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Assessment of media modification and induction of calli on coconut (*Cocos nucifera* L.) palm explants

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Abstract

The study was aimed at achieving possible successful in vitro culture of coconut palm (Cocos nucifera L.) Four (4) modified Eeuwensmedia were identified and augmented with various concentrations and combinations of plant growth regulators (auxins and cytokinins). Non- chlorophyllous immature leaves and immature inflorescences of coconut served as explants and were cultured onto the modified media and incubated under dark conditions in the growth room at 25°C. In order to ensure viable cultures, bi- weekly subcultures were carried out for eight (8) weeks. The results showed that calli was formed on the immature inflorescences explants in the modified Eeuwens medium augmented with 2,4-Dichlorophenoxy acetic acid (2,4-D) at 60mg/l in combinations with 2 mg/l6-Benzyl amino purine (BAP) after six (6) weeks of culture. The results obtained showed that calli generation was not possible from both explants at 2,4-D concentrations between 0 - 10 mg/l and otherplant growth regulators used for media supplementation.

Keywords: *Coconut*, explant, auxin, cytokinins culture, *in vitro*..

Introduction

Plant tissue culture techniques are an incentive for basic research as well as a technology with potential and established applications in agriculture. Plant tissue culture offers a wide range of applications, including vector-based and vector-free genetic cell transformation, embryo rescue, somatic embryogenesis, and commercial clonal propagation[1]. Many articles document how simple adjustments to the circumstances, particularly chemical components of the culture medium, enable previously stubborn cultures to operate as expected[2]. In vitro culture has found widespread application in crop enhancement biotechnology. The culture of live materials (explants) in vitro on a predetermined nutritional medium under sterile circumstances is referred to as in vitro culture[3]. The use of the developing factor on younger seedlings has resulted in the development of many offshoots[4]. The expense of those tests, however, cannot be instantaneously subordinated in order to propagate leading armaments. Conversion of inflorescences or vegetation into asexual buds is rarely acknowledged. As a result, tissue culture is a viable strategy for vegetative breeding of coconut. One of the most important tree crops in Nigeria is the coconut palm (*Cocos nucifera* L.). It is a major source of vegetable oil for meals. The palm tree produces a huge oval brown seed with a strong woody husk, crinkled with edible white meat, and holding a perfect liquid known as coconut water[4]. However, there are several constraints associated with this crop. These have prompted tissue culture clonal propagation. The medium composition and morphological responses of cultures must be determined in order to develop embryogeniccalli capable of regeneration into full plants. The goal of this study is to look at the impact of explants on various modified Euewens media and plant growth regulator concentrations.

Materials and methods

This study was carried out in the Plant Physiology and Tissue Culture Division, Nigerian Institute for Oil Palm Research (NIFOR). Benin City, Nigeria.

Plant material used: Coconut tissues from two different sources, young leaves and immature inflorescences, were obtained from Dwarf Green coconut palms from Plant Breeding (PB) field NIFOR, Benin City. The leaf explants were obtained from coconut seedlings of about 1-2 years old. Unopened spear leaf tissues taken from the apical growing section of the palm were used. The immature inflorescence explants were obtained from leaf axils (frond). Following the methods adopted by Steinmacher[5] for peach palm, the inflorescences used were very young. The length of the external spa was 7-8cm. After collection of explants, 70% ethanol was used to spray the explant before being wrapped in aluminum foil and put in a sampling bag for protection and then taken to the laboratory.

Culture and media preparation: Eeuwens[6] medium were modified into four different ways and supplemented with various concentrations of auxins and cytokinins, (Table-1,4) in

order to investigate the effect of *in vitro* culture of coconut palm. Eeuwens[6] medium which was modified with putrescine, was used as the basal stock. The medium was supplemented with growth regulators, 2,4-dichlorophenoxy acetic acid (2,4-D). 6-Benzyl amino purine (BAP) and picloram at concentration of 5mg/l each as started in Table-1. The second trial was Eeuwens[6] medium which was modified with Murashige and Skoog[7] macro and iron salts were used as the basal stock. The basal medium was supplemented with growth regulators, 2,4-dichlorophenoxy acetic acid (2,4-D). 1-naphthalene acetic acid (NAA), thidiazuron (TZD) and picloram (PIC) at concentrations 60mg/leach. The medium was also supplemented with 6-Benzyl amino purine (BAP). Table-2 at concentration of 2mg/l each. Following the third media modified Eeuwens[6] medium with high amount of sucrose (40g/l) was used as the basal stock. The medium was supplemented with growth regulators as shown in Table-3. Finally, Table-4 consisted of half strength of Eeuwens[6] 1978 (medium) and was supplemented with growth regulators, 2,4-dichlorophenoxy acetic acid (2,4-D) at concentration 2 µm plus 6-Benzyl amino purine (BAP) and 6-y dimethylallyl amino purine (2iP) at concentration of50 µm each.

Sterilization and inoculation of explants: After washing the inflorescence explants with detergent, they were immersed in flowing tap water for 10 minutes. Under aseptic conditions, the exterior spathes of the inflorescence were removed. Both explants (leaf and inflorescence) were immersed in a (3% sodium hypochlorite) solution for 5 minutes and gently stirred. After discarding the disinfectant solution, the explants were rinsed three times with sterile distilled water. The sterilized explants were immersed in culture medium. The initiated cultures were incubated in a growth environment at 252 ^{o}C for two weeks before being subcultured into fresh culture media. The cultures were subjected for 12-14 and 8-10 hours under dark and light photo periods, respectively.

Data analysis: Data were subjected to descriptive and inferential statistical analyses for all treatments. Measurable variables were reported as a mean of three replicates, while observable and categorical data were presented as a modal representation of three observations. The Duncan Multiple Range Test had separated the means at p 0.05. For statistical analyses, the Statistical Package for Social Science (SPSS) software version 21 and PAST version 2 were used.

Table-1: Eeuwens Medium[6] with Putrecscine.

Treatments	PUT (mg/l)	2,4-D (mg/l)	BAP (mg/l)	PIC (mg/l)
A	10. 0	5. 0	-	-
B	10. 0	-	5. 0	-
C	10. 0	-	-	5. 0

Table-2: Eeuwens Medium[6] with Murashige and Skoog[7] macro, Iron.

Treatments	2,4-D (mg/l)	BAP (mg/l)	NAA (mg/l)	TDZ (mg/l)	PIC (mg/l)
A	60. 0	2. 0	-	-	-
B	-	2. 0	60. 0	-	-
C	-	2. 0	-	60. 0	-
D	-	2. 0	-	-	60. 0

Table-3: Modified Eeuwens[6] Medium with TDZ and high sucrose.

Treatments	TDZ (mg/l)	2IP (mg/l)	2,4-D (mg/l)	BAP (mg/l)
A	1. 0	1. 0	-	-
B	-	1. 0	5. 0	-
C	-	-	10. 0	1. 0

Table-4: Eeuwen's Medium with low Hormone[6].

Treatment	2IP (µm)	2,4-D (µm)	BAP (µm)
A	50. 0	2. 00	50. 0

Results and discussion

Table-5 shows the results achieved when leaf explants were cultivated in modified Eeuwens media with putrescine. The medium was supplemented with 10mg/l putrescine +5.0mg/l 2, 4-D, and a total of 15 test tubes were inoculated, with 46.7% contaminated at the second week and 40.0% and 13.3% contaminated at the fourth and sixth weeks, respectively. As the weeks passed, the browning of the explants increased. There was no callus growth as all the cultures got infected by the 8th week. Within the first two weeks of start, 33.3% of the 15 cultures inoculated in medium supplemented with 10mg/l putrescine and 5.0mg/l BAP were infected. In the fourth and sixth weeks, 13.3% and 20.0% of the cultures were infected, respectively. Browning of cultures was less noticeable in this therapy, and explants in culture were viable until the eighth week. Contamination was 46.7% by the sixth week in the medium supplemented with 10mg/l putrescine + 5.0mg/l picloram, and browning was detected in the second week of inoculation. In all treatments, there was no callus formation from leaf explants within the first 8 weeks of culture.

Table-6 shows the response of inflorescence explants in modified Eeuwens media. In each of the treatments, 12 test tubes were started in Eeuwens medium plus putrescine. A medium supplemented with 10mg/l Put + 5.0mg/l 2, 4-D reported about 8.3% contamination but no contamination within the first two weeks. By the fourth week, 25.0% of these cultures had become polluted, with evidence of browning visible, while 16.7% of the cultures had become contaminated by the sixth week. The cultures that remained eventually turned dark brown. Browning did not occur in cultures treated with 10mg/l putrescine +5.0mg/l BAP. In the second week, 16.7% of the cultures were contaminated, while in the sixth week, 25.0% of the cultures were contaminated, and the culture size increased. However, indications of browning and 25.0% contaminated cultures were seen in medium containing 10mg/l putrescine +5 on the second week. Following the other week, 0mg/l picloram was used. Furthermore, by the eighth week, the majority of the cultures had turned dark brown.

Table-7 shows modified Eeuwens media with MS macro and iron on leaf explants. In each treatment, 14 test tubes were inoculated according to this methodology. Cultures in medium supplemented with 60mg/l of 2, 4-D +2.0mg/l BAP exhibited no contaminations, although browning was noticed at the second week, along with an increase in the size of the leaf explants. However, by the fourth week, 50.0% of the cultures were infected, and by the sixth week, 21.4% of the cultures were contaminated. Browning was also noticed. At week 2, cultures engorged in media supplemented with 60mg/l NAA +2.0mg/l BAP, with a trace of browning. Contamination was lower, although browning became apparent by the sixth week. However, in the medium containing 60mg/l TDZ and 2.0mg/l BAP, the cultures were browning-free between the second and fourth weeks, with minimal contamination. Meanwhile, by the sixth week, there were more infected cultures, and some cultures had gone necrotic. At the second week, leaf explants size decreased and necrosis was observed in leaf culture supplemented with 60mg/l PIC +2.0mg/l BAP, and contamination of 42.9% and 30% cultures was observed at the fourth and sixth weeks, respectively. However, no callus was formed from leaf explants, and by the eighth week, the majority of the cultures had gone dark brown.

The reaction of inflorescences explants in Eeuwens medium with MSmacro & iron on achieving callogenesisis presented in Table-8. Medium was augmented with 60mg/l 2, 4-D + 2. 0mg/l BAP, 12 tubes were inoculated. There were traces of browning and no contaminated culture in 2 weeks. By the 6th week, 16.7% cultures were contaminated and callus was induced Figure-1. Cultures later became dark brown after 8 weeks. There was no contaminated culture reported in inflorescence explants in medium complemented with 60mg/l NAA + 20mg/l BAP, at 4th week. By the 8th week, however, necrosis was present in the cultures. However, in medium complemented with 60mg/l TDZ+ 2.0mg/l BAP, contaminated cultures were 16.7% out of 12, test tubes initiated with browning at 2 weeks. Cultures turned dark brown, without callusing for 8 weeks. Whereas in medium with 60mg/l PIC + 2.0mg/l BAP, cultures were free from browning in the 2nd week, while, contaminated cultures and browning were observed in the 4th week.

Table-5: The effects of modified Eeuwens medium with putrescine supplemented with 2, 4-D, BAP and PIC separately on callus formation, explants colour and % contamination from leaf explants.

Treatments	Growth Regulators And Concentrations (mg/L)				Response of leaf explants at different bi-weekkly regimes											
					2nd Week			4th Week			6th Week			8th Week		
	PUT	24D	BAP	PIC	Callus F.	Explant C.	% Con	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.
1	10	5. 0	-	-	NIL	Light brown	46. 7	NIL	Light brown	40. 0	NIL	Browning	13. 3	NIL	Browning	-
2	10	-	5. 0	-	NIL	Light brown	33. 3	NIL	Light brown	13. 3	NIL	Light brown	20. 0	NIL	Browning	-
3	10		-	5. 0	NIL	Light brown	20. 0	NIL	Light brown	13. 3	NIL	Browning	46. 7	NIL	Browning	-

PUT: Putrescine, 2,4-D: 2,4 Dichlorophenoxy Acetic acid, BAP: 6 - Benzylamino purine, PIC: Picloram, Callus F. : Callus formation, Explant C. : Explant colour, %Con. : Percentage contamination.

Table-6: The effects of modified Eeuwens medium with putrescine supplemented with 2, 4-D, BAP and PIC separately on callus formation, and explants colour and % contamination from inflorescence explants.

Treatments	Growth Regulators And Concentrations (mg/L)				Response of inflorescence explants at different bi-weekly regimes											
					2nd Week			4th Week			6th Week			8th Week		
	PUT	24D	BAP	PIC	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.
1	10	5. 0	-	-	NIL	Normal	8. 3	NIL	Light brown	25. 0	NIL	Brown	16. 7	NIL	Light brown	-
2	10	-	5. 0	-	NIL	Normal	16. 7	NIL	Normal	-	NIL	Light brown	25. 0	NIL	Light brown	-
3	10		-	5. 0	NIL	Light brown	25. 0	NIL	Light brown	16. 7	NIL	Brown	16. 7	NIL	Browning	-

PUT: Putrescine, 2,4-D: 2,4 Dichlorophenoxy Acetic acid, BAP: 6 - Benzylamino purine, PIC: Picloram, Callus F.: Callus formation, Explant C. : Explant colour, % Con. : Percentage contamination.

Table-7: The effects of modified Euewens medium with MS macro andFe augmented with cytokininsalone and auxinsseparately Combined on callus formation, explant colour and % contamination from leaf explants.

Treatments	Growth Regulators And Concentrations (mg/L)					Response of leaf explants at different bi-weekkly regimes											
						2nd Week			4th Week			6th Week			8th Week		
	2,4D	BAP	NAA	TDZ	PIC	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con	Callus F.	Explant C.	% Con.
1	60. 0	2. 0	-	-	-	NIL	Brown	-	NIL	Light brown	50. 0	NIL	Brown	21. 4	NIL	Brown	-
2	-	2. 0	60. 0	-	-	NIL	Light brown	14. 3	NIL	Brown	-	NIL	Brown	7. 14	NIL	Brown	7. 14
3	-	2. 0	-	60. 0	-	NIL	Normal	21. 4	NIL	Normal	-	NIL	Light brown	42. 9	NIL	Brown	21. 4
4	-	2. 0	-	-	60. 0	NIL	Brown	-	NIL	Brown	42. 9	NIL	Brown	28. 6	NIL	Dark brown	-

2,4-D: 2,4 Dichlorophenoxy Acetic acid, BAP: 6 - Benzylaminopurine, NAA: 1-Naphthaleneacetic acid, TDZ: Thidiazuron, PIC: Picloram, Callus F. : Callus formation, Explant C. : Explant colour, % Con. : Percentage contamination.

Table-8: The effects of modified Euewens medium with MS macro and Fe augmented with cytokinins alone and auxinsseparately combined on callus formation, explant colour and % contamination from inflorescence explants

Treatments	Growth Regulators And Concentrations (mg/L)					Response of inflorescence explants at different bi-weekly regimes											
						2nd Week			4th Week			6th Week			8th Week		
	2,4D	BAP	NAA	TDZ	PIC	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.	Callus F.	Explant C	% Con.	Callus F.	Explant C.	% Con.
1	60. 0	2. 0	-	-	-	NIL	Brown	-	NIL	Light brown	50. 0	Calli formation	Brown	21. 4	Callus	Brown	-
2	-	2. 0	60. 0	-	-	NIL	Light brown	14. 3	NIL	Brown	-	NIL	Brown	7. 14	NIL	Brown	7. 14
3	-	2. 0	-	60. 0	-	NIL	Normal	21. 4	NIL	Normal	-	NIL	Light brown	42. 9	NIL	Brown	21. 4
4	-	2. 0	-	-	60. 0	NIL	Brqwn	-	NIL	Brown	42. 9	NIL	Brown	28. 6	NIL	Dark brown	-

2,4-D: 2,4 Dichlorophenoxy Acetic acid, BAP: 6 - Benzylaminopurine, NAA: 1-Naphthaleneacetic acid, TDZ: Thidiazuron, PIC: Picloram , Callus F. : Callus formation, Explant C. : Explant colour, % Con. : Percentage contamination.

Figure-1: Mass ofcallus from coconut Inflorescence explants.

Induction of callus from leaf explants in Eeuwens medium with high sucrose is presented in Table-9: Eeuwens medium plus 60 g of sucrose, supplemented with 1. 0mg/l TDZ + 1.0mg/l 2ip on leaf cultures showed no browning effect within 8 weeks of initiation. However, 11.1% and 33.3% contaminated cultures out of 18 test tubes inoculated were recorded between 6th and 8th week respectively. There was no callus production. Cultures initiated in the Eeuwens medium supplemented with 5. 0 mg/l2, 4-D +1.0mg/l 2ip, showed that 11.1% cultures out of 18 inoculated cultures were contaminated by the 2nd week following inoculation. There was no browning of cultures, whereas medium supplemented with10mg/l 2,4-D + 1mg/l BAP had contaminated cultures at weeks 4 and 6. There was no callusing and browning observed by the 6th week. Increase in size of explants in cultures was noticed in leaf explants in all the treatments at the 2nd week. By the 8th week, most of the cultures were still clean.

Table-10 shows inflorescences explants in Modified Eeuwens medium with sucrose. In the medium supplemented with 1.0mg/l TDZ + 1.0mg/l 2ip, 16.7% inflorescence cultures out of 12 inoculated test tubes were contaminated and traces of browning were observed at the 2nd week. Most of the cultures became dark brown, with 25.0% contaminated cultures by the 6th week. However, in the medium supplemented with 5.0mg/l 2,4-D + 1.0mg/l 2ip, cultures were free from browning, with 33.3% contaminated cultures at 2 weeks. An increase in the size of cultures was reported in inflorescence explants in the 4th week. Furthermore some cultures showed traces of browning and 16.7% contaminated cultures in medium supplemented with 10mg/l 2,4-D + 1mg/l BAP, at 2 weeks. By the 4th week, browning was obvious, with 25.0% contaminated cultures. Whereas, by the 8th week, there were no callusing and most of the cultures turned brown, with contamination.

Medium augmented with 2.0µm 2,4-D+50µm 2ip + 50µm BAP, 13.3% leaf cultures were contaminated out of 15 tubes inoculated (Table-11). There was no callus induction. Browning of culture was not observed until after 8th week in the basal medium. However, by the 8th week, 53.3% cultures were contaminated of the cultures had traces of browning.

The response of inflorescences explants in Eeuwen's medium with low hormones on callus formation is presented in Table-12. Medium supplemented with 2.0µm, 2,4-D + 50µm 2ip + 50µm BAP, 33.3% cultures out of 15 test tubes were contaminated, without traces of browning by the 2nd week. Traces of browning were observed at the 4^{t}h week and 25.0% contaminated cultures were observed at the 6th week. Furthermore, there was no induction of callus from inflorescence cultures within 8 weeks of inoculation.

Table-9: The effects of Euewens medium augmented with high amount of sucrose with combination of auxins and cytokininson callus formation explant colour and % contamination from leaf explants.

Treatments	Growth Regulators And Concentrations (mg/L)				Response of leaf explants at different bi-weekkly regimes											
					2nd Week			4th Week			6th Week			8th Week		
	TDZ	24D	2IP	BAP	Callus F.	Explant C.	% Con.	Callus F.	Explant C	% Con.	Callus F.	Explant C	% Con.	Callus F.	Explant C.	% Con.
1	1. 0	-	1. 0	-	NIL	Normal	-	NIL	Normal	-	NIL	Normal	11. 1	NIL	Normal	33. 3
2	-	5. 0	1. 0	-	NIL	Normal	11. 1	NIL	Normal	-	NIL	Normal	22. 2	NIL	Normal	16. 7
3	-	10. 0	-	1,0	NIL	Normal	-	NIL	Normal	16. 7	NIL	Light brown	22.2	NIL	Light brown	16. 7

TDZ: Thidiazuron, 2,4-D: 2,4 Dichlorophenoxy Acetic acid, BAP:6 - Benzylamino purine, 2iP: 6- γ, y-Dimethylallylamino purine, Callus F. : Callus formation, Explant C. : Explant colour, % Con. : Percentage contamination.

Table-10: The effects of Euewens medium augmented with high amount of sucrose with combination of auxins and cytokininson callus formation explant colour and % contamination from inflorescence explants.

Treatments	Growth Regulators And Concentrations (mg/L)				Response of inflorescence explants at different bi-weekkly regimes											
					2nd Week			4th Week			6th Week			8th Week		
	TDZ	24D	2IP	BAP	Callus F.	Explant C.	% Con.	Callus F.	Explant C	% Con.	Callus F.	Explant C	% Con.	Callus F.	Explant C.	% Con.
1	1. 0	-	1. 0	-	NIL	Normal	-	NIL	Normal	-	NIL	Normal	11. 1	NIL	Normal	33. 3
2	-	5. 0	1. 0	-	NIL	Normal	11. 1	NIL	Normal	-	NIL	Normal	22. 2	NIL	Normal	16. 7
3	-	10. 0	-	1,0	NIL	Normal	-	NIL	Normal	16. 7	NIL	Light brown	22. . 2	NIL	Light brown	16. 7

TDZ: Thidiazuron, 2,4-D: 2,4 Dichlorophenoxy Acetic acid, BAP:6 - Benzylamino purine, 2iP: 6- γ, y-Dimethylallylamino purine, Callus F. : Callus formation, Explant C. : Explant colour, % Con. : Percentage contamination.

Table-11: The effects of Euewens medium augmented with low amount of auxins and cytokininson callus formation explant colour and % contamination from leaf explants

Treatments	Growth Regulators And Concentrations (µm)			Response of leaf explants at different bi-weekkly regimes										
				2nd Week			4th Week			6th Week			8th Week	
	24D	2IP	BAP	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.	Callus F.	Explant	% Con.	Callus F.	Explant C.	% Con.
1	2. 0	50	50	NIL	Normal	13. 3	NIL	Normal	-	NIL	Normal	-	NIL	Light brown	53. 3

2,4-D: 2,4 Dichlorophenoxy Acetic acid, BAP: 6 - Benzylamino purine, 2iP: 6- γ, y-Dimethylallylamino purine , Callus F.: Callus formation, Explant C. : Explant colour, % Con. : Percentage contamination.

Table-12: The effects of Euewens medium augmented with low amount of auxins and cytokininson callus formation explant colour and % contamination from inflorescence explants.

Treatments	Growth Regulators And Concentrations (µm)				Response of inflorescence explants at different bi-weekly regimes									
					2nd Week			4th Week			6th Week		8th Week	
	24D	2IP	BAP	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.	Callus F.	Explant C.	% Con.
1	2. 0	50	50	NIL	Normal	33. 3	NIL	Light brown	-	NIL	Brown	25. 0	NIL	Brown	25. 0

2,4-D: 2,4 Dichlorophenoxy Acetic acid, BAP:6 - Benzylamino purine, 2iP: 6- γ, y-Dimethylallylamino purine, Callus F.: Callus formation, Explant C. : Explant colour, % Con. : Percentage contamination.

Discussion: For many years, clonal propagation of coconut palm has been investigated as a potential method of developing high-yielding collections while bypassing the long generation period required by standard breeding techniques. The focus of this research was on changing the medium for in-vitro coconut palm growth in order to reduce browning and contamination and so achieve effective in-vitro culture. As a result, a series of studies including 4 (four) modified medium and hormone combinations, including (Table-1,4) were carried out in order to establish an appropriate callus-initiating methodology. Under the conditions utilized, interactions between auxins and cytokinins enhanced morphogenetic response in coconut inflorescence explants but not in leaf explants. The auxins 2,4 Dichlorophenoxy Acetic acid (2,4-D) in combination with cytokinins significantly increased callus initiation and growth (BAP). In 6 weeks of medium culture, optimal callus initiation and growth was obtained at a 2,4-D dose of 60mg/l with 2mg/l BAP. This relatively high 2,4-D concentration has been shown to be effective in phoenix dactylifera inflorescence explants (personal communication). The cytokinin BAP was added to a culture medium containing near optimal amounts of 2,4-D, which increased callus development and friability. A friable callus was formed from inflorescence explants of Phoenix dactylifera, according to a similar research[8]. However, when used to treat equimolarbasis, BAP combined with NAA orpicloram was less effective than BAP combined with 2,4-D. This is consistent with the findings of (Eke[10]), who found that 2,4-D with BAP was more effective than NAA or Picloram with BAP in encouraging explant and callus growth in Phoenix dactylifera. Callus formation was not achievable from either explant or other plant growth regulator employed for media supplementation at 2,4-D concentrations ranging from 0 to 10mg/l. It is worth noting that the relatively high amount of 2,4-D (60mg/l) that successfully initiated callus in inflorescence explants did not initiate callus in leaf explants. This variation in responsiveness to 2,4-D appears to be due to the reduced embryonic potential of coconut leaf explants. According to Karunaratne[9], the embryonic potential of coconut leaf explants is quite low (10%). Furthermore, they hypothesized that the embryogenesis of the leaf explants is brief, limiting the use of coconut leaf for clonal multiplication.

Although the callus that was began utilizing inflorescence in the medium containing 2,4-D and BAP at 6 weeks was only around 5%, the rate of induction was swift and fast within 6 weeks. This was to be expected given that extremely young inflorescence explants were employed. Belt[11] stated that young inflorescence explants are more promising than leaves since they have more meristamatic sites. Contamination and browning of cultured explants are also important factors in callogenesis. There has been little research into this element of study, particularly with the coconut palm. However, a solid working method for inoculating coconut explants with no sterilants or sterilizing agents was discovered. Perhaps the sterilizing methods used were harmful or damaging to callogenesis.

In addition to microbial contamination, browning of explants in culture occurred immediately after commencement. Browning is caused by the oxidation of phenols within the tissue. According to Alkhateeb and Al-Khateeb[12], explant damage causes the release of phenolic chemicals into the culture medium. This halts the development of the initial explants, resulting in tissue death. The phenolic compound (causative agent for browning) in this culture medium were the inflorescence explants callused profusely and swiftly (within 6 weeks) had very low values (> 0.01). This most likely accounted for the explants' rapid callogenesis in the culture medium.

Conclusion

This study looked at four different media for in-vitro culture of coconut palm leaf and inflorescence explants. The results showed that the majority of the cultures lasted for 8 weeks and were still appearing viable, with callus generated using a modified Euewens medium protocol supplemented with 2,4-D and BAP (60mg/l and 2mg/l, respectively). Immature inflorescence was successfully used as a source of explants in this study. The utilization of inflorescence as a source of explants has the benefit of being non-destructive, as the mother palm can be sampled consecutively.

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