

## The Influence of Growth Hormones and *Cocos nucifera* Water on the *In Vitro* Propagation of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill.

Gbadamosi I. T.\* and Sulaiman M. O.

Department of Botany, University of Ibadan, Ibadan, Nigeria

\*Corresponding author: Dr. Idayat T. Gbadamosi

Department of Botany, University of Ibadan, Ibadan, Nigeria; 08035505173 / 07056114030;

[gita4me2004@yahoo.com](mailto:gita4me2004@yahoo.com)

**Abstract:** *Irvingia gabonensis* fruit has high nutritional, therapeutic and commercial value. Despite its economic importance, this plant is threatened in Nigeria due to over collection in the wild and its very slow growth via natural method of propagation. In view of this, the *in vitro* culture of *I. gabonensis* was studied to promote its conservation and sustainable use in Nigeria. Embryos from fresh and ripe seeds of *I. gabonensis* were cultured aseptically on MS-basal media supplemented with varied concentration of four different growth hormones and *Cocos nucifera* (coconut) water. The development of *I. gabonensis* in culture was evaluated using standard growth parameters: viability; number of shoot; shoot length; number of root; root length; number of leaf primordia; and % callus formation. Data were analysed statistically. *Irvingia gabonensis* demonstrated varied growth patterns in cultures. Medium IG02 (1/4MS + 0.05mg/L NAA + 20.0% coconut water) gave the highest viability (60%) and best enhanced root formation (1.67 roots). Medium IG05 (1/4MS + 0.05mg/L BAP + 0.05mg/L KIN + 0.05 mg/L IBA + 10.0% coconut water) supported shoot (2.17 shoots) and leaf (6.00 leaves) formation. The least growth of *I. gabonensis* was recorded on the control medium (1/4MS only), although it best supported root elongation (58.67 mm). The experiments are easily reproducible and generated prototypes of the parent plant. It was concluded that pathogen-free *I. gabonensis* plantlets could be produced via tissue culture to supplement natural propagation. The improved variety of the plant could be produced from wild varieties via *in vitro* propagation and biotechnology to combat the slow growth of naturally propagated germplasms.

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### Introduction

*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill is indigenous to the humid forest zone of west and central Africa. It is found in southwestern Nigeria and southern Cameroon, and also in Côte d'Ivoire, Ghana, Togo and Benin. It is a valuable plant, being source of food, medicines, fodders, dye, building materials and item of commercial within and outside Nigeria (Tchoundjeu and Atangana, 2007).

The fruit kernel is an important food additive; used to thicken soups and stews. Edible oil extracted from the seed is used in cooking and as a substitute for cocoa butter. Matos *et al.* (2009) reported that the margarine based on *I. gabonensis* oil is an alternative to the trans-fatty acids obtained during hydrogenation and other reactions used in marginary. Unlike the fruit pulp of most other *Irvingia* spp. which is bitter, the pulp of the fruit of *I. gabonensis* is juicy and sweet and eaten fresh. It can be used for the preparation of juice, jelly, jam and wine. The kernels are also made into a cake called 'dika bread' or 'odika bread' for year-round preservation and easy use (Tchoundjeu and Atangana, 2007).

Traditionally, *I. gabonensis* bark is used as analgesic, antiseptic, antipyretic and also in the treatment of diarrhoea and hernia. The leaf extract is used as a febrifuge and the kernels in the management of diabetes (Burkill, 1985; Okolo *et al.*, 1995). The kernel fat has found application in weight loss (Ngondi *et al.*, 2005). George *et al.* (2007) stated that 2,3,8-tri-O-methyl ellagic acid isolated from the stem bark of *Irvingia gabonensis* showed significant antimicrobial activity on the pathogens causing diarrhoea/dysentery and typhoid fever. Raji *et al.* (2001) reported that the methanol extract of its stem bark has anti-diarrhoeal and antiulcer properties in rats. It has been used wholly or as supplement in the treatment of type II diabetics and in reducing obesity (Omoruyi *et al.*, 1994). Oben *et al.* (2008) stated that *Irvingia gabonensis* seed extract (IGOB131) resulted in a significant inhibition of intracellular triglycerides. A clinical test has shown that IRWINOL® LS 9319 a botanical butter extracted from the edible fruit of *Irvingia gabonensis* has a secondary activity in anti-age care and also function as a moisturizer ([www.docstoc.com](http://www.docstoc.com)).

The oil is solid at ambient temperatures and it is used for soap making. The pressed cake is a good cattle feed. The pulp has also been used to prepare a black dye for cloth. The wood is used locally for heavy construction work and for making ships' decks, paving blocks and planking. Young trees are used for making poles and stakes, while branches are made into walking sticks or thatched roof supports. Dead branches are used as firewood (Tchoundjeu and Atangana, 2007). The tree is usually preserved on farms to provide shade for crops such as Coffee and Cocoa and is reported to restore the soil fertility (Shiembo *et al.*, 1996). Fruit is only traded locally, but kernels are widely and extensively traded domestically, from the forest zone to the savanna zone and between countries in West and Central Africa. They are exported to Europe. Cameroon being the main exporter (Tchoundjeu and Atangana, 2007).

Sahoré *et al* (2012) reported that the *I. gabonensis* seed is rich in lipid and contains appreciable levels of carbohydrates, protein and mineral matter (K and Ca). The raw fruit pulp contains 61 Calories food energy, 81.4% moisture, 0.9 g/100g protein, 0.2 g/100g fat, 15.7 g/100g carbohydrates, 0.4 g/100g fibre, 1.8 g/100g ash, 49 mg phosphorous (P) and 1.8 mg iron (Fe) (Ola-Adams and Onyeachusim, 1992). Other authors also reported the proximate or nutritional constituents of *I. gabonensis* (Matos *et al.*, 2009; Womeni *et al.*, 2006; Adamson *et al.*, 1990).

In Nigeria, intensification of cultivation, bush-burning, lumbering and over exploitation of forest resources, urbanization, boundary dispute and rapid population growth are factors responsible for forest loss at an alarming rate (Okafor, 1992). Okafor (1992) reported that *I. gabonensis* is an important forest food plant that is progressively in the process of being lost. He stated that the plant is a suitable species for commercial production of jams and jellies, fruit juices,

oil, cosmetics, pharmaceutical and soup condiment "ogbono cubes". Ola-Adams and Onyeachusim (1992) also listed the plant among species threatened with extinction due to destruction of their habitats in Nigeria.

In view of the economic values of the plant and its conservation status being vulnerable to extinction, this study established protocols for its *in vitro* propagation to supplement natural propagation, promote its conservation and sustainable use as an economic plant in Nigeria. The study also explores the benefit of *Cocos nucifera* water in media for the *in vitro* propagation of *I. gabonensis*.

## Materials and Methods

### Collection and Identification of Plant

**material:** Fresh fruits of *Irvingia gabonensis* were collected during the raining season from University of Ibadan campus. The seeds were removed and stored in glass bottles for further use in experiments. The plant was identified at species level at the University of Ibadan Herbarium (UIH).

**Media preparation:** The experiment was performed in the tissue culture laboratory of National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria in 2011. The media constituents are presented in Table 1. Media IG01, IG02, IG03 and IG05 were used to study the effects of auxins, cytokinins and coconut water on the growth of *I. gabonensis* in culture. Medium IG04 was used to observe the influence of coconut water only on the plant growth and IGC was the control culture of 1/4 MS only. The pH of each medium was adjusted to 5.7 with 1M NaOH or 1M HCl prior to the addition of 0.7% agar (Difco, USA). Media and instruments were sterilized by autoclaving for 15 – 30 min at 121°C (1 atm) (Gbadamosi and Egunyomi, 2010).

**Table 1: The Media Components Used for the *In-Vitro* Growth of *Irvingia gabonensis***

Media code	Components						
	1/4 MS	BAP (mg/L)	NAA (mg/L)	KIN (mg/L)	Coconut water % (v/v)	IBA (mg/L)	Sucrose (mg/L)
IG01	+	0.65	0.05	-	20.00	-	30.00
IG02	+	-	0.05	-	20.00	-	30.00
IG03	+	0.50	-	0.05	-	-	30.00
IG04	+	-	-	-	25.00	-	30.00
IG05	+	0.50	-	0.05	10.00	0.05	30.00
IGC (Control)	+	-	-	-	-	-	30.00

**Legends:** + = Present; - = Absent; MS = Murashige and Skoog salt base (Murashige and Skoog, 1962); NAA = 1-naphthalene acetic acid; BAP = Benzyl aminopurine; KIN = Kinetin; IBA = 1H-indole-3-butyric acid.

**Aseptic inoculation of explants in media:** The surface sterilization of explants (seed) was done using

standard methods (Gbadamosi and Egunyomi, 2010). The sterile explants were inoculated in cultures in a

laminar flow hood. The embryos were removed from seeds and implanted vertically on the sterile media in glass tubes (20 x 150 mm) using forceps. The glass tubes were sealed with paraffin wax and labeled. The cultures were maintained at  $27 \pm 1^\circ\text{C}$  with a photoperiod of 16 h at an intensity of 10 - 20  $\mu\text{mol m}^{-2} \text{S}^{-1}$  (Phillips ThD 36W/84) in the growth room for a period of 30 - 90 days. Each treatment was replicated three times.

*In vitro* plantlets were repeatedly sub-cultured on MS supplemented with NAA and BAP media at 4-week intervals for six months. Rooted plantlets in MS supplemented with NAA and BAP media were washed and transferred to plastic chambers containing sterile mixture of soil and sand (1:1). The plastic chambers were covered with a plastic cap that was gradually opened during the acclimatization period of fifteen days.

**Evaluation of growth of *Irvingia gabonensis* in cultures:** Growth of the plant was assessed weekly. The growth factors used were viability, shoot length, root length, number of leaf primordia, root number, shoot number and % callus formation. Growth was observed, measured and recorded.

**Data analysis:** The data were statistically analysed using ANOVA and Duncan's multiple range tests. Significant differences between means were determined at  $p < 0.05$ .

## Results and Discussion

Table 2 shows the influence of growth hormones and various concentrations of coconut water on the development of *I. gabonensis* in cultures. The highest viability (60%) of the plant in culture was recorded on IG02 and IG04, followed by 50% viability on IG01 and IG03, and the (least 10%) was on the control medium (IGC). Furthermore medium IG04 supported rapid shooting (Plate 1). IG02 and IG04 contained 20% and 25% coconut water respectively, the coconut water significantly enhanced viability and shooting. Shoot production was best enhanced on medium IG05 (Table 1 and Plate 2) which contained two types of cytokinins (0.50 mg/L BAP and 0.05 mg/L KIN) with 2.17 shoots, other media including the control medium produced 1 shoot only. Cytokinins are known to promote cell division, cell differentiation, and shoot production (Razdan, 2003). Although cytokinins are primarily involved in cell growth and differentiation, they also affect apical dominance, axillary bud growth, and leaf senescence (Kieber, 2002). The longest shoot (50.00 mm) was obtained from medium IG02, followed by 49.50 mm on IG05 and the least (23.67 mm) on IGC. Root formation was best on IG02 with 1.67 roots, followed by 1.50 roots on IG01 and medium IG05 did not support root

formation. Medium IG02 contained 0.05 mg/L NAA and 20% coconut water. Auxins (NAA) have characteristic features of promoting cell division, stem elongation and rooting (Razdan, 2003). The longest root was recorded on the control medium (IGC) with 58.67 mm (Plate 3) and the shortest (9.05 mm) on IG01. Medium IG05 best enhanced leaf formation with 6 leaves, followed by IG01 with 5.17 leaves and least (3.17 leaves) was recorded on IG04. There was no callus formation on all the media used.

Medium IG04 contained 25% coconut water only and it supported viability, shooting, shoot elongation, rooting, root elongation and leaf formation. Coconut water as an organic supplement in culture media had significant effect on growth and shoots production. Razdan (2003) reported that the success achieved with the use of coconut water in tissue culture is 5 - 20 % and it is reasonably significant. Ge *et al.* (2004) reported the presence of zeatin-O-glucoside and dihydrozeatin-O-glucoside, a cytokinin in coconut water. The shoot inducing effect of the liquid endosperm of green coconuts has been reported by other researchers and in various online journals ([www.coconut-water-products.com](http://www.coconut-water-products.com)).

This study recorded shooting of *I. gabonensis* on  $\frac{1}{4}$  MS only (IGC) in contrast to the non-shooting of the plant recorded on the same medium by Fajimi *et al.* (2007). The observed difference might be due to the physiological condition of the explants which is determined by genetic factors (Nagarathna *et al.*; 1991). Also the use of coconut water in combination with auxins ( $\frac{1}{4}$  MS + 0.5 mg/L NAA + 20 % coconut water) in IG02 medium significantly supported multiple shooting and rooting (IG02) compared to non-shooting reported by Fajimi *et al.* (2007) on  $\frac{1}{4}$  MS + 0.1 mg/L NAA. The observed difference in growth might be due to difference in the concentration of NAA and the use of coconut water in media. Fotso *et al.* (2008) also studied the *in vitro* regeneration of *I. gabonensis* by somatic embryogenesis and recorded callus formation on media containing varied concentrations of 2-4-D and BAP. Somatic embryos were regenerated on varied concentration of BAP and NAA in media. Comparatively, the protocol for regeneration of *I. gabonensis* in this study is faster than that of Fotso *et al.* (2008), because multiple shoots were obtained directly from embryos as explants in cultures instead of calluses obtained in their study. Regeneration of plantlets from calluses could be hard and slow.

Although there are some reports on the *in vitro* propagation of Plant Genetic Resources (PGR) by previous authors: Aleo vera (Arvind *et al.*, 2010), *Acacia nilotica* (Dhabhai *et al.*, 2010), *Phyllanthus urinaria* (Kalidass and Mohan, 2009), *Taxodium distichum* (Abou Dahab *et al.*, 2010) and *Dioscorea*

*rotundata* (Ezeibekwe *et al.*, 2009). However efforts should be intensified towards conservation of reportedly threatened and endangered PGR in various

countries to ensure conservation and sustainable use of PGR globally.

**Table 2: The *In-Vitro* Growth of *Irvingia gabonensis***

Media Code	(%)Viability	Number of shoot	Shoot Length(mm)	Number of Root	Root Length(mm)	No.of Primordial	Leaf	%Callus Formation
IG01	50.00 <sup>b</sup>	*1.00±0.00 <sup>b</sup>	33.50±20.44 <sup>abc</sup>	1.50±0.55 <sup>a</sup>	9.50±3.02 <sup>d</sup>	5.17±2.23 <sup>ab</sup>		0.00 <sup>a</sup>
IG02	60.00 <sup>a</sup>	1.00±0.00 <sup>b</sup>	50.00±15.46 <sup>a</sup>	1.67±0.52 <sup>a</sup>	12.8±9.43 <sup>c</sup>	3.33±1.51 <sup>bc</sup>		0.00 <sup>a</sup>
IG03	50.00 <sup>b</sup>	1.00±0.00 <sup>b</sup>	36.33±15.46 <sup>abc</sup>	1.00±0.00 <sup>b</sup>	30.33±15.10 <sup>b</sup>	5.00±1.67 <sup>ab</sup>		0.00 <sup>a</sup>
IG04	60.00 <sup>a</sup>	1.00±0.00 <sup>b</sup>	31.17±19.11 <sup>abc</sup>	1.00±0.00 <sup>b</sup>	23.83±11.87 <sup>bc</sup>	3.17±1.47 <sup>c</sup>		0.00 <sup>a</sup>
IG05	45.00 <sup>c</sup>	2.17±0.98 <sup>a</sup>	49.50±25.23 <sup>ab</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>e</sup>	6.00±2.83 <sup>a</sup>		0.00 <sup>a</sup>
IGC	10.00 <sup>d</sup>	1.00±0.00 <sup>b</sup>	23.67±8.17 <sup>d</sup>	1.00±0.00 <sup>b</sup>	58.67±23.76 <sup>a</sup>	3.33±1.03 <sup>bc</sup>		0.00 <sup>a</sup>

\*Mean of 3 readings ± standard deviation.

\*Values in the same column followed by the same letter are not significantly different ( $p > 0.05$ ) from each other. They differ significantly ( $p < 0.05$ ) with values that do not share a similar letter. Evaluation was made after 60 days in culture.



**Plate 1.** Rapid shooting of *Irvingia gabonensis* on IG04 (1/4 MS + 25.0 % coconut water) after 14 days in culture.



**Plate 2.** Multiple Shooting of *Irvingia gabonensis* on IGO5 (1/4 MS + 0.50 mg/L BAP + 0.05 mg/L KIN + 0.05 mg/L IBA + 10.0 % coconut water) after 60 days in culture.



**Plate 3.** Root elongation of *Irvingia gabonensis* on IGC (1/4 MS only) after 30 days in culture.

### Conclusion

This study reported the protocol for the *in vitro* propagation of *I. gabonensis* and the benefit of coconut water in its *in vitro* cultures. Further research should include creation of genebank and *in vitro* bank for wild varieties of west and central Africa to preserve their germplasms. The production of genetically modified varieties from the wild varieties via biotechnology could help in overcoming slow growth of naturally propagated *I. gabonensis*.

### Corresponding author:

Dr. Idayat T. Gbadamosi  
Department of Botany,  
University of Ibadan,  
Ibadan, Nigeria;  
08035505173 / 07056114030;  
gita4me2004@yahoo.com

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