STATUS AND EFFECT OF PLANT GROWTH REGULATORS ON MORPHOGENESIS AND GROWTH RATE OF COCONUT ENDOSPERM IN VITRO

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ABSTRACT

Introduction *- Coconut is a significant palm in tropics and it gives a wellspring of occupation straightforwardly and by implication to in excess of 50 million smallholder farmers around the world. Coconut (Cocos nucifera L.) is a significant multipurpose tropical palm of the damp tropics that bolsters a huge number of individuals in coastal and island biological systems for their occupation. Individuals in excess of 93 nations flourish with coconut cultivation and related ventures.*

Aim of The Study – *The main aim of this study is to discuss the morphogenesis and growth rate of coconut endosperm in vitro.*

Methodology *– This is a qualitative report for which the Fruits of coconuts have been picked. The measurable procedure Mean, standard deviation, ANOVA and BioEstat software have been applied to test the growth of the coconut endrosperm.*

Conclusion *– It is presumed that the Coconut endosperm tissues could deliver 98.83% calli on 30 wk of culture. Endosperm position and plant growth regulators didn't fundamentally influence the growth pace of endosperm culture.*

1. INTRODUCTION

Coconut (Cocos nucifera L.) is really among the most vital tropical plant species and it is common in these areas as it can change in accordance with different nearby situations. This year, in excess of eleven million hectares of coconuts had been developed in eighty nations by more than fifty million little farmers. In the tropics, the coconut plant has most likely the biggest determination of employments. Plant territories are really used for food, oil creation, as construction data, flexibly of power, and beauty care products, among others. Despite the economic estimation of the coconut palm, productivity has been declining internationally over ongoing years because of maturing of vegetation. Restoring yields will call for growing a great deal of youthful palms of high yielding assortments just as hybrids. It will require the trading of developing materials including coconut creating nations. All things considered, developing material commonly traded utilizing nuts is very expensive because of the extraordinary size of theirs in addition to weight. Most importantly, universal trade of coconut germplasm is truly hampered, and furthermore in various examples totally prohibited, because of the odds of spreading illnesses that are in the coconut shell. Considering this, the Food just as Agriculture Organization of the United Nations just as

International Board for Plant Genetic Resources Technical Guidelines for the Safe Movement of Coconut Germplasm proposes universal trade of coconut germplasm to be led utilizing in vitro refined zygotic undeveloped organisms. Distinctive successful in vitro culture conventions have been begun for zygotic coconut embryo organisms.

1.1 Coconut (Cocos Nucifera L.) or Coconut Endosperm

Endosperm is an unmistakable tissue in its source, advancement and ploidy. It is shaped because of combination of three haploid cores—one from male gametophyte and the other two from female gametophyte. This wonder is called triple combination and is extremely normal in angiosperms. Over 80% of the blossoming plants are having endosperms in their creating seeds, which give nourishment to the developing undeveloped organism. Any variation from the norm in the advancement of endosperm may cause fetus removal of embryo organism bringing about sterile seeds. Endosperm might be completely devoured by creating embryo organism prompting the development of exalbuminous (non-endospermous) seeds or when it continues, the seed is called albuminous (endospermous). In albuminous seeds the enduring endosperm is utilized as a food source, which may contain proteins, starch or fats and the embryo organism can use this food during seed germination. Coconut Endosperm is the liquid endosperm of green coconuts Cocos nucifera. At this stage, these coconuts contain RNA-phosphorus (RNA-P), saw as especially high in youthful, green coconuts.

Coconut is a significant palm in tropics and it gives a wellspring of job legitimately and by implication to in excess of 50 million smallholder farmers around the world. It is developed in excess of 12 million ha in 90 nations for the most part in the Asian Pacific areas where Indonesia has been positioned as the biggest coconut developing locale followed by the Philippines and thirdly India. It has an assortment of conventional uses such food, drink, oil, fiber, lumber, cover, mats, fuel, and household utensils. The copra and its items produce food and non-foods which are ecologically well disposed and are utilized locally or sent out consequently turning into a wellspring of foreign trade. The virgin coconut oil is a rich wellspring of nutrients and enzymes which are utilized in the cosmetic and pharmaceutical ventures.

Coconut (Cocos nucifera L.) is a significant multipurpose tropical palm of the muggy tropics that underpins a large number of individuals in coastal and island biological systems for their business. Individuals in excess of 93 nations flourish with coconut development and related businesses. It has been generally utilized for its oil, and changed items from endosperm, nut water, delicate nut water, husk, fiber and leaves. Accentuation on item enhancement is viewed as significant in expanding productivity. Coconut hereditary assets protection and use methodologies in significant developing nations have cleared path for accomplishing the ideal outcomes in crop improvement and item expansion in coconut. India is considered as the pioneer in preservation and usage of coconut hereditary assets for advancement of improved coconut assortments for copra, oil, tender nut qualities, disease resistance and drought tolerance. The crop has been answered to display wide fluctuation for its organic products, blossoms, foliar attributes and other morpho-physiological qualities which have been broadly utilized for rearing new assortments. Item broadening and appropriation of improved assortments assume a significant job in improving the efficiency and productivity. Coconut enterprises are at present confronting difficulties in the worldwide situation attributable to the expanded expense of creation and lower costs. The status of coconut as an oilseed crop is being changed with accentuation as a food and nourishment crop in the ongoing occasions so as to build benefit

in coconut development. In coconut, various phenotypic attributes have been depicted to separate the assorted variety in coconut.

1.2 Plant growth regulators

A few synthetic compounds happening normally inside plant tissues (for example endogenously), have a regulatory, as opposed to a nutritional job in growth and improvement. These mixes, which are commonly dynamic at exceptionally low focuses, are 29 known as plant hormones (or plant growth substances). Engineered synthetic concoctions with comparable physiological exercises to plant growth substances, or mixes having a capacity to change plant growth by some different methods are typically named plant growth regulators.

There are a few perceived classes of plant growth substance. Until moderately as of late just five gatherings were perceived specifically:

- auxins
- cytokinins
- gibberellins
- ethylene
- abscisic acid

1.3 Roles of Plant Growth Regulators

- Callus Induction
- Chemical Pruning
- Defoliant
- Desiccant
- Fruit Ripener
- Germination
- Growth Inhibitor
- Growth Retardant
- Rooting
- Thinner

1.4 Plant morphogenesis

The source and improvement of plant structure and structure. Morphogenesis might be worried about the entire plant, with a plant part, or with the subcomponents of a structure. The foundation of contrasts at the two finishes of a structure is called extremity. The term morphogenesis by and large alludes to the procedures by which request is made in the creating organism. This request is accomplished as separated cells cautiously sort out into tissues, organs, organ frameworks, and eventually the organism all in all.

Growth and morphogenesis of plant tissues in vitro are to a great extent administered by the structure of the supplement medium. The fundamental prerequisites of refined plant tissue are like those of entire plants in practice. Nutrient media for plant tissue culture are intended to permit plant tissues to develop and keep up their life in a totally artificial condition.

Morphogenesis in culture continues along various pathways. Of them, two are significant pathways - organogenesis and substantial embryogenesis. Organogenesis incorporates direct beginning of extrinsic shoots or roots and by implication by means of callusing. Embryogenesis likewise has two pathways where the result contrasts in the structure "bipolar substantial undeveloped organisms" which in later stage structure singular plantlets.

A few components impact the marvel of morphogenesis impressively during society. They are: genotypes, explant, growth regulators, nutrients, different additives and physical condition.

1.5 Factor affecting Morphogenesis

- ✓ Genotype
- ✓ Explant
- ✓ Growth regulators
- ✓ Nutrient medium
- ✓ Other additives
- ✓ Culture condition
- ✓ Loss of morphogenetic capacity

1.6 Morphogenesis in vitro

Cultures in vitro fit for morphogenetic potential at first lose the capacity on the off chance that they are sub-cultured over and again. Such subcultures may bring the progressions at hereditary, epigenetic and physiological levels. Variety in ploidy level of cells cultured is the standard change happening at genetical level. Such varieties might be either polyploidy or aneuoploidy. Now and then quality changes likewise happen in the cultured cells.

The epigenetic level changes happening in culture are incompletely steady yet reversible. Adjustment to an incomplete specific part may deliver morphogenetic misfortune in vitro culture. For instance, the embryogenic cultures developed in auxin in addition to medium would create substantial undeveloped organisms when the cultures are moved to auxin free medium. The constant culturing of callus or suspensions would lose the morphogenetic potential. This might be because of higher convergence of endogenous auxin. Be that as it may, these cultures can be made to create embryo organisms by exhausting endogenous auxin level. For this the medium ought to have actuated charcoal which can possibly retain certain measure of auxin. Diminished growth rate less friability and senescence of cultures are the progressions that happen at physiological level. These progressions are impermanent and insecure. By giving ideal concoction and physical condition, such morphogenetic misfortunes can be survived. In this way, there are numerous purposes behind the loss of morphogenetic capacity by cultures, however there indicate number of procedures that will assist with diminishing, if not dispose of, the issue.

2. REVIEW OF LITERATURE

Rafaeli Aparecida Vieira de Souza, et al (2013) - The experiment was carried out to figure out the correct serving of coconut water as supplement for in vitro cultivation of zygotic embryos from nineteen olive genotypes. The isolated embryos of the olive seeds had been immersed on culture medium containing zero (control), twenty-five, fifty, along with 100mL L^{-1} of sterile and fresh coconut water and then maintained for forty-five days under controlled setting. The percentage of germination, shoot length, selection of roots, number of number as well as leaves of internodes had been assessed for those nineteen olive genotypes. The ANOVA of the parameters evaluated showed considerable genotypes x doses of coconut water interaction for shoot length, selection of number and leaves of internodes as well as the serving of 100mL L^{-1} produced the very best benefits overall as suggested by the ways of assessed parameters. Nevertheless, the study showed the benefits of figuring out the correct serving of coconut water for every genotype under consideration as proven by substantial genotype x dose of coconut water interaction effect.

Augustine Jerard Bosco, et al (2013) - The latest genetic assets explorations undertaken within Andaman Islands revealed the occurrence of coconut palms yielding gentle endosperm with the organic coconut population known as Thairu thengai or maybe Nei thengai. The characterization of Thairu thengai accession from Andaman Islands making use of the in-situ observations on 7 palms revealed that the palms belong to taller number of coconuts. The palms of this particular kind bear fruit with both soft and normal endosperm. Approximately sixteen to twenty-seven per cent of the fruits a bunch had been observed to remain with smooth endosperm as well as the remaining ones were with regular firm endosperm. The regular nuts of the identified palms created low quality copra indicating some amount of softness. The gentle endosperm fruits are actually of 3 kinds with difference in the levels of soft endosperm, nut water as well as firm endosperm. The

palms are actually discovered to take place sporadically in South Andaman. While these palms look morphologically looking much like other coconut palms except for endosperm trait, differences might be noticed for a lot of morphological and fruit component traits. The morphological and fruit component traits of Thairu thengai coconut are actually in contrast to the additional coconut populations. The methods for utilization as well as preservation of this particular novel variety of coconut are proposed.

P. I. P. Perera, et al (2009) - Coconut is actually a cross pollinating palm, propagated solely by seeds. Tissue culture is the one vegetative propagation technique readily available for coconut. Constant callogenesis was received by culturing unfertilised ovaries at -4 phase of CRI seventy-two medium containing hundred M 2,4 dichlorophenoxyacetic acid (2,4 D) as well as 0.1 % activated charcoal. Callusing was enhanced by application of nine M thidiazuron (TDZ). Embryogenic calli had been subcultured upon somatic embryogenesis induction moderate containing sixty-six M 2,4 D. Stunted growth was noticed in the somatic embryos following subculture onto CRI seventy-two medium that contains abscisic acid (ABA). Maturation of somatic embryos might be accomplished in Y_3 medium with no growth regulators. Change of somatic embryos was caused by including gibberellic acid (GA_3) to conversion medium containing five M 6 benzyladenine (BA) while 2 isopentyl adenine (2iP) enhanced the frequency of plant regeneration. A total of eighty-three plantlets was made from thirty-two cultured ovaries.

3. PROPOSED METHODOLOGY

3.1 Research design

Three assessments were made for the quantitative examination of pre-implantation embryo organism advancement (cleavage, blastocyst, and hatching) as indicated by the example set up by the International Embryo Transfer Society (IETS) Manual.

3.2 Plant material

Products of coconuts were gotten newly from coconut palm trees. Two products of one bundle were taken from one coconut palm tree. The coconuts utilized in this examination were gathered from a healthy tall assortment of coconut palm developed in the trial station.

3.3 Experimental Groups

Culumus-oocyte buildings were preserved in microcentrifuge tubes at 30°C and shielded from light with aluminium foil. Three recreates were led with 20 to 25 COCs in 1 ml preservation medium. The chose COCs were randomly doled out to the accompanying experimental gatherings.

- Control Group: in vitro develop COCs after selection.
- Groups H6, H9, and H12: COCs preserved in TCM-199 + Hepes for 6, 9, and 12 h, separately.
- Groups C6, C9, and C12: COCs preserved in coconut water arrangement comprised of 75 ml of separated coconut water, 25 ml of ultrapure water and 25 mM Hepes support (last osmolarity: 290 mOsmol/L and pH: 7.2) included with 10% FCS (v/v), pyruvate and antibiotics for 6, 9, and 12 h, individually.

3.4 In vitro maturation

Cumulus-oocyte buildings were incubated in IVM (TCM 199 enhanced with sodium bicarbonate, 10% FCS (v/v), 5 µg/ml FSH, 50 µg/ml LH, 10 µg/ml insulin, 50 µM β-Mercaptoetanol, pyruvate, and antibiotics) after explicit preservation periods. Hatching was completed in Petri dishes with 100 µl of IVM (10-13 COCs per drop) under clean mineral oil in a culture incubator with 5% CO2

in air at 38.5oC and soaked humidity. The complete IVM time was 24 h, that is, IVM for 18, 15, and 13 h and preservation for 6, 9, and 12 h, separately.

3.5 Statistical Techniques Used in This Study

The outcomes are communicated as mean ± standard deviation and were analysed utilizing the BioEstat 3.0 software. Cleavage (%), blastocyst (%), and incubated blastocyst (%) rates were changed to arcsine and assessed by analysis of difference (ANOVA - Bonferroni post-test; importance level of $P < 0.05$). Callus growth was controlled by deduction of the initial fresh weight from the last fresh weight isolated by the original weight. Growth rate was registered from callus growth partitioned by the quantity of weeks in culture. This information was examined utilizing the GLM and Contrast systems in the SAS software.

4. DATA ANALYSIS AND RESULT

4.1 Morphogenesis of Coconut In Vitro

Not many coconut seedling leaf explants created callus during the principal explore, albeit numerous explants showed impressive development in vitro. Modest quantities of callus shaped along the cut edges of some explants (Fig. 1 an), and on the surface of a couple of vascular edges. Just four of the 150 coconut leaf explants exhibited some other growth reaction, and all were on details containing both 2,4-D and DES. One leaf explant built up a few knobs on the surface of a vein on the underside of the explant. After ca. a half year, a solitary knob kept on augmenting, and built up a root (Fig. 1 b). Another single explant created various light-yellow globular structures on the two sides of the leaf explant. A portion of the globular structures expanded in size for 42 weeks, though others became hyperhydric and in the long run necrotic. A few knobs created on the two sides of the midrib and petiole of one explant and extended after some time (Fig. 1c). These nodules gave off an impression of being like the beginning phase coconut somatic embryos detailed somewhere else

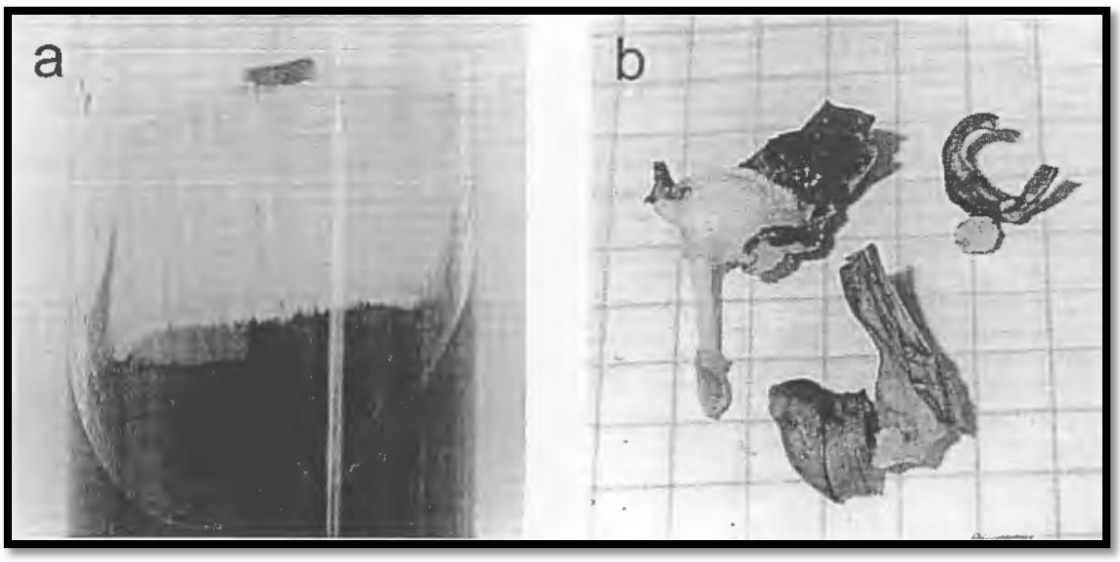

Figure l a: Callus formed in vitro on of immature coconut

Figure 1 b-c: "nodules" and roots

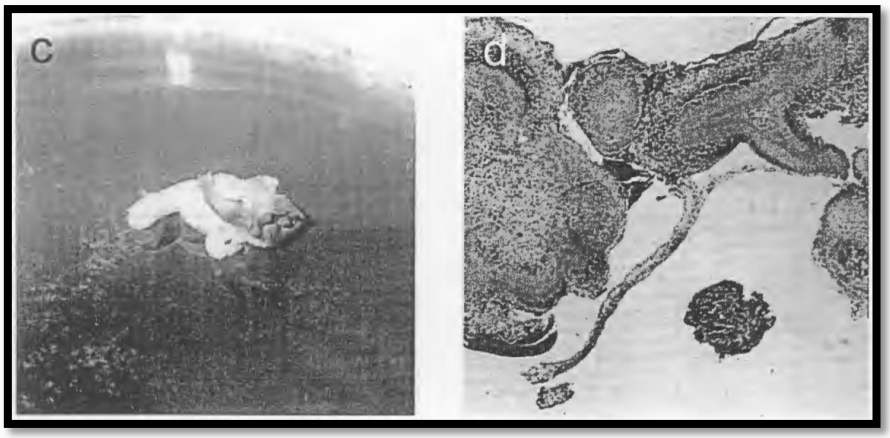

Figure l d-e: tissue differentiation and organization

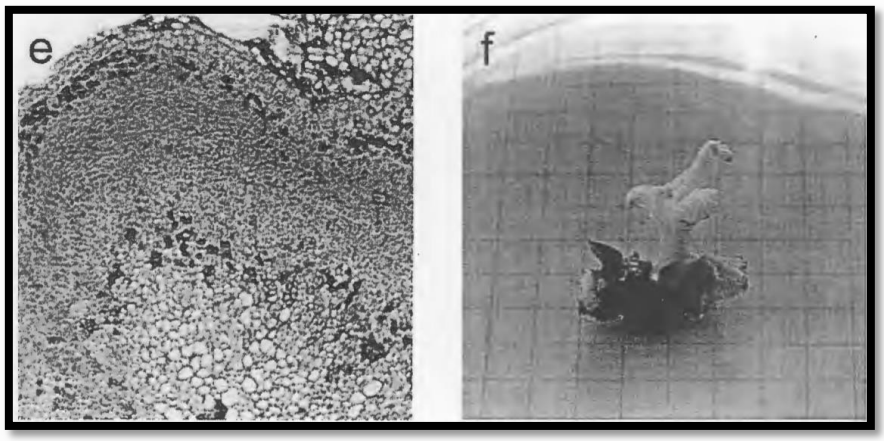

Figure l f: Adventitious shoot arising in vitm

The acceptance of embryogenic and organogenic cultures from coconut leaf explants on adjusted Y3 medium containing 2,4-D and DES has been portrayed. Albeit typical somatic undeveloped organism development and germination were not watched, this embryogenic pathway speaks to another methodology that can be explored for clonally proliferating coconut in vitro. Studies are in progress that address a portion of the issues related with development of coconut somatic embryos.

4.2 Growth rate of coconut endosperm

The growth rate of endosperm cultures expanded considerably from 8 to 30 wk, while the growth rate began to diminish on 25 wk of culture. The situation of endosperm among micropylar and antipodal tissues in coconut organic product didn't influence the growth rates of the endosperm culture. At the point when endosperm explants of coconut organic product more seasoned than 7 mth were utilized, the micropylar area was still meristematic and created callus yet the antipodal district might not have still been meristematic thus couldn't deliver callus.

There was no noteworthy distinction in the growth rate of tissues rewarded with 2,4-D contrasted with picloram. Various outcomes announced that picloram was quicker than 2,4-D for callogenesis, embryo organism acceptance and the last yield of embryos in Gasteria and Haworthia. Then again, picloram was slower than 2,4-D for callogenesis in sugarcane and 2,4-D was the best contrasted

with different auxins in coconut culture. These outcomes could have been because of contrasts in the plants or explants.

The concentrations of 2,4-D and picloram affected the growth rate of coconut calli. Auxins (2,4-D and picloram) at 1×10^{-3}M fundamentally diminished the growth rate on 9 wk of culture however the growth rate distinction was not huge in the wake of being moved to 1×10^{-6}M on 20 wk of culture. All growth regulator concentrations had comparative growth elements in that the growth rate expanded until 26 wk. From there on, the growth rate of cultures initially presented to growth regulators declined, while the growth rate of the control kept on expanding (allude Table no. 1). These outcomes vary from those of different examinations that have demonstrated that auxin at high concentrations were essential for callus enlistment, particularly on medium supplemented with 1–3 $g.L^{-1}$ AC. This was on the grounds that coconut endosperm has some auxin action, which is expanded via autoclaving and gives profoundly dynamic normal cytokinin. Expansion of AC in the media may steadily retain and discharge plant growth regulators bringing about an expansion in the viable amount of absolute plant growth regulators, which at that point restrain the growth rate of the coconut endosperm.

Table 1: Coconut endosperm's Growth rate

Source	Time (wk) 8	15	20	25	30	Average % Callogenesis
Position of Endosperm						93.94
Antipodal	62 ± 7 [a]	150 ± 14 [a]	277 ± 28 [a]	441 ± 48 [a]	184 ± 25 [a]	99.98
Micropylar	57 ± 6 [a]	140 ± 14 [a]	265 ± 26 [a]	423 ± 47 [a]	185 ± 27 [a]	99.92
Auxin:						
2,4-D	63 ± 7 [a]	149 ± 14 [a]	268 ± 27 [a]	439 ± 47[a]	189 ± 23 [a]	99.64
Picloram	56 ± 6 [a]	141 ± 14 [a]	273 ± 28 [a]	425 ± 47 [a]	180 ± 29 [a]	99.99
Concentration						
0 M	73 ± 9 [a]	133 ± 20 [ab]	263 ± 57 [a]	438 ± 99 [a]	475 ± 112 [a]	99.74
1×10^{-6}M	73 ± 9 [a]	157 ± 18 [ab]	266 ± 39 [a]	416 ± 66 [a]	152 ± 29 [b]	100.00
1×10^{-5}M	62 ± 8 [a]	167 ± 17 [a]	276 ± 38 [a]	459 ± 75 [a]	169 ± 24 [b]	99.79
1×10^{-4}M	68 ± 11 [a]	150 ± 18 [ab]	297 ± 44 [a]	438 ± 75 [a]	101 ± 12 [b]	99.79
1×10^{-3}M	23 ± 4 [b]	112 ± 26 [b]	249 ± 41 [a]	413 ± 71 [a]	170 ± 22 [b]	98.48
Cytokinin						
BAP 0 M		139 ± 12 [a]	264 ± 25 [a]	446 ± 47 [a]	201 ± 31 [a]	99.37
1×10^{-5}M		152 ± 16 [a]	279 ± 29 [a]	415 ± 47 [a]	138 ± 14 [a]	99.79

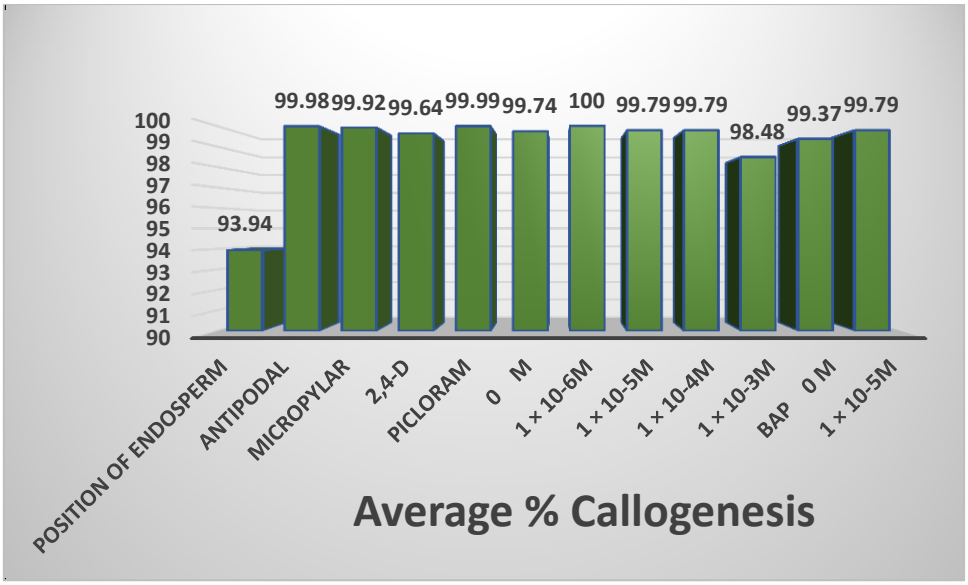

Figure 2: Coconut endosperm's Growth rate (Average % Callogenesis)

Mean qualities in a similar factor followed by a similar letter in every column are not fundamentally unique at 0.05 likelihood level.

The nearness of an undeveloped organism in the endosperm explants was plainly superfluous for callus commencement in coconut endosperm. This outcome didn't concur with Kumar et al. (1985) who recorded that callus commencement in coconut endosperm happened with an ally to the undeveloped organism. Endosperm tissue from the micropylar and antipodal position didn't contrast essentially in growth rate. This outcome didn't adjust with the work by Abraham and Mathew (1963) where the endosperm from the micropylar locale was more meristematic than that from the antipodal area.

An embryo organism like structure showed up from the antipodal situation of endosperm calli that was initially rewarded with 1×10^{-6} M picloram on 30 wk of culture. This showed antipodal tissue could create embryogenesis/organogenesis. This outcome was in opposition to Blake (1990) who inferred that drawn out culture of coconut callus couldn't be grown further. Morphogenesis happening in long haul cultures has been broadly concentrated in a few plants and demonstrated that picloram indicated potential for initiating embryogenesis/organogenesis in long haul culture in coconut endosperm calli.

The expansion of BAP didn't influence altogether the growth rate of coconut endosperm culture. Various outcomes have been accounted for that BAP could increment extraordinarily the fresh weight of coconut callus. These outcomes could have been because of contrasts in the plants or explants.

Allies for the coconut embryo and plant growth regulators were not expected to instigate callogenesis in the coconut endosperm culture in vitro. The accomplishment in accomplishing high callogenesis of the coconut endosperm culture in vitro is significant and could have been because of genotype, physiological age, shielding the explants from concoction disinfectants and media plan. The effective development of embryo-like structures on the coconut endosperm might be have been because of the media detailing, particularly the incorporation of picloram. Picloram has additionally been accounted for to keep up regenerative callus lines in long haul tissue

culture of sugarcane and to initiate somatic embryos of immature zygotic embryos on Phyla nodiflora.

4.3 Embryo development

Embryo advancement was examined in triplicate, adding up to 535 COCs over every single test gathering. There was no distinction among cleavage and hatching rates ($P > 0.05$) among medicines. There was a lower ($P < 0.05$) blastocyst rate in H9, H12, C9 and C12 when contrasted with the Control Group just as H6 and C6.

Table 2: Development of Embryo of oocytes preserved in coconut water solution In vitro

Group	Preservation + IVM (h)	Blastocyst (%)	Hatching* (%)	Cleavage (%)
Control	0+ 24	51.2 ± 9.6^{a}	80.4 ± 4.1^{a}	71.2 ± 7.0^{a}
H6	6+ 18	50.8 ± 8.2^{a}	76.8 ± 4.8^{a}	70.4 ± 6.7^{a}
H9	9+ 15	34.8 ± 9.6^{b}	75.8 ± 12.6^{a}	75.1 ± 10.2^{a}
H12	2+ 12	35.4 ± 7.1^{b}	72.1 ± 9.5^{a}	51.6 ± 7.2^{a}
C6	6+ 18	50.5 ± 5.2^{a}	81.1 ± 4.1^{a}	79.8 ± 11.6^{a}
C9	9+ 15	36.8 ± 9.8^{b}	79.2 ± 6.7^{a}	70.5 ± 13.4^{a}
C12	12+ 12	36.5 ± 6.5^{b}	76.2 ± 5.7^{a}	72.4 ± 14.8^{a}

The preservation of juvenile ox-like COCs for up to 6 h in these conditions showed that both coconut water arrangement and TCM-199 + Hepes are reasonable culture media. The preservation time frames 9 and 12 h didn't impact either cleavage or hatching rates contrasted with those of the Control Group. Be that as it may, a decline in embryo improvement up to the blastocyst stage was watched, paying little heed to the preservation medium utilized. This was most likely because of the conditions utilized in the current test. This is by all accounts amended by the utilization of hypophyseal hormones and fitting temperatures, as saw by Leivas et al. (2004), who got progressively agreeable creation of embryos after the preservation of bovine COCs in TCM 199-Hepes enhanced with FSH at 39°C for up to 12 h.

5. CONCLUSION

Coconut endosperm tissues could create 98.83% calli on 30 wk of culture. Endosperm position and plant growth regulators didn't altogether influence the growth rate of endosperm culture. A concentration of auxins at 1 × 10-3M essentially restrained growth rate until 8 wk of culture however didn't hinder from that point. The growth rate of the control was the speediest at 30 wk of culture. BAP didn't fundamentally influence the growth rate of coconut endosperm culture. Embryo-like structures were delivered from the antipodal position rewarded with 1×10^{-6}M picloram. This investigation has given the main report of embryo-like structures happening on coconut endosperm culture.

The utilization of more affordable and similarly proficient media, for example, coconut water arrangement contrasted with TCM-199 + Hepes may guarantee a noteworthy decrease in the expense of the preservation medium. In any case, further examination on the supplementation of coconut water arrangement, basically with hormones, is important for transport times longer than 6 h.

REFERENCES

1. Rafaeli Aparecida Vieira de Souza, et al (2013) – “Effect of coconut water on growth of olive embryos cultured *in vitro”,* Ciência Rural,

Print version ISSN 0103-8478, Cienc. Rural vol.43 no.2

2. Bosco, Augustine Jerard & V, Damodaran & Vittal, Niral & Kukkamgai, Samsudeen & M.K., Rajesh & Sankaran, Murugan. (2013). "Conservation and utilization of soft endosperm coconut accession from Andaman Islands", Journal of Plantation Crops. 14.
3. Engelmann F, Malaurie B, Oulo N (2011) "In vitro culture of coconut zygotic embryos. In: Thorpe T, Yeung E (eds) Plant embryo culture: methods and protocols, Methods in molecular biology series", Humana Press, Totowa, NJ, pp 63–74
4. Suranthran P, Sinniah UR, Subramaniam S (2011) "Effect of plant growth regulators and activated charcoal on in vitro growth and development of oil palm (Elaeis guineensis Jacq. var. Dura) zygotic embryo", Afr J Biotech 10:10600–10606
5. Defan AZB, Konan JL, Ablan RA, Kouamé LP (2011) "Characterization physico-chemical of almond hybrid plant vitroplants coconut palm (Cocos nucifera L.) PB121 according to stages of maturity and the shelf life of the nuts", Sci Nat 8: 63–71 (in French)
6. Yong, J.W.H., L. Ge, Y.F. Ng and S.N. Tan. (2009). "The chemical composition and biological properties of coconut (Cocos nucifera L.) water", Molecules 14: 5144–5164
7. Perera, P.I.P., Vidhanaarachchi, V.R.M., Gunathilake, T.R. *et al*. (2009) – "Effect of plant growth regulators on ovary culture of coconut (*Cocos nucifera* L.)", *Plant Cell Tiss Organ Cult* **99,** 73–81 (2009). https://doi.org/10.1007/s11240-009-9577-z
8. M.S. Cordeiro, et al (2006) – "The use of coconut water solution (Cocos nucifera) as a holding medium for immature bovine oocytes for in vitro embryo production", Anim. Reprod., v.3, n.3, p.376-379, Jul./Sept. 2006