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RESEARCH ARTICLE

GROWTH AND DEVELOPMENT RESPONSE OF CALLUS SEGMENTS OF IRVINGIA GABONENSIS AUBREY-LECOMTE, EX O' RORKE USING TISSUE CULTURE TECHNIQUES

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ABSTRACT

Growth and development response of callus segments of I. gabonensis were examined using tissue culture techniques. Explants were aseptically placed and inoculated on Murashige and Skoog (MS) callus induction medium of full-MS, 1/2 MS and 1/4 MS strengths, each supplemented with the following concentrations of plant growth regulators (PGR); To (control) - treatment without plant growth regulator, T1 - treatment containing 0.1 mg/l kinetin + 2.0 mg/l NAA, T2- treatment containing 0.2 mg/l kinetin + 4.0 mg/l NAA, T3- treatment containing 0.3 mg/l kinetin + 6.0 mg/l NAA, T4- treatment containing 0.4 mg/l kinetin + 8.0 mg/l NAA and T5- treatment containing 0.5 mg/l kinetin + 10.0 mg/l NAA. The fresh weight, dry weight, moisture contents, chlorophyll a and b contents of callus segments of the species significantly ($P< 0.05$) increased with increase in concentration of plant growth regulators and with decrease in medium strength. The optimum growth and development response were recorded at one quarter strength medium relative to full and half strengths media. Therefore, this study provides baseline information for optimum callus induction protocols and continuous domestication of this over exploited species.

INTRODUCTION

Bush mango (*Irvingia gabonensis*) grows in the tropical forests of Africa and also domesticated in farms in central and western Africa (APG, 2003; Antagana *et al*., 2001). It is regarded as the most economically important of commercially viable cultivars from forest species for fruits (Etukudo, 2003). *Irvingia gabonensis*, commonly called bush mango belongs to the family Irvingiaceae (Ude *et al*., 2004). It is characterized by sweet mesocarp, which is soft, juicy with bright orange colour, while the seeds or kernels are classified as oil seeds and usually added to soups as a thickening agent (Etukudo *et al*., 2014, Silbou *et al*., 2000).

Bush mango has potential economic and industrial applications in food, cosmetic and pharmaceutical products (Omokaro *et al*., 1999; Okafor, 2005). Medicinally, the bark, roots, leaves and kernels have been utilized in treatments of diseases such as diabetes and obesity as well as possess analgesic, antimicrobial and antioxidant properties (Fadare and Alaiyeoba, 2008; George and Zhao, 2007). Tissue culture is an area, which is attracting the attention of scientists, forest manager and conservationists (Abrie and Staden, 2001; Jain, 2002).

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Micropropagation can create or introduce new products into the market faster than conventional methods, and the speed of its development has been accelerated by its practical commercialization (Ude *et al*., 2004). Micropropagation, involves the production of plants on an artificial medium in a controlled environment, under sterile conditions (Guo *et al.,* 2007; Mondal *et al.,* 2004).Plant regeneration *in vitro* may be accomplished by employing callus, organ, cell and protoplast cultures. Though tissue explants from tree species are generally difficult to grow and differentiate *in vitro*, callus and organ cultures have been employed with varying degrees of success for micropropagation of a number of woody plants (Herman, 2006; Sarasan *et al.,* 2006). Although callus cultures were initially used for plantlets regeneration, organ cultures (embryos, cotyledons, bud meristems) are now mostly routinely employed for micropropagation (Herman, 2006; Jain and Ishii, 2003). Initiation of organized development *in vitro* is a function of various factors in, and outside the culture medium, as well as the state of the explants (Jain and Spencer, 2006; Nandwani *et al*., 2004). Thus, it is clear that although exogenous growth regulators play a pivotal role in the initiation of organized development, they do so in concert with a variety of other factors (Jain and Swennen, 2004; Siobhan *et al*., 2003; Sugiyama, 2000; Torne *et al*., 2001). Therefore, this present study was conducted to assess the growth and development characteristics of callus segments of *Irvingia gabonensis* generated through *in vitro* techniques.

Table 1. Growth Response of Callus Segments of *Irvingia gabonensis In vitro*

Growth parameters	Treatment (PGR-mg/l) Mg/l	T_0	T_1	T_2	T_3	T_4	T_5
Fresh weight (g)	MS	0.00 ± 0.00	1.29 ± 0.17	1.37 ± 0.42	1.49 ± 0.25	1.57 ± 0.11	1.77 ± 0.23
	½ MS	0.00 ± 0.00	1.82 ± 0.36	1.97 ± 0.84	2.11 ± 0.52	2.27 ± 0.54	2.44 ± 0. 63
	¼ MS	0.00 ± 0.00	2.07 ± 0.43	2.42 ± 0.27	2.67 ± 0.61	3.12 ± 0.72	3.36 ± 0.27
Dry weight (g)	MS	0.00 ± 0.00	0.36 ± 0.09	0.37 ± 0.06	0.40 ± 0.04	0.42 ± 0.04	0.45 ±0.02
	½ MS	0.00 ± 0.00	0.38 ± 0.03	0.39 ± 0.05	0.42 ± 0.02	0.45 ± 0.04	0.48 ± 0.06
	¼ MS	0.00 ± 0.00	0.42 ± 0.06	0.46 ± 0.07	0.52 ± 0.09	0.58 ±0.05	0.62 ±0.01
Moisture content (%)	MS	0.00 ± 0.00	72.09 ± 0.15	72.99 ± 0.32	73.15 ± 0.13	73.24 ±0.66	74.58 ±0.54
	½ MS	0.00 ± 0.00	79.12 ± 0.58	80.20 ± 0.33	80.09 ± 0.63	80.18 ±0.34	80.32 ±0.56
	¼ MS	0.00 ± 0.00	74.88 ± 0.63	80.99 ± 0.57	80.52 ± 0.27	81.41 ± 0.39	81.55 ± 0.26

Mean value ± standard error of 10 replicates from two determinations

Note:

- PGR- Plant growth regulators
- MS- Full strength growth medium
- ½ MS- Half strength growth medium
- ¼ MS- One-quarter strength growth medium
- To (control) – treatment without plant growth regulator
- T_1 - treatment with 0.1 mg/l kinetin + 2.0 mg/l NAA
- T_2- treatment with 0.2 mg/l kinetin + 4.0 mg/l NAA
- T_3- treatment with 0.3 mg/l kinetin + 6.0 mg/l NAA
- T_4- treatment with 0.4 mg/l kinetin + 8.0 mg/l NAA
- T_5- treatment with 0.5 mg/l kinetin + 10.0 mg/l NAA

Table 2. Chlorophyll contents of Callus Segments of *Irvingia gabonensis In vitro*

Parameters	Treatment (PGR-mg/l) Mg/l	T_0	T_1	T_2	T_3	T_4	T_5
Chlorophyll a (mg/g)	MS	0.00 ± 0.00	0.737 ± 0.07	1.105 ± 0.02	1.309 ± 0.05	1.730 ± 0.01	2.062 ± 0.03
	½ MS	0.00 ± 0.00	1.027 ± 0.32	1.249 ± 0.21	1.636 ± 0.27	2.395 ± 0.31	2.707 ± 0. 74
	¼ MS	0.00 ± 0.00	2.072 ± 0.73	2.407 ± 0.64	2.764 ± 0.14	3.406 ± 0.24	3.982 ± 0.79
Chlorophyll b (mg/g)	MS	0.00 ± 0.00	0.526 ± 0.06	0.852 ± 0.04	1.105 ± 0.02	1.547 ± 0.23	1.821 ± 0.14
	½ MS	0.00 ± 0.00	0.741 ± 0.02	0.930 ± 0.04	1.219 ± 0.16	2.170 ± 0.22	2.312 ± 0.42
	¼ MS	0.00 ± 0.00	1.926 ± 0.14	2.215 ± 0.54	2.421 ± 0.17	3.213 ± 0.29	3.520 ± 0.31
Chlorophyll ab (mg/g)	MS	0.00 ± 0.00	1.206 ± 0.18	1.897 ± 0.27	2.402 ± 0.32	3.253 ± 0.51	3.796 ± 0.40
	½ MS	0.00 ± 0.00	1.632 ± 0.84	2.166 ± 0.36	2.843 ± 0.17	4.439 ± 0.26	5.002 ± 0.68
	¼ MS	0.00 ± 0.00	3.786 ± 0.35	4.502 ± 0.77	5.024 ± 0.31	6.503 ± 0.92	7.376 ± 0.43

Mean value ± standard error of 10 replicates from two determinations

Note:

- PGR- Plant growth regulators
- MS- Full strength growth medium
- ½ MS- Half strength growth medium
- ¼ MS- One-quarter strength growth medium
- To (control) – treatment without plant growth regulator
- T_1 - treatment with 0.1 mg/l kinetin + 2.0 mg/l NAA
- T_2- treatment with 0.2 mg/l kinetin + 4.0 mg/l NAA
- T_3- treatment with 0.3 mg/l kinetin + 6.0 mg/l NAA
- T_4- treatment with 0.4 mg/l kinetin + 8.0 mg/l NAA
- T_5- treatment with 0.5 mg/l kinetin + 10.0 mg/l NAA

MATERIALS AND METHODS

Callus Induction Medium

Apical bud explants from seedlings of *I. gabonensis* were sectioned to about 2cm in size, soaked for 5 minutes in 70% (V/V) ethanol solution, and transferred to 0.1% mercuric chloride solution for 4 minutes. Explants were aseptically placed and inoculated on Murashige and Skoog (MS) callus induction medium of full-MS, $^1/_2$ MS and $^1/_4$ MS strengths, each supplemented with the following concentrations of plant growth regulators (PGR); To (control) – treatment without plant growth regulator, T_1 - treatment containing 0.1 mg/l kinetin + 2.0 mg/l NAA, T_2- treatment containing 0.2 mg/l kinetin + 4.0 mg/l NAA, T_3- treatment containing 0.3 mg/l kinetin + 6.0 mg/l NAA, T_4- treatment containing 0.4 mg/l kinetin + 8.0 mg/l NAA and T_5- treatment containing 0.5 mg/l kinetin + 10.0 mg/l NAA. The experimental set up was maintained at a relative humidity of 80% under dark condition for 3 weeks.

Determination of Fresh Weight, Dry Weight and Moisture Content

Callus segments were removed from the culture medium and washed with sterile-distilled water using a sieve.

The fresh weight of the callus segments were measured using mettle-P-165 weighing balance. The fresh callus segments were dried in Gallen kamp oven at 65^{o}C for 2 days for the determination of dry weight. The percentage moisture content of the callus segments was measured by determining the differences between the fresh weight and dry weight of the callus segments multiplied by 100 over the fresh weight (Esenowo, 2001; Pajevic *et al*., 2004).

Determination of Chlorophyll Content

Standard methods were used for the determination of chlorophyll content in callus segments of the test plant (Ekanayake and Adeleke, 1996; Lahai *et al*., 2003). 2g of callus tissue was crushed with a mortar and homogenization of the tissue was carried out by adding appropriate quantity of 80% acetone. The supernatant was extracted with a filter paper into a 100ml volumetric flask. Repeated addition of acetone to the residue in the mortar and the extraction process was carried out. A 100ml mark was attained in the volumetric flask by using additional acetone to wash off the chlorophyll. The solution was appropriately mixed and 5ml pipette into a 50ml flask. The final solution was made to volume with 80% acetone. 80% acetone was used as blank for measurement of absorbance of the extract at 645, 663, and 652nm wavelengths using spectrophotometer for chlorophyll a, b, and ab respectively. The calculation of concentration of chlorophyll (mg/g fresh leaf weight) was carried out.

Statistical Analysis

Data analysis was carried out using analysis of variance (ANOVA) ($P < 0.05$) using the method of Ogbeibu (2005).

RESULTS

The growth responses of callus segments of *I. gabonensis* varied considerably among the various media strengths (MS, ½ MS and ¼ MS) and concentrations of plant growth regulators (T_0, T_1, T_2, T_3, T_4 and T_5). The fresh weight of callus segments of *I. gabonensis* significantly ($P< 0.05$) increased with increase in concentration of plant growth regulators and with decrease in medium strength (Table 1). The dry weight of callus segments of *I. gabonensis* significantly ($P< 0.05$) increased from 0.00 to 0.45g, 0.00 to 0.48g and 0.00 to 0.62g in the control- MS-T_0 to MS-T_5, ½ MS-T_0 to ½ MS-T_5 and ¼ MS- T_0 to ¼ MS-T_5, respectively. The highest moisture contents of 74.58, 80.32 and 81.55% were recorded at MS-T_5, ½ MS-T_5 and ¼ MS-T_5, respectively (Table 1).

The chlorophyll a content in callus segments *I. gabonensis* ranged from 0.00, 0.737, 1.05, 1.309, 1.730 to 2.062 mg/g in MS medium, 0.00, 1.027, 1.249, 1.636, 2.395 and 2.707 mg/g in ½MS medium and 0.00, 2.072, 2.407, 2.764, 3.406 and 3.982 mg/g in ¼ MS medium for T_0, T_1, T_2, T_3, T_4 and T_5 concentrations of plant growth regulators, respectively (Table 2). The values of 0.00, 0.526, 0.852, 1.105, 1.547 and 1.821 mg/g (MS medium), 0.00, 0.741, 0.930, 1.219, 2.170 and 2.312 mg/g (½ MS medium), and 0.00, 1.926, 2.215, 2.421, 3.213 and 3.520 mg/g (¼ MS medium) were recorded for chlorophyll b at T_0, T_1, T_2, T_3, T_4 and T_5 concentrations of plant growth regulators, respectively (Table 2). The total chlorophyll contents (ab) significantly increased ($P < 0.05$) with increase in the concentration of plant growth regulators and with decrease in medium strength (Table 2).

DISCUSSION

In this study, higher growth performance in terms of fresh weight, dry weight and moisture contents were recorded at lower strength growth medium (one quarter strength) than those of half and full strength media. These variations may be attributed to differences in salt concentrations of MS, ½ MS and ¼ MS media (Etukudo *et al*., 2011). Medium with higher salt concentration has been shown to induce necrotic symptoms and death of explants (Panhwar, 2005). Full strength medium promotes phenolic problems in explants due to phytotoxicity of the salt components (Bell and Reed, 2002; Etukudo *et al*., 2014). This explains the reason for lower values of fresh weight, dry weight and moisture contents at half and full strength media comparable to one quarter strength medium. In addition, callus tissue –medium- water relationship may have contributed to differences in fresh weight, dry weight and ability of callus segments to absorb water as a results of variation in osmotic potentials of the growth media (Aremu and Meshitsuka, 2005; Esenowo, 2004; Herman, 2006).

The variations in chlorophyll contents of callus segments of *I. gabonensis* among the various treatments may be due to inhibition of metabolic processes (Gupta and Gupta, 2005). In this study, higher contents of chlorophyll a, b and ab were recorded at ¼ MS medium comparable to MS and ½ MS media. This shows that changes in pH and nutrients components resulting from the chemical constituents of the growth medium might have affected the processes of cell division, cell wall, ion fluxes and plasma membrane (Esenowo, 2004, Verma and Verma, 2007). Therefore, lower chlorophyll components at higher medium strength may be attributed to inhibition of cell division, malfunction of cell wall, inhibition of ion fluxes, disruption of plasma membrane intergrity and inhibition of signal transduction pathways (Matsumoto, 2000; De-campos *et al*., 2005).

Conclusion

This study suggests that growth and development parameters of callus segments of *I. gabonensis* showed optimum response in one quarter strength MS medium relative to full and half strength MS media. Therefore, one quarter strength callus induction medium proves effective for further regeneration protocols of the species.

Conflict of interest

There was no conflict of interest among the three authors that contributed meaningfully to this research.

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