Organogenesis in *Dioscoreophyllum cumminsii* (Stapf) Diels

Happiness O. Oselebe & E.E. Ene-Obong*

Keywords: Alkaloid- Auxin- *Dioscoreophyllum cumminsii*- Caulogenesis- Cytokinin- Differentiation- Redifferentiation- Rhizogenesis- Nigeria

Summary

Dioscoreophyllum cumminsii *(Stapf) Diels (Menispermaceae) is a tropical rainforest vine that grows in the understorey of closed forests. The berries contain an intensely sweet protein syrup called monellin. The* in vitro *responses of stem explants of* D. cumminsii *were studied in the Laboratory for Plant Tissue Culture and Biotechnology, University of Nigeria, Nsukka, in 1998. Stem explants obtained from seedlings grown at the greenhouse of the Botanical Garden of the University developed brown friable callus in Arnold and Eriksson (AE), Schenk and Hildebrandt (SH), Gamborg, Miller and Ojima (B5) and Murashige and Skoog (MS) media, supplemented with 2 mg.l^{-1}of 2,4-D. Callus induction was best in MS media solidified with 10 g.l^{-1} agar and supplemented with 1 - 5 mg.l^{-1} NAA. Further addition of kinetin (1 - 5 mg.l^{-1}) resulted in more active callus formation. Kinetin in addition to an auxin was indispensable for unlimited growth and maintenance of subcultured callus, originally isolated from stem explants of* D. cumminsii*. Kinetin applied alone, however, inhibited callus formation and growth. Most frequently, rhizogenesis was observed from callus in MS medium supplemented with different combinations of auxins and kinetin. The optimum for rhizogenesis was NAA 1 mg.l^{-1} + kinetin 1 mg.l^{-1}, and NAA 2 mg.l^{-1} + kinetin* 5 *mg.l^{-1}.*

Résumé

L'organogénèse chez *Dioscoreophyllum cumminsii* (Stapf) Diels

Dioscoreophyllum cumminsii *(Stapf) Diels (Menispermaceae) est une liane de sous-bois des forêts tropicales fermées dont les fruits contiennent une protéine très sucrée dénommée monelline. Le comportement* in vitro *d'explants caulinaires de* D. cumminsii *a été étudié en 1998 au laboratoire de culture de tissu végétal et de biotechnologie de l'université du Nigeria, Nsukka. Les explants prélevés sur des plantules produites en serre au jardin botanique de l'université ont émis des calles friables de couleur brune dans les milieux de cultures Arnold et Eriksson (AE), Schenk et Hildebrandt (SH), Gamborg, Miller et Ojima (B5) et Murashige et Skoog (MS), suppléés avec 2 mg.l^{-1} de 2,4-D. Le milieu MS solidifié avec 10 g.l^{-1} d'agar et suppléé avec 1 - 3 mg.l^{-1} de 2,4-D ou 1 - 5 mg.l^{-1} de NAA a donné la meilleure induction de calles. Le rajout de kinétine (1 - 5 mg.l^{-1}) favorise la formation de calles actives et se révèle indispensable avec l'auxine pour assurer une croissance illimitée et le maintien en culture des calles issus des explants caulinaires de* D. cumminsii*. Cependant, la kinétine seule inhibe la formation et la croissance des calles. La rhizogénèse s'observe fréquemment chez les calles produites en milieu MS suppléé avec différentes combinaisons d'auxine et de kinétine. L'optimum de rhizogénèse s'observe avec rajout de soit 1 mg.l^{-1} de NAA + 1 mg.l^{-1} de kinétine soit 2 mg.l^{-1} de NAA + 5 mg.l^{-1} de kinétine*.

Introduction

Dioscoreophyllum cumminsii (Stapf) Diels, 'the serendipity berry', is a tropical rainforest vine that belongs to family Menispermaceae. It grows in dense, humid tropical forest regions of West and Central Africa (5, 11). In Nigeria, the plants grow in the relatively undisturbed rainforest areas of southern Nigeria: the Orba forest in Udenu local government area and Ugwuabor forest in Udi local government area of Enugu state; primary forest in Ntezi, Ngbo and Izzi in Ebonyi state; Otukpa Akpoto forest in Benue state and in the primary forest near the International Institute of Tropical Agriculture (IITA), Ibadan (22). In these places, *D. cumminsii* plants sprout from underground tubers or seeds germinate at the onset of the rains between April and May. From 6 to 7 weeks later, flower buds and inflorescences appear, followed by fruit production on female plants around late July and early August. The berries which are produced at basal positions along the hairy vine ripen from September through October, while the aerial vegetation dies back at the onset of dry season between November and December (22).

The berries of *D. cumminisii* contain a water-soluble substance of intense sweetness, called monellin after the Monell Chemical Sense Centre in Pennsylvania, USA (11). Monellin is a low molecular weight protein (15). This protein is the sweetest known naturally occurring substance, up to 3,000 times sweeter than sucrose (11), and approximately 100,000 times as potent as sugar on a molar basis (7, 23). The berries are, however, scarce and difficult to obtain in a large quantity from the wild (10). Cultivation is, therefore, inevitable to increase the production of the berries for large-scale extraction of the protein sweetener and subsequent commercialization.

Cultivation and mass propagation of *D. cumminsii* has been difficult due to problems associated with seed dormancy (10, 20). In natural habitats, seeds take about 4 - 7 months to germinate (1, 21). In addition, *D. cumminsii* is an ''endangered species" (20, 21), and with the disappearance of tropical forests due to human activities like lumbering, building and clearing for agriculture and industrial activities, plants may be destroyed from their natural habitats (19). It is therefore, necessary to conserve, regenerate and multiply the plant for posterity.

Substantial research results are available on traditional medicinal uses of *D. cumminisii* (12, 13), and the identification and characterization of the chemostimulatory taste-active protein, monellin (11, 16). Extensive work has also been done on the purification (15), structural determination (3), conformational transitions and crystallization of monellin (12), alkaloid production in cultured cells (2, 7) and macropropagation of *D. cumminsii* (1, 10, 20, 21). Although *in vitro* multiplication and vegetative propagation via tissue culture have been successfully applied for the production of other plants within family Menispermaceae (25), little has been done to understand the response of *D. cumminsii* to *in vitro* culture and the various conditions that would lead to organogenesis and eventual micropropagation of the plant. This experiment was initiated to study both issues, while recognizing the vital role that monellin could play in foods for diabetics and dieters.

*Laboratory for Tissue culture and Biotechnology, Department of Botany, University of Nigeria, Nsukka.
Received on 24.01.03 and accepted for publication on 24.01.06.

Materials and methods

This study was carried out in the Laboratory for Plant Tissue culture and Biotechnology, Department of Botany, University of Nigeria, Nsukka. Stem explants of *D. cumminsii* were obtained from four weeks old seedlings grown at the greenhouse of the Botanical Garden of the University.

Callus induction

Callus formation was first examined using 1 cm-long stem explants cultured in Arnold and Eriksson (AE), Schenk and Hildebrandt (SH), Gamborg, Miller and Ojima (B5) and Murashige and Skoog (MS) media, each supplemented with 2 mg.l^{-1} 2,4-D, to identify the media best suited for cultures of *D. cumminsii. In vitro* cultures were maintained at 25 ± 2 ^{0}C under white fluorescence (Ca. 500 Lux) for 12 hours per day in an air-conditioned growth room for four weeks. Stem explants of *D. cumminsii* were subsequently cultured in MS medium prepared by the addition of the elements in Table 1.

Two factorial experiments were set up to determine the effect of auxins; 2,4 dichlorophenoxyacetic acid (2,4-D) or naphthalene acetic acid (NAA) and kinetin (6-furfurylaminopurine) on callus induction from stem explants of *D. cumminsii*. 2,4-D was added in four concentrations (0, 1, 2 and 3 mg.l^{-1}) along with kinetin in 3 concentrations to give twelve 2,4-D x kinetin combinations. Similarly, NAA was added in five concentrations (0, 1, 2, 3 and 5 mg.l^{-1}) along with kinetin in 3 concentrations (0, 1 and 2 mg.l^{-1}), to give fifteen NAA x kinetin combinations. Hormones were added to the media before the pH was adjusted to 5.8 using a Kent Digital pH/Temp meter.

The media was dispensed 20 ml per pyrex glass culture tube (25 x 200 mm), capped tightly to minimize evaporation and autoclaved at 1.05 kg.cm^{-2} pressure for 20 minutes. Culture tubes were placed in slanted positions on a laboratory bench to cool, and so as to provide a surface area for the growth of cultures.

Table 1
Composition of MS nutrient medium (g.l^{-1})

Elements	Gram per litre
Major elements	
NH_4NO_3	33.00
KNO_3	38.00
$CaCL_2 2H_2O$	8.80
$M_gSO_4 7H_2O$	7.40
KH_2PO_4	3.40
Na_2-EDTA	0.75
$FeSO_4 7H_2O$	0.55
Minor elements	
H_3BO_3	0.62
$M_nSO_4.4H_2O$	2.23
$Z_nSO_4.4H_2O$	0.86
K1	0.083
$Na_2MoO_4.2H_2O$	0.025
$CaSO_4.5H_2O$	0.0025
$CaCl_2.6H_2O$	0.0025
Vitamins	
Myo-inositol	0.10
Nicotinic acid	0.05
Pyridoxine.HCl	0.05
Thiamine.HCl	0.01
Sucrose	30.00
Additional ingredients	
Casein hydrolysate	1.00
Agar	10.00

Stem explants of approximately 1 cm long were prepared in five replicates for each medium containing an auxin x kinetin combination. Explants were surface-sterilized using 50% laundry bleach (Reckitt Benckiser$_{TM}$) for 15 minutes. To increase the permeability of the sterilizing agent, one to two drops of Tween - 20 was added. After 15 minutes, bleach solution was decanted and explants washed five times with sterile distilled water before inoculation. Cultures were maintained at 25 ± 2 ^{o}C under white fluorescence (ca. 500 Lux) for 12 hours per day in an air-conditioned growth room. On 49 to 56 days after inoculation, all calluses were weighed using a sensitive balance (Sartorius).

Callus growth and organogenesis

D. cumminsii callus obtained in the above experiment was used to study callus growth and organogenesis. Callus growth and organogenesis would be indicated by an increase in cell proliferation (i.e. callus weight) and differentiation into tissues and organs. About 225 calluses, each weighing approximately 0.05 g were isolated and cultured in MS media supplemented with NAA + kinetin, IBA (indole-3-butyric acid) + kinetin or IBA + BAP (6-benzylaminopurine). Each auxin was added in one of 3 concentrations (0, 1 and 2 mg.l^{-l}), along with kinetin or BAP in one of five concentrations (0, 1, 2, 3 and 5 mg.l^{-l}). Five replicates were prepared for each medium. Cultures were maintained in a growth room under the conditions stated above. At about 42-50 days after inoculation, all calluses were examined and re-weighed. The number of rhizoids (0.2 cm and above) per tube was counted and means of replicates calculated for each hormonal combination.

Statistical analysis

Analysis of variance was done to identify hormonal combinations that were significant at stimulating callus induction, growth or organogenesis. The ratio of mean squares of various treatment effects to the error mean square was calculated to obtain the amount of variance attributable to each of the primary effects of the two auxins, kinetin and their interactions on callus induction, growth or organogenesis. Graphs were also plotted to compare the effects of different hormonal combinations.

Results

Callus induction

Several culture media were tested to determine their suitability for *in vitro* cultures of *D. cumminsii.* Light brown friable calluses were formed after 2 weeks in all media screened. Callus formation started at both ends of the explant in response to wounding, and was optimal in MS medium supplemented with 2 mg.l^{-l} 2,4-D (Table 2).

ANOVA results on the effects of two auxins and kinetin applied in combination on callus induction from young stem

Table 2
Mean weight (mean ± standard error [S.E]) of callus induced from young stem explants of *D. cumminsii* in different media formulations supplemented with 2 mg.l^{-1} 2,4-D

Treatments (media)	Callus weight (g) Mean ± SE
AE	0.32 ± 0.1^{cd}
B5	1.03 ± 0.3^{ab}
MS	1.65 ± 0.4^{a}
SH	0.68 ± 0.1^{bc}
F-LSD (0.05)	0.66

Note: AE= Arnold and Eriksson, Gamborg, B5= Miller and Ojima,s, SH= Schenk and Hildebrandt; and MS= Murashige and Skoog media.

Table 3
Results of analysis of variance on the effects of two auxins and kinetin on callus induction from young stem explants of *D. cumminsii*

Source of variation	Df	Mean squares		
		Expected	Observed	
			NAA	2,4-D
Replications	4			
Auxin (N)	4	$\sigma^2E + + r\sigma^2KN+rk\sigma^2N$	3.60**	0.31**
Kinetin (K)	2	$\sigma^2E + r\sigma^2KN+rn\sigma^2K$	1.75**	0.22
N x K	8	$\sigma^2E + r\sigma^2KN$	20.8**	0.27**
Error	60	σ^2E	0.12	0.04

explants of *D. cumminsii* are shown in table 3.
When stem explants of *D. cumminsii* were cultured in MS media supplemented with 2,4-D and kinetin, light brown friable callus developed after ten days. Results indicated that 2,4-D alone and 2,4-D x kinetin interactions were significant (P< 0.01), while kinetin was not significant in inducing callus from stem explants of *D. cumminsii.* Callus formation was optimal in a medium containing 1 mg.l^{-1} 2,4-D + 1 mg.l^{-1} kinetin. This value differed significantly from callus formation values at the other combinations of 2,4-D and kinetin. Lowest callus fresh weight was obtained when MS medium was supplemented with 1 mg.l^{-1} kinetin or when the explant was cultured in a hormone-free medium.
When MS medium was supplemented with NAA and kinetin, both substances and their interactions had significant impact (P< 0.01). Highest callus fresh weight was obtained in the medium containing 5 mg.l^{-1} NAA + 2 mg.l^{-1} kinetin (Figure 1).

This differed significantly from values obtained at other levels of treatments applied. Low auxin concentrations induced caulogenesis, while higher auxin levels suppressed callus formation, except in the presence of kinetin. The lowest callus fresh weight was obtained when MS medium was supplemented with kinetin only, or when explants were cultured in a hormone-free medium. Kinetin was therefore considered inhibitory to callus induction from stem explants of *D. cumminsii*.
Between the two auxins, NAA was better than 2-4-D for callus induction from stem explants of *D. cumminsii*, while kinetin affected callus induction only in combination with auxins. Variances attributable to kinetin were larger for explants grown in media containing NAA than in media containing 2,4-D, which was not significant (Table 4).
The interaction between NAA and kinetin (20.86) was also larger compared to the negligible interaction between 2-4-D and kinetin (6.05).

Measurement of callus growth
Combined ANOVA results on the effects of NAA or IBA, and kinetin or IBA applied in combination on growth of *D.*

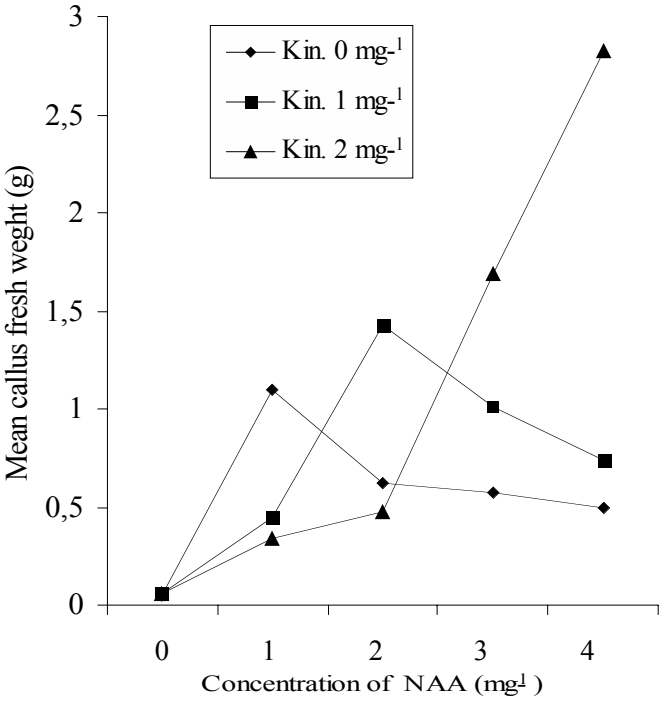

Figure 1: Effects of NAA and kinetin applied in combination on callus induction from stem explants of *D. cumminsii* after six weeks. Culture medium, MS + 3% sucrose + 100 mg.l^{-1} myo-inositol + mg.l^{-1} agar.1000 mg.l^{-1} CH. + 10,000 mg.l^{-1} agar.

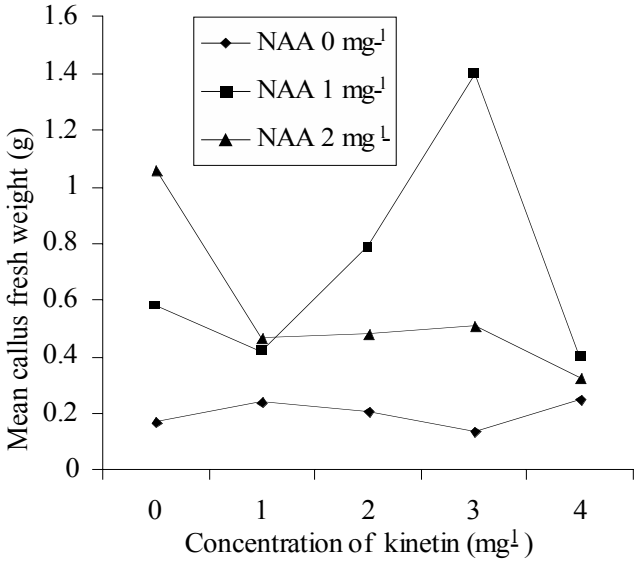

Figure 2: Effects of NAA and kinetin applied in combination on growth of *D. cumminsii* callus after 7 weeks. Culture medium, MS + 3% sucrose + 100 mg.l^{-1} myo-inositol + mg.l^{-1} agar.1000 mgl CH. + 10,000 mg.l^{-1} agar.

Table 4
Variances attributable to primary effects of auxins (σ^2_A) and kinetin (σ^2_K) and to their interaction (σ^2_I) on callus induction from young stem explants of *D. cumminsii* cultured in MS media

Blocks + (Kinetin)	Variance				Variance Ratio		
	σ^2_A	σ^2_K	σ^2_I	σ^2_E	σ^2_A/σ^2_E	σ^2_K/σ^2_E	σ^2_I/σ^2_E
NAA	3.60	1.75	2.55	0.12	29.51	14.31	20.86
2-4-D	0.31	0.22	0.27	0.04	7.09	4.95	6.05

cumminsii callus (initial weight 0.05 g) showed very highly significant results (Table 5). When NAA and kinetin were added to the media, the highest mean callus fresh weight was obtained for the combination of 1 mg.l^{-1} NAA + 3 mg.l^{-1} kinetin (Figure 2).

This was significantly different from callus fresh weight obtained in a medium containing 1 mg.l^{-1} NAA + 2 mg.l^{-1} kinetin. Lowest mean callus fresh weight was obtained in the medium containing 3 mg.l^{-1} kinetin only. This was not significantly different from the mean callus fresh weight at other levels of kinetin tested.

Similarly, when IBA and kinetin were applied to the media, highest mean callus fresh weight was obtained in the medium containing 1 mg.l^{-1} IBA + 5 mg.l^{-1} kinetin (Figure 3). This was followed by media containing 2 mg.l^{-1} IBA + 5 mg.l^{-1} kinetin; 2 mg.l^{-1} IBA + 3 mg.l^{-1} kinetin; 1 mg.l^{-1} IBA + 3 mg.l^{-1} kinetin and 1 mg.l^{-1} IBA + 2 mg.l^{-1} kinetin. Lowest mean callus fresh weight was recorded in the medium containing 3 mg.l^{-1} kinetin only. The latter did not differ significantly from the values obtained at other levels of kinetin.

When combinations of IBA and BAP were added to the media, highest mean callus fresh weight was obtained from a medium containing 1 mg.l^{-1} IBA + 2 mg.l^{-1} BAP, followed by 2 mg.l^{-1} IBA + 5 mg.l^{-1} BAP. Lowest mean callus fresh weight was obtained when MS medium was supplemented with 5 mg.l^{-1} BAP and in a hormone-free basal medium. Most of the values of other treatments were intermediate. The cytokinins therefore had an inhibitory effect on cell proliferation across all levels tested and were effective only in the presence of auxins.

Variances attributable to each of the primary effects of the two auxins and two cytokinins (Table 6) indicate that between the two auxins, NAA was more effective than IBA for callus growth. Variances attributable to kinetin were, however, greater in callus grown in IBA containing media. The interactions between IBA and kinetin were large compared to interactions between NAA and kinetin or IBA or BAP.

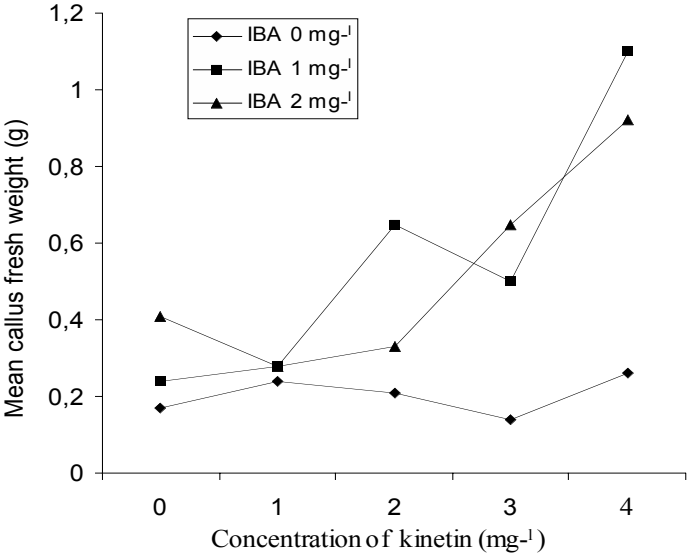

Figure 3: Effects of IBA and kinetin applied in combination on growth of *D. cumminsii* callus after 7 weeks. Culture medium, MS + 3% sucrose + 100 mg.l^{-1} myo-inositol + mg.l^{-1} agar.1000 mg.l^{-1} CH. + 10,000 mg.l^{-1} agar.

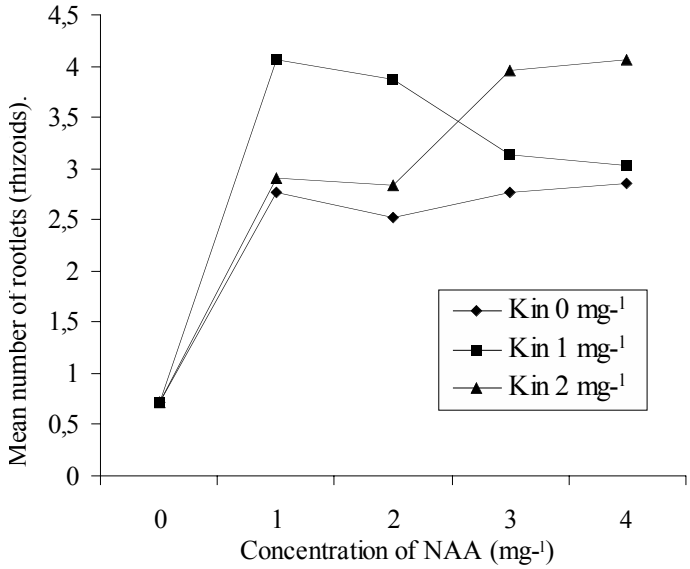

Figure 4: Effects of NAA and kinetin applied in combination on organogenesis from stem explants of *D. cumminsii* after 7 weeks. Culture medium= MS + 3% sucrose + 100 mg.l^{-1} myo-inositol + mg.l^{-1} agar. 1000 mg.l^{-1} CH. + 10,000 mg.l^{-1} agar.

Organogenesis *in vitro*

D. cumminsii calluses were mostly compact, while few were friable and could easily be broken into small fragments. The colour of the calluses ranged from brown to green, greenish yellow and yellow (Table 7).

Rhizogenesis occurred via callus of *D. cumminsii.* ANOVA results indicated a very highly significant effect (P< 0.001) of NAA or IBA and kinetin or BAP on rhizogenesis from *D. cumminsii* callus. NAA x kinetin or IBA x kinetin interactions were not significant. Comparatively, however, the highest number of rootlets was formed when 1 mg.l^{-1} NAA +1 mg.l^{-1} kinetin, and 5 mg l^{-1} NAA + 2 mg.l^{-1} kinetin were added to the media (Figure 4).

At the above concentrations, the mean number of rootlets per callus was 4.04. Lowest level of rhizogenesis was observed when MS medium was used hormone-free or when kinetin only was added as a medium-supplement.

In the media supplemented with IBA and BAP, highest rate of redifferentiation was obtained from the medium containing 2 mg.l^{-1} IBA (Figure 5). This was statistically significantly different from values obtained at other treatment

Table 5
Results of analysis of variance on the effects of two auxins and kinetin on growth of *D. cumminsii* callus

Source of variation	Df	Mean squares			
		Expected	Observed		
			NAA	IBA_1	IBA_2
Treat. combination	14				
Auxin (A)	2	$\sigma^2\rho$ + + $E\sigma^2\kappa\rho$+$NK\sigma^2N$	1.75***	1.73**	19.40**
Cytokinin (K)	4	$\sigma^2\rho$ + $E\sigma^2\nu\rho$+$NK\sigma^2K$	0.32***	1.00**	8.92**
A x K	8	$\sigma^2\rho$ + $E\sigma^2NK$	0.47***	0.66**	10.23**
Error	60	σ^2E	0.06	0.04	0.05

Note: IBA_1= media containing IBA and kinetin, IBA_2= media containing IBA and BAP.

Table 6
Variances attributable to the primary effects of auxins (A) and kinetin (K) and their interactions (I) on growth of *D. cumminsii* callus

Blocks	Variance				Variance Ratio		
	σ^2_A	σ^2_K	σ^2_I	σ^2_E	σ^2_A/σ^2_E	σ^2_K/σ^2_E	σ^2_I/σ^2_E
NAA (+ KIN.)	1.75	0.32	0.47	0.06	29.71	5.40	7.88
IBA (+ KIN.)	1.73	0.10	0.66	0.04	45.23	24.93	16.48
IBA (+ BAP)	1.01	0.46	0.53	0.05	19.40	8.92	10.23

Table 7
Weight (g), colour and nature of callus grown in MS basal medium supplemented with various combinations of auxins and cytokinins (NAA, IBA, BAP and kinetin)

Concentration of growth regulators ($mg.l^{-1}$)		Mean callus weight ± SD	Colour	Nature
NAA	Kinetin			
0	0	0.17 ± 0.09	greenish yellow	compact
	1	0.24 ± 0.05	greenish yellow	compact
	2	0.21 ± 0.06	greenish yellow	compact
	3	0.14 ± 0.04	greenish yellow	compact
	5	0.25 ± 0.02	greenish yellow	compact
1	0	0.58 ± 0.51	slightly brown	friable
	1	0.42 ± 0.02	slightly brown	compact
	2	0.79 ± 0.60	yellow	friable
	3	1.40 ± 0.42	brown	friable
	5	0.40 ± 0.42	slightly yellow	compact
2	0	1.06 ± 0.11	brown	friable
	1	0.47 ± 0.14	slightly brown	compact
	2	0.48 ± 0.08	slightly brown	compact
	3	0.51 ± 0.06	greenish yellow	compact
	5	0.33 ± 0.06	yellow	friable
IBA	Kinetin			
1	0	0.24 ± 0.11	brown	compact
	1	0.28 ± 0.06	brown	compact
	2	0.65 ± 0.28	greenish yellow	compact
	3	0.54 ± 0.26	brown	friable
	5	1.15 ± 0.24	slightly yellow	friable
2	0	0.41 ± 0.03	slightly brown	compact
	1	0.28 ± 0.06	slightly yellow	friable
	2	0.33 ± 0.04	greenish yellow	compact
	3	1.65 ± 0.17	brown	friable
	5	0.92 ± 0.50	greenish yellow	compact
IBA	BAP			
0	0	0.17 ± 0.09	greenish yellow	compact
	1	0.19 ± 0.03	green	compact
	2	0.24 ± 0.05	slightly yellow	compact
	3	0.22 ± 0.03	greenish yellow	compact
	5	0.22 ± 0.04	slightly yellow	friable
1	0	0.24 ± 0.11	brown	compact
	1	0.45 ± 0.12	slightly yellow	friable
	2	1.38 ± 0.16	green	compact
	3	0.38 ± 0.25	brown	compact
	5	0.47 ± 0.06	slightly yellow	friable
2	0	0.33 ± 0.04	slightly yellow	friable
	1	0.33 ± 0.02	greenish yellow	compact
	2	0.37 ± 0.09	yellow	compact
	3	0.42 ± 0.07	greenish yellow	compact
	5	1.08 ± 0.79	greenish yellow	compact
F-LSD (0.05)		0.13		

Note: SD= standard deviation.

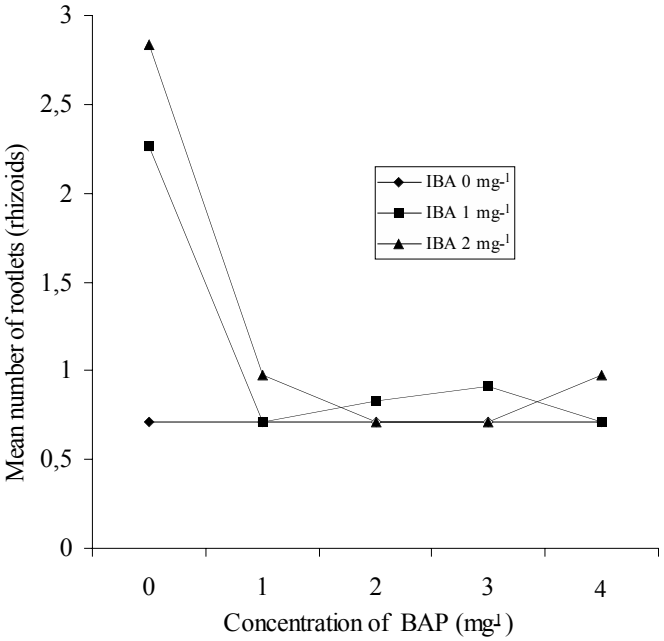

Figure 5: Effects of IBA and BAP applied in combination on organogenesis from *D. cumminsii* callus after 7 weeks. Culture medium, MS + 3% sucrose + 100 mg.l^{-1} myo-inositol + mg.l^{-1} agar.1000 mg.l^{-1} CH. + 10,000 mg.l^{-1} agar.

combinations screened. Rhizogenesis was observed in media containing 1 mg.l^{-1} IBA only, 1 mg.l^{-1} IBA + 2 mg.l^{-1} BAP, 2 mg.l^{-1} IBA + 1 mg.l^{-1} BAP, 1 mg.l^{-1} IBA + 3 mg.l^{-1} BAP, and these differed significantly from each other. No redifferentiation was observed when BAP alone was used as medium-supplement.

Discussion

The present results indicate that MS medium was better for *in vitro* cultures of *D. cumminsii* compared to all other media screened. Stem explants of *D. cumminsii* formed light brown callus in MS medium supplemented with auxins and kinetin. Among growth hormones, NAA was better than 2, 4-D for callus induction, either alone or in combination with kinetin, while kinetin induced callus formation only in the presence of auxins. The addition of kinetin resulted in more vigorous and active callus development and explains why highest proliferation of callus was recorded in MS medium containing 5 mg.l^{-1} NAA + 2 mg l^{-1} kinetin. This result is consistent with previous findings (6, 17, 18) that kinetin has a stimulatory effect on cell proliferation and differentiation, because it hastens the transition of cells from the G2 phase to mitosis. However, these effects require the presence of auxins and in this experiment, low concentrations of kinetin (0 - 2 mg.l^{-1}) promoted callus induction at high auxin levels used in this experiment.

The colour of *D. cumminsii* calluses ranged from brown to green, greenish yellow and yellow. Yellow colour was attributed to the formation of quarternary protobernerrine alkaloids from cultured cells of *D. cumminsii* (2, 7), while the greening of the callus was an indication of the morphogenetic potential inherent to the callus (8). Consequently, rhizogenesis was achieved in MS media supplemented with NAA or IBA, either alone or in combination with cytokinins. High level of kinetin with various concentrations of NAA promoted redifferentiation of roots from *D. cumminsii* callus. Auxins promoted root redifferentiation when applied alone as a medium-supplement, while cytokinins had an inhibitory effect except in combination with auxins. Similar hormonal combinations as used in this experiment have been reported to promote shoot redifferentiation in *Vigna sinensis* (14) and *T. cordifolia* Miers (25).

There are differences in morphogenetic capacity of calluses from different plant species when they occur, they are always dependent on auxin-cytokinin ratios (4). Other factors may include endogenous compounds produced in the medium and substances carried over from the original explant (6). Only a few of the calluses produced by different organisms have given rise to entire organs and organ systems. In most cases, regeneration of roots precedes that of shoots but the sequential development of the two results in the reconstitution of the whole plant (9). In this experiment, high level of kinetin with various concentrations of NAA or IBA, promoted redifferentiation of roots. Further researches are necessary to obtain the optimum combination of growth hormones for shoot redifferentiation. This will lead to micropropagation and large-scale cultivation of *D. cumminsii*. Availability of the berries will ensure large-scale extraction of monellin from the berries for use by diabetics and dieters.

Acknowledgement

The authors are grateful to the African Academy of Sciences for the award of Grant No. 201-58061 used for this work.

Literature

1. Adansi M.A. & Holloway H.L.O., 1977, Seed germination and establishment of the serendipity berry, *Dioscoreophyllum cumminsii* Diels. Acta Horticulturae, 53, 407-411.
2. Adesina S.K., Herbone J.B. & Philipson J.D., 1978, The isolation of quaternary alkaloids from the leaves of *Dioscoreophyllum cumminsii* (Stapf) Diels. Planta media, 33, 217-222.
3. Bohak Z. & Li S.L., 1976, The structure of monellin and its relation to the sweetness of the protein. Biochim Biophysics Acta, 427, 153-170.
4. Bourgin J.P., Chupeau Y. & Missonier C., 1979, Plant regeneration from mesophyll protoplasts of several *Nicotiana* spp. *Physiol. Plant.* 45, 288-292.
5. Cagan R.H., 1973, Chemostimulatory protein: a new type of taste stimulus. Science, 161, 1241-1245.
6. Esenowo G.J., 1999, Fundamentals of plant tissue culture. MEF Publishing Comp, Nigeria. 119 pp.
7. Faruya J., Takafumi Y. & Kiyohara H., 1983, Alkaloid production in cultured cells of *Dioscoreophyllum cumminsii*. Phytochemistry, 22, 1671-1673.
8. George E.F. & Sherrington P.D., 1984, Plant propagation by tissue culture. Exegetics Limited, England. 709 pp.
9. Halperin W. & Wetherell D.F., 1964, Adventive embryony in tissue culture of the wild carrot, *Daucus carota*. Amer. Jour. Bot. 27, 274-283.
10. Holloway H.L.O., 1977, Seed propagation of *Dioscoreophyllum cumminsii*, source of an intense natural sweetener. Economic Botany, 31, 17-50.
11. Inglett G.E & May J.F., 1969, Serendipity berries, source of a new intense sweetness. J. of Food Science, 34, 408-411.
12. Irvine F.R., 1961, Woody plants of Ghana with special reference to their uses. Oxford University Press, London. 578 pp.
13. Kim S.H., de vos Abraham & Ogata C., 1988, Crystal structures of two intensely sweet proteins. TIBS, 13, 13-15.
14. Matsubara S., 1975, Nutritional and hormonal requirements for growth of *Vigna sinensis* callus *in vitro*. Physiol. Plant. 34, 83-89.
15. Morris J.A. & Cagan R.H., 1972, Purification of monellin sweet principle in *Dioscoreophyllum cumminsii.* Biochem Biophysics Acta, 261, 114-122.
16. Morris J.A., Martenson R.E. & Deibler G., 1973, Characterization of monellin, a protein that tastes sweet. J. Biol. Chem. **248**, 2, 534-539.

17. Nishi T., Yamada Y. & Takahashi E., 1973, The role of auxins in differentiation of rice tissue cultured *in vitro.* Bot. Mag. Tokyo, 91, 83-91.
18. Ogihara Y., 1978, Tissue culture in Haworthia II: effects of three auxins and kinetin on greening and redifferentiation of callus. Bot. Mag. Tokyo, 92, 163-171.
19. Okonkwo S.N.C., 1996, Overview of *in vitro* technique. *In:* Proceedings of the training course on biotechnology: cell and tissue culture techniques. NASENI, Lagos. 245 pp.
20. Okoro O.O., 1980, Propagation of *Dioscoreophyllum cumminsii*. Nig. J.F. 10, 48-57.
21. Okoro O.O., 1976, Propagation of *Dioscoreophyllum cumminsii*. Nig. J.F. 6, 70.
22. Oselebe H.O., Ene-Obong E.E. & Nwankiti O.C., 2004, The biology of *Dioscoreophyllum cumminsii* (Stapf) Diels. Nigerian Jour. Bot. 17, 17-28.
23. Penarrubia L., Kim R., Giovannoni J., Kim S.H. & Fischer R.L., 1992, Production of the sweet protein monellin in transgenic plants. Bio. Technology, **10**, 5, 561-564.
24. Reddy M.K., Rao A.S., Rao M.V., 2003, *In vitro* multiplication of *Tinospora cordifolia* Miers, an Ayurvedic Medicinal plant, via somatic embryogenesis, Abstract, 3rd World Congress on Medicinal and aromatic plants for human welfare Chiang Mai, Thailand, pp. 267.
25. Shimada T., Sasakuma T. & Tsunewaka K., 1969, *In vitro* culture of wheat tissues callus formation, organ redifferentiation and single cell culture. Can. J. Genet. Cytol. II, 294-304.

Happiness O. Oselebe, Nigerian, B.Sc. (Education Biology), M.Sc. (Plant and Breeding Genetics). Graduate student, Laboratory for Tissue Culture and Biotechnology, Department of Botany, University of Nigeria, Nsukka, Nigeria.

E.E. Ene-Obong, Nigerian, Ph.D., Professor of Plant Breeding and Genetics, Department of Botany, University of Nigeria, Nsukka, Nigeria.

AVIS

Nous rappelons à tous nos lecteurs, particulièrement ceux résidant dans les pays en voie de développement, que TROPICULTURA est destiné à tous ceux qui oeuvrent dans le domaine rural pris au sens large.

Pour cette raison, il serait utile que vous nous fassiez connaître des Institutions, Ecoles, Facultés, Centres ou Stations de recherche en agriculture du pays ou de la région où vous vous trouvez. Nous pourrions les abonner si ce n'est déjà fait.

Nous pensons ainsi, grâce à votre aide, pouvoir rendre un grand service à la communauté pour laquelle vous travaillez.

Merci.

BERICHT

Wij herrineren al onze lezers eraan, vooral diegenen in de ontwikkelingslanden ,
dat TROPICULTURA bestemd is voor ieder die werk verricht op het gebied van het platteland en dit in de meest ruime zin van het woord.

Daarom zou het nuttig zijn dat u ons de adressen zou geven van de Instellingen, Scholen, Faculteiten, Centra of Stations voor landbouwonderzoekvan het land of de streek waar U zich bevindt. Wij zouden ze kunnen abonneren, zo dit niet reeds gebeurd is.

Met uw hulp denken we dus een grote dienst te kunnen bewijzen aan de gemeenschap waarvoor u werkt.

Dank U.