GROWTH RESPONSE OF EXPLANTS OF *Irvingia gabonensis* (O'RORKE, BAILL) TO *IN VITRO* TREATMENT

[*1] Etukudo, M. M., [2] Nkang, A. E., [3] Edu, E. A., [4] Osim, S.E. and [5] Udo, J. I.
[1&5] Department of Plant Science and Biotechnology, University of Port Harcourt
[2&3]Department of Botany, University of Calabar
[4]Department of Biological Sciences, Cross River State University of Technology

ABSTRACT
Growth response of explants of Irvingia gabonensis to in vitro treatment was investigated using full, half and one quarter strength mineral components based on Murashige and Skoog medium. Plant growth regulator (kinetin-Kin) with concentration levels of 0, 1, 2, 3, 4 and 5mg/l were used for shoots initiation, while axillary formed shoots were rooted in various concentration levels of 0, 1, 2, 3, 4 and 5mg/l of indole butyric acid (IBA). One quarter strength growth medium containing 3mg/l kinetin, which supported the highest growth performance was used to study the effect of explant source and number of leaf primordia on shoot initiation of the species. Shoot length of axillary bud explant significantly ($P<0.05$) increased in one quarter strength medium than those of half and full strength media. Axillary bud explants with 1-2 leaf primordia gave optimum response in terms of regeneration frequency and coefficient of velocity of bud burst. High regeneration frequency and coefficient of velocity of bud burst with low percentage contamination were recorded by seedling and stump sprout explants. This study suggests that axillary bud explants of Irvingia gabonensis can respond to in vitro treatment under optimum culture conditions.

Key words: *Growth response, explants, Irvingia gabonensis, in vitro*

INTRODUCTION

Irvingia gabonensis (O'Rorke, Baill) belongs to the family Irvingiaceae, and is considered as one of the over exploited species in the African humid and sub-humid tropics due to high demand for them by individuals and organizations (Etukudo, 2003; Okafor, 2005). The non-timber and timber products generated from *Irvingia* are useful both for domestic and industrial purposes, hence source of food and income to the teaming population (Nya *et al*., 2000; Okafor, 2005). Advances in micropropagation are aimed at rejuvenating forest resources, which are disappearing at an unprecedented rate. Central to this current effort is the need for extensive systematic germplasm collection, screening, evaluation and conservation of *Irvingia* species (Okafor and Ujor, 1994). *In vitro* culture techniques have been reported to record numerous applications to both temperate and tropical fruits crops (Rathhore *et al.*, 2006). Though micropropagation techniques have been developed for some woody species and have been successfully produced over the last few years, scanty reports exists on the micropropagation of *Irvingia gabonensis*. Thus, the use of *in vitro* techniques in practical forestry with this plant is limited at present.

Therefore, this study was aimed at evaluating the response of *Irvingia gabonensis* explants to *in vitro* treatments as a basis for producing large number of propagules of the species to compensate for the low propagation rate of conventional vegetative methods (Antangana *et al*., 2001; Ude *et al*., 2004).

MATERIALS AND METHODS

All the experiments were performed with axillary bud explants collected from *Irvingia gabonensis* grown in the field in Igbere, Bende Local Government Area of Abia State,

Nigeria. Explants of (0.5-1.5cm) *Irvingia gabonensis* were pretreated for 5minutes in 70% (v/v) ethanol solution, and surface disinfected for 4minutes in 0.1% mercuric chloride solution and rinsed 3-4 times in steriled distilled water. Single explant was placed on various strength, full (MS+Kin), half (½ MS + Kin) and one quarter (1/4 MS + Kin) of MS (Murashige and Skoog, 1962) medium supplemented with different levels (0, 1, 2, 3, 4 and 5mg/l) of kinetin (Kin). The pH of all media was adjusted to 5.8± 0.1 prior to the addition of 8g agar and dispensing (10ml) into culture tubes (test tubes) and then autoclaving. All cultures were maintained in the culture room at 28± 1 ^{o}C and 80% relative humidity with 16-h photo period under white fluorescent light for shoot initiation for 12 weeks. Shoots were transferred to fresh MS medium devoid of growth regulators for shoots elongation and maintained for 8 weeks. Axillary formed shoots were rooted in various levels(0, 1, 2, 3, 4, and 5mg/l of indole butyric acid (IBA) to enhance rooting and this was maintained for the development of plantlets for 12weeks.

One quarter strength growth medium with 3mg/l of kinetin (Kin), which gave the best growth performance was used to study, the effects of explant source (seedling, stump sprout, middle aged tree, and adult tree) on shoot initiation of the species. Shoot length (cm), regeneration frequency (%), coefficient of velocity of bud burst and contamination (%) were examined. Each treatment was replicated 10 times, repeated 2 times and the mean value expressed. The experimental set-up was a 3 X 6 factorial arrangement fitted into a randomized complete block design. Standard error of the mean values were calculated for the replicate readings and data were subjected to analysis of variance (ANOVA), where the differences in the means were tested using the least significant difference (LSD) at 0.05 level of probability.

RESULTS

One quarter strength MS medium with 3mg/l of kinetin was optimum for shoot initiation of the species. There were significant ($P<0.05$) differences in shoot length among treatments with full (MS+Kin), half (1/2 MS+Kin) and one quarter (1/4 MS +Kin) strength media. The shoot length of *I. gabonensis* increased with decrease in medium strength. The shoot length of *I. gabonensis* in MS, ½ MS and ¼ MS media increased from 1.84 ± 0.12, 2.56 ± 0.32 and 2.90± 0.53cm (control) to 2.47 ± 0.34 (5mg/l Kin), 4.22 ± 0.52 (5mg/l Kin) and 6.02 ± 0.72cm (3mg/l Kin), respectively (Table 1). The shoot length of *I. gabonensis* increased with decrease in medium strength (Table 2). Indole butyric acid supported the rooting of *I. gabonensis* with the best response being recorded at 5mg/l concentration (Table 3). In terms of response to concentration of plants growth regulators, the shoot length of the species increased with increase in concentration of plant growth regulator with 3mg/l kinetin recording the highest value in ¼ MS medium (Table 1, 2 and 3).

Regeneration frequency (RF) and coefficient of velocity of bud burst (CV) of explants of *I. gabonensis* cultured on ¼ MS medium with 3mg/l kinetin decreased from 75.00 ± 1.02% (RF) and 0.30 ± 0.04 (CV) in treatment with one (1) leaf primordium to 30.00±0.04% (RF) and 0.16±0.09 (CV) in treatment with four (4) leaf primordia comparable to the control treatment (having all leaf primordia intact) with a value of 25.00 ±0.03% (RF) and 0.15± 0.03 (CV) (Table 4).

High regeneration frequency of 80.00± 0.67 and 75.00 ± 0.43% were recorded by explants from seedling and stump sprout, respectively, while explants from middle aged

tree and adult tree of *I. gabonensis* recorded a regeneration frequency of 60.00 ± 0.51 and 45.00± 0.33%, respectively (Table 5).
Contamination percentage of *I. gabonensis* increased with increase in age of explants source with the lowest value of 10.00 + 0.32 in seedlings explants and highest value of 35.00 + 1.24 in explants from adult tree (Table 5). Coefficient of velocity of bud burst decreased with increased in age of explants source from 0.32± 0.02 in seedling explants to 0.15+ 0.02 in explants from adults tree (Table 5).

Table 1: Shoot length (cm) of Irvingia gabonensis as affected by various strength of Murashige and Skoog Medium (MS) at varying concentration of kinetin during shoot initiation stage maintain for 12 weeks

concentration of kinetin (mg/l)	0	**1**	**2**	**3**	**4**	**5**
Growth medium						
MS	1.84 ± 0.12	1.96 ± 0.17	2.07 ± 0.21	2.24 ± 0.13	2.40± 0.72	2.47 ± 0.34
MS	2.56 ± 0.32	3.60 ± 0.21	3.62 ± 0.27	3.80 ± 0.17	3.72 ± 0.66	4.22 ± 0.52
MS	2.90 ± 0.53	4.80 ± 0.40	4.94 ± 0.36	6.02 ± 0.22	5.09 ± 0.49	5.24 ± 0.37
Mean	2.43	3.45	3.54	4.02	11.21	3.98
LSD (P<0.05)	0.1	0.1	0.1	0.2	0.1	0.2

*Mean value ± standard error of 10 replicates from two determinations

Table 2: Shoot length(cm) of Irvingia gabonensis as affected by various strength of Murashige and Skoog(MS) medium during shoot elongation sgae maintain for 8 weeks

Pre-concentration levels of kinetin (mg/l)	0	**1**	**2**	**3**	**4**	**5**
Growth medium						
MS	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.00
MS	2.94 ± 0.41	4.26 ± 0.22	4.87 ± 0.11	5.66 ± 0.26	5.04 ± 0.62	5.01 ± 0.32
MS	3.66 ± 0.23	5.92 ± 0.13	6.50 ± 0.24	6.90 ± 0.22	6.24 ± 0.31	6.02 ± 0.06
Mean	2.20	3.39	3.79	4.19	3.76	3.68
LSD (P<0.05)	0.1	0.2	0.2	0.2	0.2	0.1

*Mean value ± standard error of 10 replicates from two determinations

Table 3: Root number of Irvingia gabonensis as affected by various strength of Murashige and Skoog(MS) medium at varying concentration of indole butyric acid during root initiation stagemaintain for12 weeks

concentration of indole butyric acid (mg/l)	0	1	2	3	4	5
Growth medium						
MS	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.00
MS	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MS	0.00 ± 0.00	1.05 ± 0.21	2.13 ± 0.04	3.52 ± 0.33	5.33 ± 0.29	6.45 ± 0.66
Mean	0.00	0.35	0.71	1.17	1.78	2.15
LSD (P<0.05)	0.0	0.1	0.1	0.1	0.1	0.2

*Mean value ± standard error of 10 replicates from two determinations

Table 4: Effects of number of leaf primodiaon regeneration frequencyR F (%), and coefficient of bud burst (CV) o*I rvingia gabonensis* explants cultured on 1/4 MS with 3mg/l Kinetin

Number of leaf Primordia	Regeneration Frequency RF (%)	Coefficient of Velocity of bud burst (CV)
0	25.00 ± 0.23	0.15 ± 0.03
1	75.00 ± 1.02	0.30 ± 0.04
2	60.00 ± 0.13	0.24 ± 0.09
3	45.00 ± 0.24	0.19 ± 0.03
4	30.00 ± 0.15	0.16 ± 0.07
Mean	47.00	0.21
LSD (0.05)	1.72	2.03

*Mean value ± standard error of 10 replicates from two determinations

Table 5: Effects of explants source on regeneration frequency RF (%),c ontamination (%) and coefficient of velocity of bud burst (CV) of *Irvingia gabonensis* explants cwlitred on h 3mg/l Kinetin MS

Explant source	Regeneration Frequency (%)	Contamination (%)	Coefficient of velocity of bud burst
Seedling	80.00 ± 0.67	10.00 ± 0.32	0.32 ± 0.02
Stump sprout	75.00 ± 0.43	15.00 ± 0.41	0.30 ± 0.06
Middle aged tree	60.00 ± 0.51	20.00 ± 0.30	0.21 ± 0.01
Adult tree	45.00 ± 0.33	35.00 ± 01.24	0.15 ± 0.02
Mean	65.00	20.00	0.25
LSD (0.05)	1.38	1.24	0.03

*Mean value ± standard error of 10 replicates from two determinations

DISCUSSION

Explants of *Irvingia gabonensis* cultured on one quarter strength medium exhibited optimum growth response than those of half and full strength media. The low regeneration frequency of explants of *Irvingia gabonensis* on media with full strength concentration was apparently due to higher salt concentrations that induced necrosis and death of the explants. This is consonance with the works of Panhwar, 2005, and Ozyigit *et al*.(2007) that full strength medium promoted the release of phenols due to an apparent phytotoxicity. The working out of a suitable culture medium and effective hormonal balance in favour of cytokinins has been shown to stimulate the breaking of bud explants, while those initiated in favour of auxins tends to promote the production of roots (Cheong and Pooler, 2003, Cline, 2000).

The percentage contamination of culture in this study was affected by the use of explants from field grown plants. Problems of contamination and necrosis during explants establishment have been reported (Bindu *et al*., 2007; Debnath, 2003). Choice of juvenile explants has been reported to reduce contamination rate (Cassells and O'Herlihy, 2003) and necrosis (Arnaldos *et al*., 2001).

The decreased regeneration frequency and coefficient of velocity of bud burst of *I. gabonensis* with increase in number of leaf primordia may be due to high meristematic activity being localized in specific directions, such that some parts in the explants were regenerative, while others were dormant. This agrees with the work of Nhut *et al*., (2003) who reported that the ability of explants to regenerate may be influenced by the thickness of the explants. This suggests that organogenesis in plant tissues cultured *in vitro* is often associated with localized cell division in specific directions (Rathhore *et al*., 2006). The low growth performance of explants of *I. gabonensis* generated from some stock plants may be attributed to the chronological age of the explant tissue (seedling, stump sprout, juvenile and adult tree) since they influence the extent of differentiation of the cells. This result agrees with the work of Vila *et al*., (2004) who reported that cells of adult woody plants have cells walls that are fully lignified,

as a result are virtually impossible to culture *in vitro*. Thus, a pre-existing low organogenic competence cannot be completely compensated for by manipulating the composition of the medium (Rodriguez and Vendrome, 2003).

CONCLUSION

This study shows that the response of axillary bud explants of *I. gabonensis* can be enhanced by utilization of optimum culture conditions. The starting materials, position, and age at the time of bud isolation are of great importance in influencing the extent of proliferation of bud explants *in vitro*.

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