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In Vitro Morphogenetic Responses of *Dioscoreophyllum cumminsii* (Stapf) Diels Seed Explant

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Abstract

This study was undertaken to investigate the *in vitro* morphogenetic responses of *Dioscoreophyllum cumminsii* (Stapf) Diels (serendipity berry) using seed explant. A combination of different plant growth regulators (PGRs) were used; five concentrations of Naphthalene acetic acid (NAA) and five concentrations of 6-Benzyl amino purine (BAP) or Kinetin, to supplement Murashige and Skoog (MS) medium. The highest callus intensity was observed in the media containing 25mg/l NAA + 1mg/l kinetin and also in the media containing 5mg/l NAA + 2mg/l BAP. With regard to organogenesis, it was observed that 2.5mg/l NAA alone produced the highest number of rootlets and 2mg/l BAP alone produced taller plantlets. The results obtained in the study indicated that morphogenetic responses of *D. cumminsii* could be by NAA in combination with BAP or Kinetin, to undergo callogenesis, shoot formation or/and rhizogenesis based on the ratio of the growth regulators.

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Introduction

Dioscoreophyllum cumminsii (Stapf) Diels is a member of the Menispermaceae (Moonseed) family. It is a climbing vine with heart-shaped leaves (Inglett, 1981). The species have a complex life cycle; the males propagate asexually by subterranean tubers, which might have impacted on the species genetic variability, while the females reproduce sexually by seeds (Obioh and Isichei, 2007). *D. cumminsii* sprouts from underground tubers or seeds which germinate at the onset of the rainy season between April and May From 6 to 7 weeks later, flower buds and inflorescence 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 (Oselebe *et al.,* 2004).

The plant is indigenous to Tropical West Africa. Common in Guinea, Cameroon and in Central Africa (Inglett, 1981). In Nigeria, it grows in the relatively undisturbed rainforest areas of Southern Nigeria: the Orba forest in Udenu Local Government Area of Enugu State, 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, Oyo State (Oselebe *et al.,* 2004). In Edo State it is also found in Sakpoba forest reserve and the free area in Ugo - Niyekorhionmon town, along Benin - Sapele Road and the Nigerian Institute for Oil Palm Research (NIFOR), Benin City.

The plant is highly understudied and underutilized. It has been found to have some unique properties. It is a potential source of non-carbohydrate sweetener because of the constituent active ingredient known as monellin, which is 3000 times sweeter than sugar and is useful in low calorie diets for diabetics and dieters, combating tooth decay and as a protein sweetener in the food industry (Holloway, 1977). The total carotenoid content of *D. cumminsii* indicates that it has various health benefits and physiological effects of vitamin A, particularly its benefits on vision, disease resistance, cell integrity, bone re-modeling and reproduction (IITA, 2008). The vitamin C content of *D. cumminsii* according to Abiodun and Akinoso (2014) is 12.80 mg/100g, which is more than the vitamin C of local orange, watermelon and banana (Tee *et al.,* 1988). It was reported that the fruit is useful for human health and could be substituted for citrus fruits (Abiodun and Akinoso, 2014).

The major challenge facing this unique plant is its propagation through seed. Several studies have been carried out on seed propagation of *D. cumminsii* (Adansi and Holloway, 1977; Holloway, 1977; Okoro, 1976; Okoro 1980). Cultivation and mass propagation of *D. cumminsii* have been difficult due to problems associated with seed dormancy (Holloway, 1977; Okoro, 1980). In natural habitats, seeds take about 4 - 7 months to germinate (Adansi and Holloway, 1977; Okoro, 1976). The use of *in vitro* procedure for propagating *D. cumminsii*, thereby bypassing the dormancy period of the seeds is being explored. There is a report of the *in vitro* culture using stem explants (Oselebe and Ene-Obong, 2007). The aims of this study were to establish an *in vitro* culture of *D. cumminsii* using seed explant and also to investigate the type and concentration of phytohormones (NAA, BAP and Kinetin) in relation to its *in vitro* morphogenetic responses.

Materials and methods

Plant material

Fruits of *D. cumminsii* were collected from the free area and second pipeline area of the Sakpoba Forest Reserve (Latitude 06º04¹22.3"N and Longitude 005º55¹58"E); (Latitude 06º04¹19"N and Longitude 005 º55¹59.3"), 75 m to 76 m above sea level, located in Ugo - Niyekorhionmon town, along Benin - Sapele Road, Benin City, Edo State.

Media preparation and sterilization of instruments

The study was carried out using the facilities of the Plant tissue culture laboratory of the Nigerian Institute for Oil Palm Research, Benin City, Edo State. Murashige and Skoog (MS) medium, which consists of macro nutrients, micro nutrients, Iron, vitamins, Casein hydrolysate and other supplements was used. The media were prepared based on the laboratory protocol (Murashige and Skoog, 1962). Phytohormones were added to the media; pH was adjusted to 5.8 and properly dispensed into Mc-Cartney bottles and test tubes. These were covered with foil paper and sterilized along with forceps, petri dishes and foil papers by autoclaving at 121ºC, 15 psi for 30 minutes. After autoclaving, the media were taken to the cooling room and allowed to cool.

Sterilization of explants and establishment of in vitro culture

The plant/fruits were collected and surface sterilized by rinsing thoroughly with 3.5% Sodium hypochlorite and distilled water to

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remove any trace of debris from the field and covered with foil paper. The fruits were then transferred to the Laminar flow hood where the mesocarps were removed and the seeds were sterilized using 3.5% Sodium Hypochlorite and Tween-20 for 1 minute. Sterile distilled water was used to rinse away the Sterilant 3 to 4 times, to prevent damage to the cells of the explants. After sterilization, the seeds were placed on a petri dish using a sterile forceps and dissected into 2 to 4 parts with a surgical blade. Sterilized explants were then inoculated on the surface of the semi-solid MS media. All these activities were carried out in a laminar flow hood. The *in vitro* cultures were incubated in the dark growth room at 25 ± 2ºC, 50 to 60% relative humidity until emergence of shoot or root, after which they were transferred to the light growth room at 25 ± 2ºC, 50 to 60% relative humidity with 16 hours of photoperiod (light and dark regime was created artificially in growth room).

Experimental design

Two 5×5 factorial experiments was set up to determine the effect of Napthalene acetic acid (NAA) in five concentrations (0, 2.5, 5, 7.5, 10 mg/l) in combination with Benzyl amino purine (BAP) or Kinetin both in five concentrations (0, 0.5, 1, 1.5, 2 mg/l) to give twenty-five NAA × Kinetin combinations and twenty-five NAA × BAP combinations. Four replicates were prepared for each medium.

Results

The results obtained when *D. cumminsii* cotyledons were cultured in MS medium supplemented with NAA and Kinetin alone and in combinations (Table 1) show that the cotyledons formed callus within 40 to 43 days at 2.5 - 7.5 mg/l of NAA in culture medium and estimated mean weight of callus was between 0.09 g to 0.18 g. Callus was only induced when 0.5 mg/l of Kinetin was used alone, at an average period of 42 days with estimated mean weight of 0.18 g. At concentration 5 – 10 mg/l of NAA in combination with 0.5 mg/l of Kinetin, callogenesis was induced between 30 to 45 days and estimated mean weight of callus was between 0.15 to 0.25 g. At concentration 2.5, 7.5, and 10 mg/l of NAA in combination with 1 mg/l of Kinetin, callogenesis was induced between 30 to 45 days and estimated mean weight of callus was between 0.12 g to 1.05 g. Callogenesis was also induced at 10 mg/l of NAA in combination with 1.5 mg/l of Kinetin in 30 days, estimated mean weight was 0.51 g. At 7.5 and 10 mg/l of NAA in combination with 2 mg/l of Kinetin, callus was induced between 30 to 45 days; estimated mean weight of callus was between 0.24 g to 0.26 g. The highest callus fresh weight was obtained at 2.5 mg/l of NAA and 1 mg/l of Kinetin. The lowest callus fresh weight was obtained at 7.5 mg/l of NAA alone. *D. cumminsii* calli were mostly friable while a few were compact. Their colours ranged from white, milky, yellow and brown.

Table 1: Time of response and estimated mean callus weight of callus grown in MS basal medium supplemented with combinations of NAA and Kinetin (mg/l).

Phytohormone		Response	Average time of response (days)	Estimated mean weight of callus	Colour	Nature	Intensity
NAA	Kinetin						
0	**0**	-	-	-	-	-	-
2.5		Callus	40	0.18 ± 0.06	White	Friable	++
5		Callus	40	0.18 ± 0.05	White, Yellow, Brown	Friable, Compact	++
7.5		Callus	43	0.09 ± 0.02	White	Friable	+
10		-	-	-	-	-	-
0	**0.5**	Callus	42	0.18 ± 0.03	Yellow	Friable	++
2.5		-	-	-	-	-	-
5		Callus	45	0.25 ± 0.15	Yellow	Friable	+++
7.5		Callus	45	0.15 ± 0.04	Brown	Compact	++
10		Callus	30	0.20 ± 0.04	White	Friable	+++
0	**1**	-	-	-	-	-	-
2.5		Callus	45	1.05 ± 0.24	White, yellow, brown	Friable, compact	+++++
5		-	-	-	-	-	-
7.5		Callus	45	0.12 ± 0.07	White, brown	Friable, compact	++
10		Callus	30	0.16 ± 0.05	Brown	Compact	++
0	**1.5**	-	-	-	-	-	-
2.5		-	-	-	-	-	-
5		-	-	-	-	-	-
7.5		-	-	-	-	-	-
10		Callus	30	0.51 ± 0.12	Milky, white	Friable	++++
0	**2**	-	-	-	-	-	-
2.5		-	-	-	-	-	-
5		-	-	-	-	-	-
7.5		Callus	30	0.26 ± 0.07	Milky, white	Friable	+++
10		Callus	45	0.24 ± 0.05	White	Friable	+++

Note: Estimated mean weight of callus ± SD (g); n = 4

Key: (-) = No callus formation; (+) = Not profuse; (++) = Slightly profuse; (+++) = Profuse; (++++) = Very profuse; (+++++) = Highly profuse

When the cotyledons were cultured in MS medium supplemented with NAA and BAP alone and in combinations, it was observed (Table 2) that the cotyledons formed callus within 20 to 47 days at 2.5 - 10 mg/l of NAA in culture medium and estimated mean weight of callus was between 0.09 to 0.19 g. Callus was only induced when 0.5 – 2 mg/l of BAP was used alone, at an average period of 30 to 50 days with estimated mean weight of 0.08 to 0.34 g. At concentration 2.5, 7.5 and 10 mg/l of NAA in combination with 0.5 mg/l of BAP, callus was induced between 25 to 30 days and estimated mean weight of callus was between 0.03 to 0.14 g. At concentration 5 - 10 mg/l of NAA in combination with 1 mg/l of BAP, callus was induced between 12 to 27 days and estimated mean weight of callus was between 0.15 g to 0.40 g. Callogenesis was also induced at 10 mg/l of NAA in combination with 1.5 mg/l of BAP in 33 to 43 days, estimated mean weight was 0.31 to 0.39 g. At 2.5 to 10 mg/l of NAA in combination with 2 mg/l of BAP, callus was induced between 19 to 33 days; estimated mean weight of callus was between 0.17 g to 0.51 g.

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Table 2: Time of response and estimated mean weight of callus grown in MS basal medium supplemented with combinations of NAA and BAP

Phytohormone		Response	Average time of response (days)	Estimated mean weight of callus	Colour	Nature	Intensity
NAA	BAP						
0	**0**	-	-	-	-	-	-
2.5		Callus	34	0.09 ± 0.04	Brown	Compact	+
5		Callus	47	0.19 ± 0.14	White	Friable, Compact	++
7.5		Callus	20	0.09 ± 0.01	Milky, Brown	Friable	+
10		Callus	45	0.09 ± 0.04	White, Yellow	Friable, Compact	+
0	**0.5**	Callus	50	0.08 ± 0.05	White	Friable, Compact	+
2.5		Callus	25	0.03 ± 0.02	White, Yellow, Brown	Friable, Compact	+
5		-	-	-	-	-	-
7.5		Callus	31	0.11 ± 0.02	White, Yellow	Friable	++
10		Callus	30	0.14 ± 0.03	White	Friable	++
0	**1**	-	-	-	-	-	-
2.5		-	-	-	-	-	-
5		Callus	27	0.40 ± 0.49	Brown	Compact	++++
7.5		Callus	12	0.23 ± 0.02	White, Brown	Friable, Compact	+++
10		Callus	14	0.15 ± 0.04	Milky, Brown, White	Friable, Compact	++
0	**1.5**	Callus	30	0.34 ± 0.03	Yellow, White	Friable	+++
2.5		-	-	-	-	-	-
5		Callus	43	0.39 ± 0.17	White	Friable, Compact	+++
7.5		Callus	33	0.31 ± 0.07	White	Friable	+++
10		Callus	36	0.31 ± 0.18	White, Brown	Friable, Compact	+++
0	**2**	Callus	31	0.23 ± 0.01	White	Friable	+++
2.5		Callus	19	0.38 ± 0.24	White	Friable	+++
5		Callus	19	0.51 ± 0.30	White	Friable	++++
7.5		Callus	33	0.27 ± 0.19	White	Friable	+++
10		Callus	28	0.17 ± 0.10	White	Friable	++

Note: Estimated mean weight of callus ± SD (g); n = 4

Key: (-) = No callus formation; (+) = Not profuse; (++) = Slightly profuse; (+++) = Profuse; (++++) = Very profuse; (+++++) = Highly profuse; MS = Murashige and Skoog; NAA = Napthalene acetic acid and BAP= Benzyl Amino Purine

Table 3: Periods of shoot, root production and time of leaf emergence in MS basal medium supplemented with combinations of NAA and Kinetin

Phytohormone		Response	Period of Shooting (days) ± SD	Period of Rooting (days) ± SD	Time of Leaf Emergence (days) ± SD
NAA	Kinetin				
0	**0**	-	-	-	-
2.5		OR	-	69 ± 4.43	33 ± 1.71
5		OR	-	100 ± 2.58	33 ± 4.08
7.5		-	-	-	-
10		OR	32 ± 2.58	-	-
0	**0.5**	OR	28 ± 3.50	-	-
2.5		-	-	-	-
5		-	-	-	-
7.5		-	-	-	-
10		OR	98 ± 2.16	-	74 ± 3.16
0	**1**	-	-	-	-
2.5		-	-	-	-
5		OR	-	32 ± 3.77	-
7.5		-	-	-	-
10		-	-	-	-
0	**1.5**	-	-	-	-
2.5		-	-	-	-
5		-	-	-	-
7.5		-	-	-	-
10		OR	-	28 ± 2.94	-
0	**2**	-	-	-	-
2.5		-	-	-	-
5		-	-	-	-
7.5		-	-	-	-
10		-	-	-	-

Key: (-) = No response; OR = Direct organogenesis; MS = Murashige and Skoog and NAA = Napthalene acetic acid

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The highest callus fresh weight was obtained at 5 mg/l of NAA and 2 mg/l of BAP.

The initial time of shoot production, root production and time of leaf emergence from *D. cumminsii* cotyledons cultured in MS basal medium supplemented with NAA and Kinetin in combinations and alone is presented in Table 3. The earliest time of shoot production was in the medium supplemented with 0.5 mg/l of Kinetin alone, which was 28 days; shoot was also produced in the medium supplemented with 10 mg/l of NAA alone and 10 mg/l of NAA and 0.5 mg/l of Kinetin. The earliest time of root production was in the medium supplemented with 10 mg/l of NAA and 1.5 mg/l of Kinetin Rooting was also noticed in the medium supplemented with 2.5 and 5 mg/l of NAA alone, and also in 5 mg/l of NAA and 1 mg/l of kinetin. The earliest leaf emergence was, noticed in the medium supplemented with 2.5 and 5 mg/l of NAA alone, which was in 33 days. Also, leaf emergence was noticed in the medium supplemented with 10 mg/l of NAA and 0.5 mg/l of Kinetin.

Initial time of shoot production, root production and time of leaf emergence from *D. cumminsii* cotyledons cultured in MS basal medium supplemented with NAA and BAP in combinations and alone. The earliest time of shoot production was in the medium supplemented with 1 and 2 mg/l of BAP alone and 10 mg/l of NAA and 1.5 mg/l of BAP in combination, which was 11 days. The earliest time of rooting was in the medium supplemented with 5 mg/l of NAA and 1 mg/l of BAP. The earliest time of leaf emergence was in 2 mg/l of BAP alone. The effect of NAA and BAP in combination and alone on plantlet height was uniform after 42 days (6 weeks) of culture initiation (Table 5). Within 12 to 19 weeks, the plantlet heights of the various combinations of NAA and BAP started to differ. The results showed that medium containing only BAP (2 mg/l) produced the tallest plantlets.

Table 4: Period of shoot, root production and time of leaf emergence in MS basal medium supplemented with combinations of NAA and BAP

Phytohormone		Response	Period of Shooting (days) ± SD	Period of Rooting (days) ± SD	Time of Leaf Emergence (days) ± SD
NAA	BAP				
0	**0**	-	-	-	-
2.5		-	-	-	-
5		OR	27 ± 4.32	-	-
7.5		-	-	-	-
10		-	-	-	-
0	**0.5**	-	-	-	-
2.5		-	-	-	-
5		-	-	-	-
7.5		OR	14 ± 3.37	-	-
10		-	-	-	-
0	**1**	OR	11 ± 1.83	116 ± 2.63	80 ± 2.58
2.5		-	-	-	-
5		OR	-	11 ± 3.30	-
7.5		-	-	-	-
10		OR	-	20 ± 2.16	-
0	**1.5**	-	-	-	-
2.5		-	-	-	-
5		-	-	-	-
7.5		-	-	-	-
10		OR	11 ± 2.83	14 ± 3.50	-
0	**2**	OR	11 ± 3.30	19 ± 3.40	26 ± 4.40
2.5		OR	-	21 ± 3.16	-
5		OR	-	21 ± 4.40	-
7.5		-	-	-	-
10		-	-	-	-

Key: (-) = No response; OR = Direct organogenesis; MS = Murashige and Skoog; NAA = Napthalene acetic acid and BAP = Benzyl Amino Purine

Table 5: ***Effect of NAA and BAP on plant height of Dioscoreophyllumcumminsii at various stages of culture initiation.***

NAA (mg/l)	BAP (mg/l)	Plantlet height (mm), 6 weeks after Inoculation	Plantlet height (mm), 12 weeks after inoculation	Plantlet height (mm), 19 weeks after inoculation
0	**1**	1	5	12
10	**1.5**	1	10	15
0	**2**	1	10	34

Key: NAA = Napthalene acetic acid, BAP = Benzyl Amino Purine

Discussion

Plant growth and developmental processes, such as germination, stem elongation, leaf growth and development, flowering, fruit set, fruit growth and ripening are controlled by plant growth regulators (Gaba, 2005). In this study, the morphogenetic responses of *D.cumminsii* cotyledons cultured in MS medium supplemented with NAA, BAP or/and Kinetin (Plate 1A-E) followed direct (organs developed directly from the cotyledons) and/or indirect (explants developed into calli, which in turn gave rise to organs) pathways to induce organogenesis.

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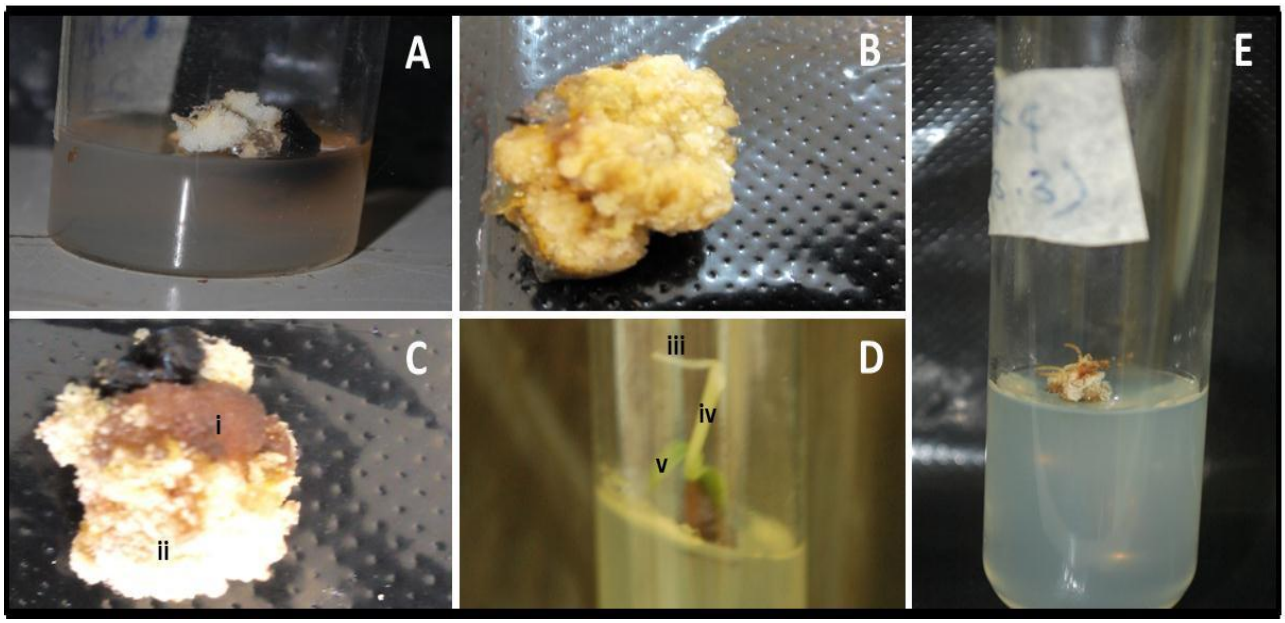

Figure 1: *In vitro* responses of *Dioscoreophyllum cumminsii* cotyledons cultured in MS medium

A) Callus in medium supplemented with NAA and BAP after 36 days of culture initiation.
B) Callus in medium supplemented with NAA and Kinetin after 40 days of culture initiation.
C) Callus in medium supplemented with NAA and BAP after 40 days of culture initiation.
D) Plantlet in medium supplemented with NAA and BAP after 21 days of culture initiation.
E) Rootlet in medium supplemented with NAA and Kinetin after 21 days of culture initiation.

(i) = Compact, (ii) = Friable, (iii) = Rootlet, (iv) = Shoot, (v) = Leaf

Among the growth hormones under study, high amount of NAA alone stimulated callogenesis. This observation is in agreement with the statement of Gaba (2005) that a greater than optimum concentration of auxin often causes callus production. The findings of Hussain *et al.,* (2012) also confirmed that high amount of auxin in MS medium induced callogenesis. Similar trend was also noticed in the medium containing 0.5 mg/l of kinetin alone. MS media containing 2.5 mg/l NAA + 1 mg/l Kinetin was optimal for the production of higher fresh weight of callus compared to other combinations. Also, MS media containing 5 mg/l NAA + 2 mg/l BAP was optimal for the production of higher fresh weight of callus compared to other combinations. NAA and Kinetin combination and NAA and BAP combination were better than when they were used alone based on the callus intensity. This is in agreement with the report of Oselebe and Ene-Obong (2007), which suggested that highest callus fresh weight was obtained when they cultured stem explants of *D. cumminsii* in MS medium supplemented with NAA and Kinetin. The above observation is in conformity with the report by Skoog and Miller (1957) on tobacco; they reported that when plant growth regulators were combined, cell division activity was enhanced.

The results of this study indicated that medium containing NAA (2.5 mg/l) alone produced the highest number of rootlets. Similar observation was noticed in the report of Seyyed *et al.* (2013), when they cultured *Alstroemeria* in MS medium supplemented with NAA; this gave rise to the highest number of rootlets.BAP (2 mg/l) produced taller plantlets than other interactions between NAA and BAP, as earlier reported by Ammirato (2004) that in *Dioscoreabulbifera* and *Dioscoreaalata*, cytokinin at moderate concentrations enhanced shoot development. Similarly high and intermediate levels of NAA in combination with various levels of BAP gave rise to organogenesis. The common form of organogenesis for NAA and BAP combination was plantlet formation. A possible explanation to the morphogenetic responses observed that explants' response to plant growth regulators depends on the genotype (species and/or cultivar), the state of the plant tissue, growth conditions, the nutrition of the source plant, the class, chemical structure and concentration of the plant growth regulators (Gaba, 2005).

Conclusion

The results obtained in the study showed that from callus intensities, NAA in combination with Kinetin was more effective for callogenesis when compared to NAA in combination with BAP. High and intermediate levels of NAA in combination with various levels of Kinetin gave rise to organogenesis, but the most common form of organogenesis was rhizogenesis (root formation). The future direction to this study is to optimize *in vitro* conditions required for the in vitro regeneration of *D. cumminsii* which is aimed towards its domestication and large scale production.

References

Abiodun, O.A. and Akinoso, R. (2014). Physico-chemical properties of serendipity berry (*Dioscoreophyllum cumminsii)* fruit. *Journal of Applied Science and Environmental Management*, 18 (2) 218-221.

Adansi, M.A. and Holloway, H.L.O. (1977). Seed germination and establishment of the serendipity berry, *Dioscoreophyllum cumminsii*. Diels. Acta Horticulturae, 53:407-411.

Ammirato, P.V. (2004).Yams.*In:* Ammirato, P.V., Erans, D.A., Sharp, W.R. and Yamada, Y. (eds). Handbook of Plant cell culture, Vol. 3. Macmillan, New York, pp. 329-354.

Ata, J.K.B.A. and Hammonds, T.W. (1976). A note on oil extracted from Serendipity berry (*Dioscoreophyllum cumminsii.* Diels), Ghana Journal of Agricultural Science, 9:63-64.

Gaba, V.P. (2005). Plant growth regulators in plant tissue culture. *In*: Trigiano, R.N. and Gray, D.J (eds). Plant development and biotechnology. CRC press, Boca Raton, London, New York, Washington D.C. 348p.

Holloway, H.L.O. (1977). Seed propagation of *Dioscoreophyllum cumminsii,* source of an intense sweetener. Economic Botany. 31:17-50.

Hussain, A., Qarshi, I.A., Nazir, H. and Ullah, I. (2012). Plant tissue culture: Current status and opportunities. *In:* Leva A. and

International Journal of Basic Science and Technology October 2015, Volume 1, Number 1, Pages 1 - 6

Rinaldi L.M.R. (eds) Recent advances in plant *in vitro* culture. In Tech, Rijeka, p. 1-28

IITA. (2008). Yam research for development, IITA publication. 1:1-10.

Inglett, G.E. (1981). Unusual sweeteners of plant origin.Food technology, 35 (3) 31-41.

Murashige, T. and Skoog, F. (1962).A realized medium for rapid growth and bioassays with tobacco cultures.*Plant Physiology*, 15: 473 – 497.

Obioh, G.I.B and Isichei, A.O. (2007).A population viability analysis of serendipity berry (*Dioscoreophyllum cumminsii)* in a semi-deciduous forest in Nigeria. Ecological modeling. 201:558-562.

Okoro, O.O. (1976). Propagation of *Dioscoreophyllum cumminsii.* Nigerian Journal of Forestry, 6:70.

Okoro, O.O. (1980). Propagation of *Dioscoreophyllum cumminsii.* Nigerian Journal of Forestry, 10:48-57.

Oselebe, H.O., Ene-Obong, E.E. and Nwankiti, O.C. (2004). The biology of *Dioscoreophyllum cumminsii* (Stapf) Diels. Nigerian Journal of Botany, 17:17-28.

Oselebe, H.O and Ene-Obong, E.E. (2007). Organogenesis in *Dioscoreophyllum cumminsii* (Stapf) Diels. Tropicultura, 25(1) 37-43.

Seyyed R. S.,Behzad K. and Naghi P.D. (2013). The effect of different concentrations of NAA and BAP on micropropagation of *Alstroemeria.* European Journal of Experimental Biology, 3(5):133-136.

Skoog, F. and Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissue cultured *In-vitro*. Symp. Soc. Exper. Biol. 11:118-131.