



Effects of Woody Plant Media (WPM) and Plant Growth Regulators (PGRs') on Micropropagation of *Morinda lucida* Benth., Using Shoot Systems and Seeds.

Ajongbolo Febisola Bukola², Oyetunji Olusola Jacob¹

¹ Department of Botany, University of Ibadan, Ibadan, Nigeria.

² Department of Biotechnology, National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan, Nigeria.

ABSTRACT

Morinda lucida is an economic plant of medicinal properties. Its overexploitation, limited propagation rate and dormant nature of its embryo are of major concern. This study was designed to establish the best protocol for *in-vitro* propagation of *M. lucida* using shoot systems and seeds. Woody Plant Medium was supplemented with two cytokinins [Benzene Amino Purine (BAP) and Kinetin (KIN)] varied from 1.0, 2.0, 3.0, 4.0 and 5.0mg/L for shoot formation, with constant concentration of 0.5mg/L Auxins (Indole -3-Acetic Acid and Naphthalene Acetic Acid) for root induction and 2mg/L Gibberellic Acid (GA₃) for shoots elongation respectively. Shoot systems (nodes, shoot tips and leaves) and seeds were collected, disinfected and inoculated on the prepared media, under aseptic condition. Breaking of seed dormancy was attained using scarification and hormone (30mg/L GA₃). Hardening of fully grown plantlets was achieved, using standard concentration of (2:2:1v/v/v) sterile soil components (top soil, coconut fiber and river sand). The experiment was a completely randomized design with five replicates. Results showed that excised seeds with cotyledon propagated on woody plant media supplemented with 1mg/L Kinetin+ 0.5mg/L Indole-3-Acetic Acid+ 2mg/L GA₃ showed optimum germination and growth rate with high significant difference among other treatments at (P≤0.05), while node and shoot tips cultures showed regeneration of new shoots, using 4 and 5mg/L BAP with constant concentration of 0.5mg/L IAA and 2mg/L GA₃. Leaf cultures had callus formation which inhibited growth performance. This showed that woody plant media augmented with plant growth regulators are effective constituents for *in-vitro* propagation of *M. lucida*.

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*Corresponding Author

Ajongbolo Febisola Bukola

E-mail: ajongbolofebi@gmail.com;

Phone: +2348038459639.

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INTRODUCTION

Morinda lucida Benth is commonly known as Brimstone tree. Its a tropical plant that can be found in the Africa rainforest. The plant belongs to the family of Rubiaceae (Adewole *et al.*, 2021). It is one of the most widely used plants in this region for medicinal purposes (Zapfack and Ngobo, 2002). It is an evergreen shrub of small to medium-sized tree measuring up to 18-25 m tall, with bole and branches often crooked or gnarled; bark smooth to roughly scaly, grey to brown, often with some distinct purple layers. It grows in grassland, exposed hillsides, thickets, forest, often on termite mounds, sometimes in areas which are regularly flooded, from sea level up to 1300m altitude (Ken, 2023).

Propagation of *M. lucida* is possible by seeds and cuttings, but no details are known (Jansen and Cardon, 2015). Low viability of the seeds limits natural reproduction and commercial propagation of the plant (Zimudzi and Cardon, 2005). Previous study on breaking of the seeds dormancy revealed that propagation can be attained via seeds with the pre-treatment applications of chemical (30mg/L GA₃) coupled with mechanical scarification (nicking) but no report on propagation techniques (Ajongbolo *et al.*, 2018).

Several reports describe the application of *Morinda species*; in production of anthraquinones, using cell suspension cultures (Hagendoom *et al.*, 1994), (Bassetti *et al.*, 1995). However, no previous experience on the *in-vitro* propagation of *M. lucida* has been recorded (Elio *et al.*, 2011). *Morinda lucida* plant has numerous richness in medicinal properties and health benefits. The leaves can be used to treat and manage several health problems. Several benefits and usefulness are associated with the Leaves, roots and stem bark of *Morinda lucida*, such as; Anti-inflammatory, Antibacterial, and Antiviral proper (Christian and Newman, 2013), Anti-anemia properties (Joppa *et al.*, 2019), Antipyretic properties (Nwobodo *et al.*, 2021), Anti-malarial Properties (Joseph *et al.*, 2016), and so on. The interest of the Scientific community for the use of *M. lucida* has increased, hence there is need for scaling up via *in-vitro* propagation technique to enhance mass production for field establishment (Wilken *et al.*, 2005).

MATERIALS AND METHODS

Study Location

The study was carried out in the Tissue Culture Laboratory of National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan, Nigeria (Lat. 7°23'37.69944"N and Log. 3°50'22.58916" E). The Herbarium Voucher number of the mother plant was done at the Herbarium Unit of Botany Department, University of Ibadan Nigeria, with Herbarium Voucher number; (UIH-23264).

Explant Collection

Three major shoot systems of *M. lucida* plant (Shoot tips, Nodes, Leaves) and Seeds were collected from NACGRAB medicinal gardens inside vials, containing water as transport media and brought to the Laboratory. Thorough rinsing of the explants was attained, using running tap water with tween twenty.

Shoot Systems Disinfection

Disinfections of Shoot tips, Leaves and Nodes were achieved by double disinfection techniques, using 70% ethanol for 5minutes as surface sterilization and later used 3% and 1% w/v sodium hypochlorite (NaOCl) for 20 and 10minutes respectively, with final rinsing in sterile water for 3 to 4 times prior excising, under the Laminar flow cabinet. Each disinfected shoot system were inoculated on the prepared media supplemented with different concentrations of cytokinins (Benzene Amino Purine and Kinetin) and constant concentration of Auxins (Indole-3- Acetic Acid and Naphthalene Acetic Acid) and Gibberellic acid (GA₃). They were later transferred to the growth room for further growth and development. Some were cultured on ordinary Woody Plant Media without any plant growth regulators (PGR's), as controls.

Seeds Disinfection and Pre-treatment Application

The seeds were subjected to different disinfection protocols, but the best was attained by using 2% w/v disinfectant (NaOCl), with 70% ethanol and was later rinsed thrice, with sterile water. Thereafter, the seeds were soaked in 30mg/L Gibberellic Acid (GA₃) for 5minutes prior inoculation to break the seed dormancy. The disinfected seeds soaked with Gibberellic Acid (GA₃) were divided into two fractions;

The first fraction was scarified prior inoculation (Nicking method).

The second fraction was not scarified prior inoculated (without Nicking)

These were cultured on Woody Plant (WP) media supplemented with Plant growth regulators, while some were cultured on ordinary Woody Plant media as controls.

Media Constituents and Plant Growth Regulators

In-vitro propagation of *M. lucida* was achieved, using the modified method described by (Elio *et al.*, 2011), on *Morinda royoc*. Media preparation for propagation of *Morinda lucida* shoot systems (Nodes, Shoot tips and Leaves) and Seeds were achieved with the usage of Woody Plant Media basal constituents; WPM powder

(2.30g/L), inositol (10mg/L), vitamin (5ml /L), sucrose (3%w/v), gelling agent (0.5% /w/v), plant preservative mixture (2.5mls/L) , pH (5.7) supplemented with two major cytokinins (Benzyl Amino purine and Kinetin) respectively, at different standard concentrations, varying from 1.0mg/l to 5.0mg/L, with Auxins (Indole-3-Acetic Acid and Naphthalene Acetic Acid) at a constant concentration of 0.5mg/l, 2mg/L constant concentration of GA₃ and control, which was ordinary WP media without adding plant growth regulators. Sterilization was achieved at 121°C for 15 minutes at 1.05kg/cm².

Inoculation of Explants and Growth Room Condition

Morinda lucida explants were excised and cultured on prepared media under the Laminar flow cabinet prior proper arrangement inside growth room condition. This was a controllable environment with photoperiods of 16hrs day light and 8hrs darkness. Temperature is 25°C±2°C, relative humidity of 50-55% and illumination was between 3000 to 4000 lux. The cultures were subjected to this condition for proper growth and development.

Data collection and Experimental Design

M. lucida seeds and shoot systems cultured and excised via *in-vitro* propagation were monitored on daily basis and the data were recorded fortnightly. Experimental design was a Completely Randomized Design with five replicates.

Statistical Analysis

Data collected were subjected Analysis of Variance (ANOVA), using Statistical Tool for Agriculture and Research (STAR). Means were separated by Duncan Multiple Range Test (DMRT) at 5% significant level.

RESULTS

Effect of Woody Plant Media (WPM) Fortified with Plant Growth Regulators on In-Vitro Propagation of *M. lucida* Shoot Systems and Seeds.

The Woody Plant media basic constituents augmented with Plant growth regulators (PGRS), revealed effects of different concentrations of cytokinins (BAP and KIN)

Gibberellic acid (GA₃) and auxins (NAA and IAA) on growth performances and morphological characterization of *in-vitro* propagated *M. lucida* shoot systems and seeds (Table 1). The highest growth performance of plantlets propagated was obtained in seeds propagated on Woody Plant media, fortified with (1mg/LKIN+2mg/LGA₃+0.5mg/LIAA), as shown in (Plate 1b). *M. lucida* shoot systems and seeds propagated on ordinary Woody Plant media (control) did not respond to regeneration. Instead of growth, virtually all the cultures turned brownish and contaminated. It was observed that shoot tips, nodes, leaves and seeds of *M. lucida* can't be propagated *in-vitro* on ordinary Woody Plant media, compared to media fortified with plant growth regulators.

Analysis of variance for morphological characters of the *in-vitro* propagated shoot systems and seeds of *M. lucida*, using Woody Plant media augmented with Plant growth regulators (PGRs').

The result of analysis of variance shown in Table 1.1 revealed that, there were significant differences among the morphological characters of propagated shoot systems and seeds via *in-vitro* technique at P≤0.05 (Table 1.1).

Correlation Associated with morphological characters of the *in-vitro* propagated shoot systems and seeds of *M. lucida*, using Woody Plant media augmented with Plant growth regulators (PGR's).

The result of the correlation associated with the morphological characters of propagated shoot systems and seeds propagated via *in-vitro* technique, using woody Plant media with Plant growth regulators indicated positive responses and effects with significant differences (P≤0.05) as shown in (Table 1.2). There were strong correlation among the morphological characters.

Hardening and Transplanting of *in-vitro* Propagated *M. lucida* Plantlets.

Plates 2a shown successful hardening technique of fully grown *in-vitro* propagated plantlets under *ex-vitro* condition with no mortality rate, while plate 2b indicated effective transplanting procedure in screen house condition prior field establishment for further utilization. Both hardening and transplanting techniques gave positive effects under *ex-vitro* condition Plates 2a and 2b.

Table 1. Effect of Woody Plant Media (WPM) Fortified with Plant Growth Regulators on *In-Vitro* Propagation of *M. lucida* Shoot Systems and Seeds.

Treatment	Plant Height (cm)	Number Of New Shoot	Height Of New Shoot (cm)	Number Of Leaf	Number Of Root	Callus Formation
1mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (NODE)	0.98±0.11b	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (SHT TIPS)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (LEAF)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (NODE)	0.62±0.10d	0.36±0.10bc	0.07±0.02f	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (SHT TIPS)	0.18±0.05fg	0.32±0.10c	0.07±0.02f	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (LEAF)	0.56±0.09de	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.48±0.10c
2mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (NODE)	0.13±0.05fg	0.08±0.06e	0.01±0.01f	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (SHT TIPS)	0.08±0.05gh	0.04±0.04e	0.01±0.01f	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (LEAF)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.16±0.07de
3mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (SEED)	0.60±0.26d	0.32±0.10c	0.59±0.26d	0.24±0.12cd	0.00±0.00g	0.24±0.09d
4mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (NODE)	0.48±0.09e	0.48±0.10ab	0.08±0.02f	0.00±0.00e	0.00±0.00g	0.48±0.10c
4mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (SHT TIPS)	0.36±0.10ef	0.16±0.07de	0.10±0.05ef	0.00±0.00e	0.00±0.00g	0.00±0.00f
4mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (LEAF)	0.20±0.08f	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.20±0.08d
4mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (SEED)	0.70±0.26cd	0.32±0.10c	0.70±0.26cd	0.28±0.10cd	0.32±0.15ef	0.00±0.00f
5mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (NODE)	0.12±0.04fg	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
5mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (SHT TIPS)	0.36±0.08ef	0.32±0.10c	0.12±0.04ef	0.00±0.00e	0.00±0.00g	0.00±0.00f
5mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (LEAF)	0.46±0.08e	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.60±0.10bd
5mg/LBAP+2mg/LGA ₃ +0.5mg/LIAA (SEED)	0.26±0.08f	0.32±0.10c	0.22±0.08e	0.16±0.10d	0.24±0.12ef	0.32±0.10cd
1mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (NODE)	0.29±0.09f	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (SHT TIPS)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (LEAF)	0.44±0.10e	0.00±0.00f	0.00±0.00g	0.12±0.07d	0.00±0.00g	0.00±0.00f
1mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (NODE)	0.21±0.09f	0.08±0.06e	0.02±0.01f	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (SHT TIPS)	0.14±0.07fg	0.08±0.06e	0.02±0.01f	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (LEAF)	0.44±0.10e	0.00±0.00f	0.00±0.00g	0.12±0.07d	0.00±0.00g	0.00±0.00f
2mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (NODE)	0.25±0.09f	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.24±0.09d
3mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (SHT TIPS)	0.08±0.04gh	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (LEAF)	0.20±0.08f	0.00±0.00f	0.00±0.00g	0.04±0.04de	0.00±0.00g	0.00±0.00f
3mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
4mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (NODE)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
4mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (SHT TIPS)	0.14±0.06fg	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
4mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (LEAF)	0.23±0.09f	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
4mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f

5mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (NODE)	0.16±0.07fg	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
5mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (SHT TIPS)	0.04±0.03gh	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
5mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (LEAF)	0.16±0.07fg	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
5mg/LBAP+2mg/LGA ₃ +0.5mg/LNAA (SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (NODE)	0.30±0.08ef	0.24±0.09cd	0.05±0.02f	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (SHT TIPS)	0.14±0.06fg	0.12±0.07de	0.02±0.01f	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (LEAF)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (SEED)	1.49±0.36a	0.64±0.10a	1.49±0.36a	1.04±0.39a	3.28±0.93a	0.00±0.00f
2mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (NODE)	0.14±0.05fg	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (SHT TIPS)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (LEAF)	0.08±0.06gh	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.04±0.04e
2mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (SEED)	0.94±0.31bc	0.36±0.10bc	0.94±0.31b	0.60±0.24b	2.24±0.87b	0.00±0.00f
3mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (NODE)	0.12±0.04fg	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (SHT TIPS)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (LEAF)	0.58±0.09de	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.64±0.10b
3mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (SEED)	0.87±0.25c	0.40±0.10b	0.87±0.25c	0.28±0.12cd	1.84±0.76c	0.00±0.00f
4mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (NODE)	0.17±0.05fg	0.04±0.04e	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.32±0.10cd
4mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (SHT TIPS)	0.23±0.06f	0.20±0.08cd	0.06±0.03f	0.00±0.00e	0.00±0.00g	0.44±0.10c
4mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (LEAF)	0.64±0.10d	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.64±0.10b
4mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (SEED)	0.73±0.22cd	0.48±0.10ab	0.73±0.22cd	0.44±0.20bc	1.56±0.50cd	0.00±0.00f
5mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (NODE)	0.17±0.06fg	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.24±0.09d
5mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (SHT TIPS)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
5mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (LEAF)	0.76±0.07cd	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.72±0.10a
5mg/LKIN+2mg/LGA ₃ +0.5mg/LIAA (SEED)	0.34±0.19ef	0.16±0.07de	0.34±0.19e	0.20±0.12cd	0.12±0.09f	0.00±0.00f
1mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (NODE)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (SHT TIPS)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (LEAF)	0.11±0.06fg	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
1mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (SEED)	0.13±0.07fg	0.12±0.07de	0.13±0.07ef	0.20±0.12cd	0.60±0.34e	0.00±0.00f
2mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (NODE)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (SHT TIPS)	0.20±0.08f	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (LEAF)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
2mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (NODE)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (SHT TIPS)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (LEAF)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
3mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
4mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (NODE)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
4mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (SHT TIPS)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
4mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (LEAF)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
4mg/LKIN+2mg/LGA ₃ +0.5mg/LNAA (SEED)	0.26±0.13f	0.16±0.07de	0.26±0.13e	0.32±0.16c	0.72±0.36e	0.00±0.00f

5mg/LKIN+2mg/LGA₃+0.5mg/LNAA (NODE)	0.14±0.05fg	0.08±0.06e	0.01±0.01f	0.00±0.00e	0.00±0.00g	0.00±0.00f
5mg/LKIN+2mg/LGA₃+0.5mg/LNAA (SHT TIPS)	0.15±0.08fg	0.12±0.07de	0.04±0.02f	0.00±0.00e	0.00±0.00g	0.00±0.00f
5mg/LKIN+2mg/LGA₃+0.5mg/LNAA (LEAF)	0.32±0.11ef	0.16±0.07de	0.12±0.06ef	0.00±0.00e	0.00±0.00g	0.00±0.00f
5mg/LKIN+2mg/LGA₃+0.5mg/LNAA (SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
WPM ONLY control(NODE)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
WPM ONLY control(SHT TIPS)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
WPM ONLY control(LEAF)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f
WPM ONLY control(SEED)	0.00±0.00h	0.00±0.00f	0.00±0.00g	0.00±0.00e	0.00±0.00g	0.00±0.00f

Values are means and standard errors of five replicates. Values with different letters are significantly different at $P \leq 0.05$, using Duncan Multiple Range Test. BAP= Benzene Amino Purine, KIN= Kinetin, IAA=Indole Acetic Acid, NAA=Napthaline Acetic Acid, WPM= Woody Plant Media

Table 1.1 Analysis Of Variance For *In-Vitro* Propagation of *M. lucida* Shoot systems and Seeds, Using Woody Plant Media Fortified With Plant Growth Regulators (PGRs').

Parameter	Sources	Df	Sum Of Square	Mean Of Square	F-Value	Pr (>F)
Plant Height (Cm)	Treatment	83	167.5836	2.0187	8.80	0.0000
	Error	2016	462.6116	0.2295		
	Total	2099	630.1652			
Number Of New Shoot	Treatment	83	14.0381	0.1691	13.53	0.0000
	Error	2016	25.2000	0.0125		
	Total	2099	39.2381			
Height Of New Shoot	Treatment	83	47.3424	0.4885	9.54	0.0000
	Error	2016	102.1600	0.0507		
	Total	2099	142.7067			
Number Of Leaves	Treatment	83	47.3424	0.5704	5.40	0.0000
	Error	2016	212.8000	0.1056		
	Total	2099	260.1424			
Number Of Root	Treatment	83	530.7100	6.3941	19.69	0.0000
	Error	2016	1652.8000	0.8198		
	Total	2099	2183.5100			
Callus Formation	Treatment	83	60.0457	0.7234	19.69	0.0000
	Error	2016	74.0800	0.0367		
	Total	2099	134.1257			

Table 1.2 Correlation Of Characters Associated with *In-Vitro* Propagation Of *M. lucida*, Using Woody Plant Media With Plant Growth Regulators (PGRs').

Parameter	Plant Height	Number Of New Shoot	Height Of New Shoot	Number Of Leave	Number Of Root
Number Of New Shoot	0.67**				
Height Of New Shoot	0.76**	0.80**			
Number Of Leave	0.72**	0.68**	0.95**		
Number Of Root	0.70**	0.64**	0.92**	0.97**	
Callus Formation	0.41	0.26	0.14	0.09	0.09

Correlation of growth parameters associated with in-vitro propagation of *M. lucida*, using shoot systems and seeds. Highly significant at $P \leq 0.01$ and significant at $P \leq 0.05$.

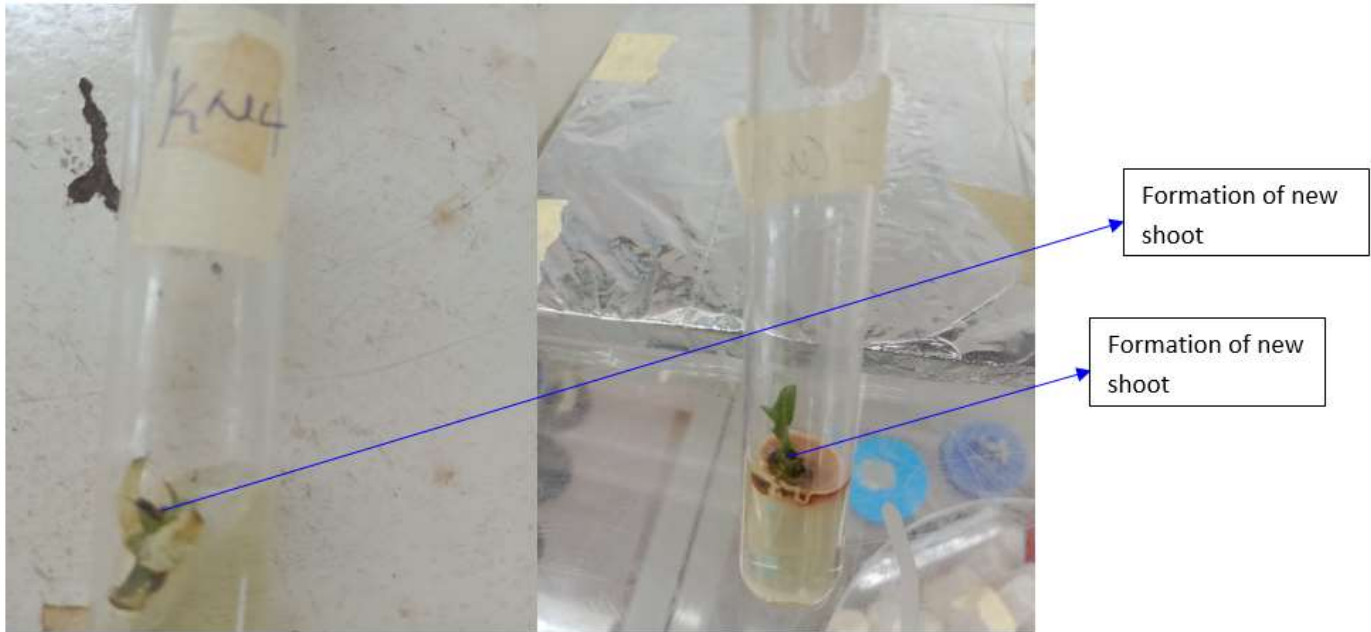


Plate 1a: *Morinda lucida* Node and Shoot tips cultures, propagated on Woody Plant Media augmented with Plant growth regulators (PGR's).

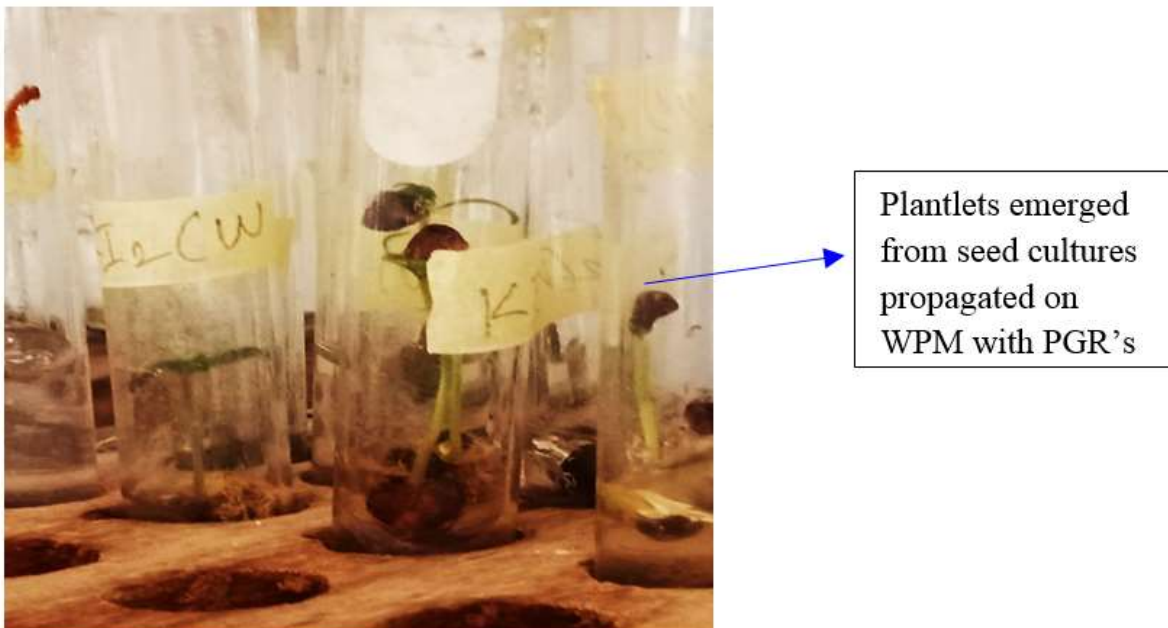


Plate 1b: Plantlets emerged from *M. lucida* seed cultures propagated on Woody Plant Media, supplemented with KIN, GA_3 and IAA (PGR's). WPM=Woody Plant Media, PGR's=Plant growth regulators, KIN=Kinetin, GA_3 =Gibberellic Acid, IAA=Indole -3-Acetic Acid.



Plantlets
hardened

Pla 2a: Hardening of fully grown *in-vitro* propagated plantlets of *M. lucida*, under acclimatization chamber.



Seedlings
Propagated via
in-vitro

Plate 2b: Transplanted seedlings of *M. lucida* propagated via *in-vitro* techniques.

DISCUSSION

In-vitro propagation of *M. lucida* shoot systems and seeds, using Woody Plant media (WPM) as basic constituents, revealed high significant differences in plant height, number of shoot, number of leaf and number of root in treatment K11 (1mg/L KIN +2mg/L

GA₃+ 0.5mg/L IAA). This was similar to the report of Afolabi *et al.* (2020), which stated that best shoot regeneration was obtained from Noni plant (*Morinda citrifolia*) at the presence of 1.0 to 2.0mg/L BAP/KIN. The leaf cultured on Woody Plant media did not response to noticeable regeneration but rather induced callus at higher concentrations of Plant growth regulators. This

report was concurred with the observation of De-Klerk (2002), on callus formation as a mechanical barrier to nutrient and water uptake for *in-vitro* cultures.

In-vitro propagation of shoot tips, node and leaf on ordinary Woody Plant Media showed no response to regeneration, this might be as a result of inadequate chemical nutrients in the media. This supported the observation affirmed by Shahina and Anwar (2014), in their report which stated that nutrients composition and other factors have influence on the induction and proliferation of multiple shoots.

Highest germination and growth performance was obtained from *M. lucida* seeds cultured on WPM fortified with lower concentration of Kinetin(1mg/L), GA₃(2mg/L) and IAA(0.5mg/L). This was in agreement with the findings of Fletcher *et al.*, (2000) and Redemacher (2000) which stated that plant growth hormones are known to stimulate seed germination.

Also, the result gotten from the study showed that Woody Plant media, supplemented with kinetin and Indole-3- Acetic Acid are good plant growth regulators for the germination of *M. lucida* seed cultures. This was concurred with the work reported by Fletcher *et al.*, (2000), that growth influences in plant may be explained with Indole-3-Acetic Acid (IAA), which had the highest germination percentage.

The *M. lucida* (seed cultures) excised embryo with cotyledon propagated on Woody Plant Media basic constituents with different concentrations of Plant Growth Regulators (PGRs') revealed good and better germination with growth performance compared to the one propagated on ordinary Woody Plant media. This report was in accordance with the report of Afolabi *et al.* (2023), which stated that higher germination of cultured matured Noni embryo was favored by high levels of KIN and BAP combinations.

For seeds cultured on ordinary Woody Plant Media, the study showed that both scarified and not scarified seeds has no suitable basic constituents and appropriate plant growth regulators that can enhance germination and growth. This may be as a result of exposure of seeds to improper chemical constituents *in-vitro* (<https://www.PlantCellTechnology.Com>, (2022). Woody Plant Media basic constituent supplemented with Plant Growth Regulators revealed optimum regeneration, germination and growth performance of seeds and shoot systems via *in-vitro* technique. This showed that Woody Plant media was very effective for propagation of *M. lucida* shoot systems and seeds. This was in agreement with the report of Takuya *et al.*, 2008 which stated that Woody Plant Media is more effective for propagation of tree plants.

Hardening and transplanting of fully grown *in-vitro* propagated plantlets of *M. lucida* were successful with no mortality rate via *ex-vitro* condition. This was in accordance with the previous study on hardening of *in-vitro* propagated wine Grape by Rahul *et al.* (2019) and Ajongbolo *et al.* (2024).

CONCLUSION AND RECOMMENDATIONS

In-vitro propagation technique can be utilized as alternative means of conservation and mass production for *M. lucida* shoot systems and seeds. This technique will also enhance all year propagation of *M. lucida* within a short period of time for future availability and utilization. Woody Plant media fortified with 1mg/LKIN+2mg/LGA₃+0.5mg/LIAA are recommended with pretreatment applications (scarification and soaking in 30mg/L GA₃) for propagation of *M. lucida* seeds. Propagation of nodes and shoot tips are also attainable via *in-vitro* technique, using Woody Plant media augmented with 4 and 5mg/LBAP with constant concentration of GA₃ (2mg/L) and 0.5mg/L IAA.

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