoculation of coconut zygotic embryo was liquid (Table 1, Plates 1 and 2). Out of the 15 test tubes inoculated, 60% successfully germinated. This was determined on the basis of initial root emergence. In the solid medium, only 26.7% of the inoculated embryos germinated. Further, 46.7% of the germinated rooting embryos successfully developed shoots within 2 – 4 weeks, amounting to a 77.8% rate, compared to 50% shooting success rate in the solid medium.

The findings of the present study are similar to the earlier report of El Rosario and De Guzman (1976) on the comparative outcome of the use of liquid and solid media to initiate Makapuno embryos. Similarly, Thuzar *et al*. (2012) reported the use of MS medium for rapid plant regeneration of oil palm zygotic embryos.

Table 1: Effect of culture in Eeuwems medium for coconut embryos germination between

Medium	Total number of test tube inoculated	Percentage zygotic embryo germination at 2 – 4 wks (%)	Successful shoot emergence of zygotic embryos b/w 2- 4 weeks (%)	Successful shoot establishment (%)	No. of contaminated culture (percentage, %)
Liquid medium	15	60.0	46.7	77.8	2 (13.3)
Solid medium	15	26.7	13.3	50.0	8 (53.2)

Plate 1: Zygotic embroyo in liquid medium

Plate **2**: Zygotic embryo in solid medium

As presented on Table 2 and Plates 3 – 5, there were shoot formations from immature zygotic embryos in some treatments between 4 – 6 weeks. The shoots were induced in liquid medium containing 1.5 mg/l of NAA, with the most shoot presence (60%) and the least shoot presence (20%) at 1.0mg/l concentration. There were root formations in all the treatments except 2.5mg/l. The zygotic embryo cultures in IBA hormone with 2.5mg/l concentration produced shoots with roots (20%); while those in 0.5, 1.0, 1.5 and 2.0 mg/l had no shoot and root formation. A similar observation was reported by Venkatesh *et al.* (2009) on the effects of auxins and concentration on groundnut.

These results probably suggest that the liquid medium provided optimum uptake of nutrients by the germinating embryos and also improved gaseous exchange than solid medium. Embryos were considered germinated when the plumule sprouts and the radicle shows signs of emergence as reported by Danson *et al*. (2009). By way of comparing; NAA was more preferred in shoot and root formation from 1.0 -1.5mg/l concentration than IBA.

Table 2: Effect of NAA and IBA concentrations on embryos germination in liquid medium between 4-6 weeks

Media	Total number of embryos inoculated	Percentage (%) successful of Shoot development	Root yields
NAA mg/l			
0.5	15	0.0	+
1.0	15	20.0	++
1.5	15	60.0	+++
2.0	15	0.0	+
2.5	15	0.0	-
IBA mg/l			
0.5	15	0.0	-
1.0	15	0.0	-
1.5	15	0.0	-
2.0	15	0.0	-
2.5	15	20.0	++

+ scanty roots, ++ slightly profuse roots, +++ presence of highly profuse roots. – absence of roots

Plate 3: Plant from zygotic embryo in liquid medium containing NNA (Tall)

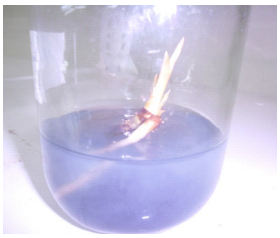

Plate 4: Plant from zygotic embryo in liquid medium containing NNA (Dwarf)

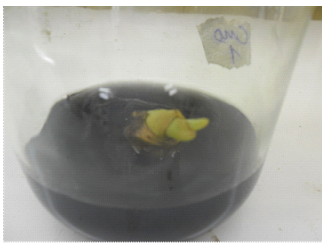

Plate 5: Plant from zygotic embryo in liquid medium containing IBA (Tall)

Conclusion: The preference of liquid media in embryonic growth initiation has been established in this study. Media supplementation with 1.0 -1.5mg/l NAA is also preferred. Germinated embryos may then be transferred onto solid medium.

REFERENCES

Campbell-Falck, D; Thomas, T; Falck, TM; Tutuo, N; Clem, K (2000).The intravenous use of coconut palm. *American Journal of Emergency Medicine* 18(1):108-111.

Child, R (1974). Coconut Tropical Agricultural Series. Second edition. Longman Group Ltd, London.

Cutter, VM; Wilson, KS (1954). Effect of coconut endosperm and other growth stimulants upon the development *in vitro* of embryos of *Cocos nucifera Botanical Gaz* (Chicago) 115: 234-239.

Danson, K; Quaicoe, R; Dery, S; Owusu-Nipah, J; Amiteye, S; Malaurie, L (2009). *In vitro* germination responses and plantlets development of healthy and diseased zygotic embryos of coconut accession. *International Journal of Botany* 7 (1):26-31.

Eeuwens, CJ (1976). Mineral requirements for growth and callus initiation os tissue explants excised from mature coconut palms (*Cocos nucifera*) and cultured *in vitro*. *Physiologia Plantarum* 36: 23-28.

El Rosario, AG; De Guzman, EV (1976). The growth of coconut 'makapuno' embryos *in vitro* as affected by mineral composition and sugar level of the medium during the liquid and solid cultures. *The Philippines Journal of Science* 105:215-222

Molla, MMH; Bhuiyan, MSA; Dilafroza, KM; Pons, B (2004). *In Vitro* coconut (*Cocos nucifera* L) embryo culture in Bangladesh. *Biotechnology* 3(1):98-101.

Muhammed, NR (2013). Zygotic embryo *in vitro* culture of *Cocos nucifera* L.(sv. East African Tall variety) in the coastal lowlands of Kenya. *African Journal of Biotechnology* 12(22):3435 - 3440.

Thuzar, M; Vanavichit, A; Tragoonrung, S; Jantasuriyarat, C (2012). Recloning of regenerated plantlets from elite oil palm (ElaeisguineensisJacq.) cv. Tenera. *African Journal of Biotechnology* 11(82): 14761 - 14770.

Venkatesh, K; Rani, AR; Baburao, N; Padmaja, G (2009). Effect of auxins and auxin polar transport inhibitor (TIBA) on somatic embryogenesis in Groundnut (*Arachis hypogaea* L.). *African Journal of Plant Science* 3(12):288-293.

Waaijenberg, H (1994). Mijikenda Agriculture in Coast Province of Kenya: Peasants in between Tradition, Ecology and Policy. Doctoral Thesis. Wageningen Agricultural University, Wageningen, the Netherlands. 307p