
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

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 primordial; 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.

Keywords: Irvingia gabonensis, embryo, tissue culture experiment, growth hormones, Cocos nucifera water, Nigeria.

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 margarinery. 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).
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 and its branches are used as walking sticks or thatched roof supports. Dead branches are used as firewood and its branches are used as walking sticks or thatched roof supports. Dead branches are used as firewood and its branches are used as walking sticks or thatched roof supports. Dead branches are used as firewood and its branches are used as walking sticks or thatched roof supports.

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 ¼ 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) (Gbadamos and Egunyomi, 2010).

<table>
<thead>
<tr>
<th>Media code</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4 MS</td>
<td>BAP (mg/L)</td>
</tr>
<tr>
<td>IG01</td>
<td>+</td>
</tr>
<tr>
<td>IG02</td>
<td>+</td>
</tr>
<tr>
<td>IG03</td>
<td>+</td>
</tr>
<tr>
<td>IG04</td>
<td>+</td>
</tr>
<tr>
<td>IG05</td>
<td>+</td>
</tr>
<tr>
<td>IGC</td>
<td>+</td>
</tr>
</tbody>
</table>

**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 (Gbadamos and Egunyomi, 2010). The sterile explants were inoculated in cultures in a

Table 1: The Media Components Used for the In-Vitro Growth of *Irvingia gabonensis*
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 ± 1°C with a photoperiod of 16 h at an intensity of of 10 - 20 μmol m-2 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 subcultured 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 primordial, 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 cytokinins (0.50 mg/L BAP and 0.05mg/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 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 feature 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).

This study recorded shooting of I. gabonensis on ¼ 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 (¼ 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 ¼ 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 urinavias (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

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

*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.

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**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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