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RESEARCH ARTICLE

GROWTH STUDIES OF BUSH MANGO- *IRVINGIA WOMBOLU* MILDBR USING *IN VITRO* TECHNIQUES

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ABSTRACT

Growth studies of Bush mango- *Irvingia wombolu* Mildbr was investigated using *In vitro* techniques with full, half and one quarter strength mineral components of Murashige and Skoog medium. This research was designed into 2 experiments. Experiment 1 was aimed at assessing the best medium strength and concentration of plant growth regulator for optimum growth of explants (axillary bud) of the species, using the concentration levels of 0, 1, 2, 3, 4, and 5mg/l of kinetin (Kin) and indole butyric acid (IBA) for shoot and root initiation, respectively. Experiment 2 was aimed at assessing the growth performance of explants of the species using the best medium strength (one quarter strength with 3mg/l kinetin) for further growth studies. In experiment 1, significant ($P< 0.05$) increase in shoot length of axillary bud explants was recorded in one quarter strength medium, while those of half and full strength media showed no significant increases. In experiment 2, leaf primordia of 1-2 layers as well as seedling and stump sprout explants were effective for optimum regeneration of the species. This study shows that optimum growth response of *Irvingia wombolu* explants can be achieved at lower medium strength ($^1/_4$ MS).

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INTRODUCTION

Irvingia wombolu (Mildbr) belongs to the family Irvingiaceae, and is regarded as one of the most economically viable cultivars from forest species for fruits (Etukudo, 2003; Etukudo *et al*., 2010; Okafor, 2005). It is one the *Irvingia* species found in Nigeria, and displays close similarity in many fundamental characters with *Irvingia gabonensis* (Ladipo *et al*., 1994). Its economic importance derives from the range of non-timber and timber products obtainable from the tree, which are useful both for domestic and industrial purposes (Etukudo, 2003; Nya *et al*., 2000). The kernel, which is widely known as Ogbono in some parts of Nigeria is dried, macerated and added to soups as a thickener with mucilaginous consistency (Etukudo, 2003). This species belongs to the group of over exploited plants requiring techniques involving the management of genetic resources through conservation, selection, breeding and propagation (Laferla *et al*., 2002; Sarasan *et al*., 2006). Advances in micropropagation are aimed at conservation of forest resources, which are disappearing at an unprecedented rate. *In vitro* technology involves the production of plants on an artificial medium in a controlled environment, under sterile conditions (Guo *et al*., 2007; Mondal *et al*., 2004). *In vitro* culture techniques can rapidly increase the number of individuals for endangered species with reproductive problems and extremely reduced population. Thus, this technique has been useful in the conservation of some species (Rout and Jain, 2004; Okafor, 2005). In view of the current efforts of rejuvenating *Irvingia* species, this technique was used in this study to assess the growth response of explants of *Irvingia wombolu* to *in vitro* treatments.

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MATERIALS AND METHODS

Experiment 1

Axillary bud explants were collected from *Irvingia wombolu* grown in the field in Igbere, Bende Local Government Area of Abia State, Nigeria. Explants of 0.5cm in size were pretreated for 5 min in 70% (v/v) ethanol solution, and for 4min in 0.1% mercuric chloride solution. The disinfected explants were rinsed 3-4 times in sterile distilled water. Explants (one explants per test tube) was aseptically placed on full -MS+ Kin, half - $^1/_2$ MS + Kin and one quarter - $^1/_4$MS+ Kin strength of MS (Murashige and Skoog, 1962) medium supplemented with 0, 1, 2, 3, 4 and 5mg/l of kinetin (Kin). The pH of the media was adjusted to 5.8±0.1 followed by the addition of 8g agar. 10ml of the culture medium was dispensed into test tubes and autoclaved. The condition of the culture room was maintained at a temperature of 28± 1^{o}C, relative humidity of 80% and 16-h photo period under white fluorescent light for shoot initiation for 12 weeks. Shoot elongation medium (MS medium devoid of growth regulators) was used to raise

shoot-lets transferred from shoot initiation stage, for 8 weeks. Axillary formed shoots were placed on rooting medium containing - 0, 1, 2, 3, 4, and 5mg/l of indole butyric acid (IBA) and maintained for plantlets development for 12 weeks (Etukudo *et al*., 2014).

Experiment 2

The effects of number of leaf primordium (0, 1, 2, 3, and 4 leaf layers), and explants source (seedling, stump sprout, middle aged tree, and adult tree) on shoot initiation of the species were assessed using the best growth medium (one quarter strength growth medium with 3mg/l of kinetin-Kin), (Etukudo *et al*., 2011). The following growth parameters were examined; shoot length (cm), regeneration frequency (%), coefficient of velocity of bud burst and contamination (%). Each treatment was replicated 10 times, repeated 2 times and the mean value expressed. Standard error of the mean values were calculated for 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 (Obi, 2002; Wahua, 1999).

RESULTS

Optimum growth response was obtained at 3mg/l of kinetin for shoot initiation of *I. wombolu*. 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. wombolu* increased with decrease in medium strength. The shoot length of *I. wombolu* in MS, $^1/_2$ MS and $^1/_4$ MS media increased from 1.84 ± 0.10, 2.24 ± 0.26, and 2.68 ± 0.45 cm (control) to 2.42 ± 0.36 (5mg/l Kin), 3.86 ± 0.34 (4mg/l Kin) and 4.45 ± 0.57 (4mg/l Kin), respectively (Table 1). The shoot length of *I. wombolu* increased with decrease in medium strength (Table 2). Treatments with full (MS + IBA), half ($^1/_2$ MS + IBA) and the control (0mg/l IBA- in all levels of growth medium) did not support the rooting of *I. wombolu.* However, the root response of *I. wombolu* in one quarter strength medium containing indole butyric acid ($^1/_4$ MS + IBA) increased from 1.00 ± 0.00 (1mg/l IBA), 2.20 ± 0.21 (2mg/l IBA), 2.83 ± 0.25 (3mg/l IBA), 3.10 ± 0.17 (4mg/l IBA) to 4.50 ± 0.22 (5mg/l IBA) (Table 3).

In terms of response to plant 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 $^1/_4$ MS medium (Table 1, 2 and 3). Regeneration frequency (RF) and coefficient of velocity of bud burst (CV) of explants of *I. wombolu* cultured on $^1/_4$ MS medium with 3mg/l kinetin decreased from 65.00 ± 0.32% (RF) and 0.28 ± 0.09 (CV) in treatment with one (1) leaf primordium to 20.00 ± 0.23% (RF) and 0.14 ± 0.08 (CV) in treatment with four (4) leaf primordia comparable to the control treatment (having all leaf primordia intact) with a value of 20.00 ± 0.43% (RF) and 0.14 ± 0.08 (CV) (Table 4). High regeneration frequency of 75.00 ± 0.43 and 75.00 ± 0.27% were recorded by explants from seedling and stump sprout, respectively, while explants from middle aged tree and adult

Table 1. Shoot length (cm) of *Irvingia wombolu* 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±0.10	1.92±0.14	2.02±0.19	2.17±0.32	2.32± 0.41	2.42 ± 0.36
$^1/_2$ MS	2.24 ± 0.26	3.34 ± 0.28	3.50 ± 0.19	3.70 ± 0.51	3.86 ± 0.34	3.80 ± 0.42
$^1/_4$ MS	2.68± 0.45	4.20 ± 0.33	4.73 ± 0.24	5.94 ± 0.39	4.45 ± 0.57	4.40 ±0.46
Mean	2.25	3.15	3.42	3.94	3.54	3.54
LSD ($P< 0.05$)	0.1	0.1	0.2	0.2	0.2	0.1

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

Table 2. Shoot length (cm) of *Irvingia wombolu* as affected by various strength of Murashige and Skoog medium (MS) during shoot elongation stage maintain for 8 weeks

Pre-Concentration 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
$^1/_2$ MS	2.82 ± 0.21	4.60 ± 0.29	4.83 ± 0.37	5.20 ± 0.42	5.24 ± 0.44	4.97 ± 0.36
$^1/_4$ MS	3.48 ± 0.46	5.72 ± 0.24	6.58 ± 0.53	8.52 ± 0.48	6.40 ± 0.37	5.98 ±0.44
Mean	2.10	3.44	3.80	4.57	3.88	3.65
LSD ($P< 0.05$)	0.1	0.2	0.2	0.1	0.2	0.1

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

Table 3. Root number of *Irvingia wombolu* as affected by various strength of Murashige and Skoog medium (MS) at varying concentrations of indole butyric acid (IBA) during root initiation stage maintain for 12 weeks

Concentration of IBA (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
$^1/_2$ 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
$^1/_4$ MS	0.00 ± 0.00	1.00 ± 0.00	2.20 ± 0.21	2.83 ± 0.25	3.I0 ± 0.17	4.50 ±0.22
Mean	0.00	.033	0.73	0.94	1.70	2.17
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 primordia on regeneration frequency –RF (%), and coefficient of bud burst (CV) of *Irvingia wombolu* cultured on $^1/_4$ MS with 3mg/l kinetin

Number of leaf primordia	Regeneration Frequency-RF (%)	Coefficient of velocity of bud burst (CV)
0	20.00± 0.43	0.14±0.08
1	65.00± 0.32	0.28 ± 0.09
2	55.00± 0.17	0.23 ± 0.03
3	30.00 ±0.15	0.15 ± 0.03
4	20.00 ± 0.23	0.14 ± 0.08
Mean	39.00	0.19
LSD (P< 0.05)	1.64	2.07

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

Table 5. Effects of explants source on regeneration frequency – RF (%), and coefficient of bud burst (CV) of *Irvingia wombolu* cultured on $^1/_4$ MS with 3mg/l kinetin

Explants source Regeneration	Contamination (%)	Coefficient of velocity of bud burst (CV)	Frequency-RF (%)
Seedling	75.00 ± 0.43	15.00 ± 0.41	0.31 ± 0.04
Stump sprout	75.00 ±0.27	15.00 ± 0.24	0.29 ± 0.05
Middle aged tree	40.00± 0.35	20.00 ± 0.14	0.20 ± 0.02
Adult tree	40.00± 0.35	45.00 ± 0.19	0.14 ± 0.07
Mean	63.75	23.75	0.24
LSD (P< 0.05)	1.27	1.56	1.92

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

tree of *I. wombolu* recorded a regeneration frequency of 65.00 ± 0.36 and 40.00 ± 0.35%, respectively (Table 5). Contamination percentage of *I. wombolu* increased with increase in age of explants source with the lowest value of 15.00 ± 0.41 and 15.00 ± 0.24% in seedlings and stump sprout explants, respectively, and highest value of 45.00 ± 0.19% in explants from adult tree (Table 5). Coefficient of velocity of bud burst decreased with increase in age of explants source from 0.31 ± 0.04 in seedling explants to 0.14 ± 0.07 in explants from adults tree (Table 5).

DISCUSSION

In this study, explants of *Irvingia wombolu* showed optimum regeneration capacity at one quarter strength medium comparable with those of half and full strength media. It has been demonstrated that tissue explants from a number of tree species can be grown and induced to undergo organogenesis and plantlet regeneration *in vitro* (Jain and Ishii, 2003; Nandwani *et al*., 2004; Watt *et al*., 2003), as shown in this study. According to Rout and Jain (2004), organogenesis involves differentiation of micro-shoots and roots during plantlet development. Organogenesis involves induction of micro-shoots or tissues in a cytokinin-enriched medium, and subsequent rooting of the micro- shoots in an auxin-enriched medium to give rise to plantlets (Bhalla-Sarin *et al*., 2003; Shishkova *et al*., 2007). Necrosis and death of explants due to oxidation of phenolic compounds and high medium strength have been reported as one of the factors that lead to low regeneration frequency in some woody species *in vitro* (Etukudo *et al*., 2014; Panhwar, 2005). Therefore, this explains the reason for low proliferation of explants at half and full strength media. In this study, regeneration potential of explants was affected by the developmental state of the explants source. Regeneration of woody plants has been reported to be achievable in juvenile tissues, stump sprouts, sprouts from pruned trees, zygotic embryos or seedling parts (epicotyl, hypocotyl and cotyledon) (Mondal *et al.,* 2004; Rout and Jain, 2004). Similarly, chronological age of the potential explant tissue and season of the year that the explant is obtained are important factors that influence the extent of differentiation of the cells and their physiological age (Kurata and Kozai, 1992; Vila *et al*., 2004). This suggests that the quality of the initial explants is of profound importance in encouraging proliferation of tissues *in vitro* (Jain and Ishii, 2003). In general, growth response of explants to *in vitro* treatment has been shown to be greatly influenced by the genotype, physiological state of the explant, age of the explant, and the *in vitro* environment, both the light and temperature regimes, and the constitution of the medium with the hormone concentrations in particular (Bhalla-Sarin *et al*., 2003; Dhaliwal *et al*., 2003; Sharma *et al*., 2005).

Conclusion

This study shows that the regeneration potential of axillary bud explants of *I. wombolu* is a function of optimum culture conditions. Therefore, the initiation of organized development *in vitro* is influenced by various factors in, and outside the culture medium, as well as the state of the explants.

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