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Full Length Research Article

In-Vitro Growth Enhancement of *Cedrela odorata* L. using Benzyl Amino Purine and A-Naphthalene Acetic Acid

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ABSTRACT
Studies were conducted in order to develop protocol for mass-production of *Cedrela odorata* seedlings. Clean-plantlets were initiated on four media at full and half strengths. Shoot-regeneration consisted two media types (Murashige & Skoog (MS) and Woody Plant Medium (WPM)) and four Benzyl Amino Purine (BAP) concentrations: 0.0, 1.0, 2.0 and 3.0 mg/l. Root-induction involved three MS media strengths (25, 50 and 100 % basal salts) and two Naphthalene Acetic Acid (NAA) levels (0.0 and 0.5 mg/l) all in factorial combinations and laid out in completely randomised design (CRD). The results at initiation showed that MS at full strength enhanced *in vitro* seeds growth better in terms of root lengths (6.50 cm) and number of leaves (7.6) while WPM gave better shoot length (6.24 cm) at 3 Week after inoculation (WAI). Shoots were best regenerated on MS basal medium supplemented with 1.0 mg/l BAP for shoot length at 4 WAI while the effects of BAP levels were comparable but higher than control for number of leaves and axillary shoots. Highest number of roots (3.92) induced and root length (2.93 cm) obtained were from 100% MS medium at 8 WAI. Hence, MS medium (100% basal salt) with 1.0 mg/l BAP with or without 0.5 mg/l NAA are considered suitable protocol for *in vitro* propagation of *C. odorata.*
Keywords: Clean-plantlets, Benzyl amino purine, Root-induction, Shoot-regeneration

INTRODUCTION
Cedrela odorata L, commonly known as Cedar Wood belongs to family Meliaceae and originated from the tropical America (Pennington and Muellner 2010; Rolando *et al.*, 2011). The species is one of the most valuable world's timber and highly overexploited. Every part of the tree has been reported for various medicinal uses while it is useful for agroforestry, afforestation and furniture making. The species is attacked by a pest called Hypsipyla and consequently becomes so scarce and has been listed among the endangered tree species (Carlos *et al.,* 2002; Perez and Esquivel 2008). Conventional method of propagating such trees like this with long gestation period is not adequate to establish its commercial plantation (Perez *et al*., 2006; Carola and Michael 2013). Therefore, it is expedient and needful to propagate the tree species in large numbers through alternative means in order to catch up with its high demand and avoid its extinction (Pennington and Muellner 2010; Ken 2019).

Tissue culture technique (*in vitro* propagation) is a modern method of propagating woody trees and has provided a rapid means of multiplying them in a shorter time under limited space with *ex situ* conservation of their germplasms. However, tissue culturing of woody plant is noted to be difficult owing to its slow regeneration ability (Husain *et al.,* 2008; Nitish and Reddy 2011; Rolando *et al*., 2011). Perez *et al*. (2002) reported that *in vitro* propagation of *C. odorata* has not been thoroughly done. Although, there have been reports on the use of its nodal segments and apical buds collected from juvenile plants raised from seeds (Perez *et al*., 2002; Maruyama, 2006). Notwithstanding, it has been observed that *C. odorata* is recalcitrant to tissue culture as a result of oxidative processes of fungi and bacteria after disinfection which leads to a less morphogenic response of the explants. Such challenges can be overcome by supplementing the media with cytokinins and auxins at different rate (Perez *et al.,* 2002; Rolando *et al*., 2011). Consequently, the aims of this study was to assess the effect of

different media supplemented with growth regulators on growth performance of *C. odorata* in order to ensure its *in vitro* mass propagation in Nigeria.

MATERIALS AND METHODS

Study Location

The research was conducted in the Biotechnology section, Bioscience Department, Forestry Research Institute of Nigeria (FRIN), Ibadan, located on the longitude 07°23'18 N to 07°23'43N and latitude 03°51'20E to 03°23'43E (FRIN, 2018, unpublished). The location is 199 m above sea level and the climate is tropical. It has average annual temperature of 25.9 °C while precipitation is about 1467 mm annually (CDO, 2019)

Experimental Design and Treatments

The following sequence of experiments were conducted to achieve *in vitro* regeneration of *C. odorata* plantlets: culture initiation, shoot regeneration and root induction. The culture initiation comprised of 4 x 2 factorial treatments with five replicates. The first factor were four media types (Murashige and Skoog (MS), Driver and Kuniyuki (DKW), Preece (P) and Woody Plant Medium (WPM)) while second factor were two media strengths (Full and half i.e. 100 and 50% of their basal salts, respectively). Shoot regeneration experiment consist of 2 x 4 factorial treatments with six replicates. First factor for shoot regeneration were two media types (MS and WPM) while second factor were benzyl amino purine (BAP) levels (0.0 (control), 1.0, 2.0 and 3.0 mg/l). The rooting experiment involved 3 x 2 factorial treatments with six replicates. The first factor for root induction were three MS media strengths (25, 50 and 100% basal salts) while the second factor were two levels of naphthalene acetic acid (NAA) (0.0 (control) and 0.5 mg/l). All the experiments were laid out in a completely randomised design (CRD).

Media Preparation and Explant Sterilization

All the media were prepared in accordance with standard procedures, MS; (Murashige and Skoog, 1962), WPM; (Lloyd and McCown, 1980), DKW; (Driver and Kuniyuki, 1984; McGranahan *et al.,* 1987) and Preece; (Preece *et al.,* 1989). The growth regulators were added according to the treatments and experiments. The media pH was all adjusted to 5.8, add up and gelled with 9.0 g/l of agar (Sigma Aldrich, Lot 83112) then 20 ml of prepared media was dispensed per tube and autoclaved for 15 minutes at 121 °C and 15 psi. Matured seed of *C. odorata* were collected from Forestry Research Institute of Nigeria, Arboretum. The seeds were de-winged and sterilized as follows: the seeds were dipped in fungicide mixture (5 g/l Z-force + 5 g/l cibaplus + 0.4 g/l amoxicillin) for 60 minutes, rinsed thrice with sterile distilled water then dipped in 70 % ethanol for 5 minutes. The seeds were rinsed thrice then dipped in 10 % hypochlorite solution containing two drops of tween 20. The seeds were then rinsed four times and blotted on sterilized petri-dish laid filter paper before inoculation. The shoot tips and nodal segment of the seed plantlets were subcultured 4 Weeks after inoculation (WAI) into the shoot regeneration media. After 5 WAI of growth, the plantlets were subcultured into root induction media.

Data Collection and Analysis

Data collected included shoot and root lengths (cm) with the aid of a meter rule. Number of leaves and roots were determined by manually counting plantlets. Replicates were subjected to analysis of variance while means for the different treatments were separated using Duncan Multiple Range Test at 95% confidence level.

RESULTS

Seed Culture Initiation

The results of *in-vitro* propagation of *C. odorata* seeds using different media types and strengths at 3 weeks after inoculation (WAI) are presented in Table 1. There was no significant difference ($p>0.05$) in the main effect of the factors and their interaction on shoot length of the plantlets. The shoot length ranged from the highest (6.24 cm) in WPM at full strength to the lowest (4.38 cm) in

MS medium at half strength. At the same time, effect of media strengths and interactive effect of both factors on root lengths and number of leaves were significantly different ($p<0.05$). The average root lengths (5.07 cm) and number of leaves (7) produced from full strength media were significantly higher than those of half strength media having 4.01 cm and 5.5, respectively at 3 WAI (Figures 1 and 2). The average root length (6.50 cm) from full strength MS medium was significantly higher than observed for Preece (4.20 cm), WPM (3.80 cm) and half strength MS (3.14 cm) media but comparable to WPM and DKW at full strength (Table 1). Similarly, average number of leaves of 7.6 obtained from MS at full strength was higher than MS at half strength but similar to other media irrespective of their strength (Table 1).

Table 1: Effect of Different Media and Strengths on Growth of *in vitro* Seed Germination of *Cedrela odorata* at 3 (WAI)

FACTORS		SHOOT LENGTH (CM)	ROOT LENGTH (CM)	NUMBER OF LEAVES
MEDIA TYPE	**MEDIA STRENGTH**			
MURASHIGE AND SKOOG	Full	6.16	6.50a	7.6a
	Half	4.38	3.14b	3.6b
PREECE	Full	5.12	4.50ab	5.2ab
	Half	5.60	4.20b	6.0ab
WOODY PLANT MEDIUM	Full	6.24	4.76ab	7.6a
	Half	5.90	3.80b	7.2a
DKW	Full	6.06	4.50ab	7.6a
	Half	5.40	4.90ab	5.2ab
L.S.D @ p ≤ 0.05				
Media types		1.12	1.3	1.79
Media strength		0.79	0.9*	1.26*
Media types x Media strengths		1.59	1.84*	2.53*

* indicates means difference significant at p≤0.05, (n=40)

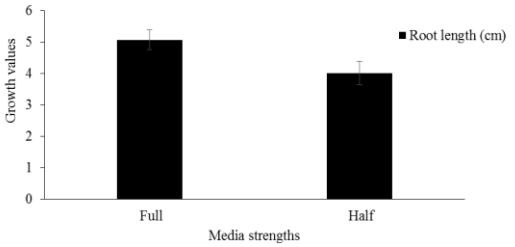

Figure 1: Effect of media strengths on root length of *in vitro* propagated seeds of *Cedrela odorata* at 3 WAI; (n=20)

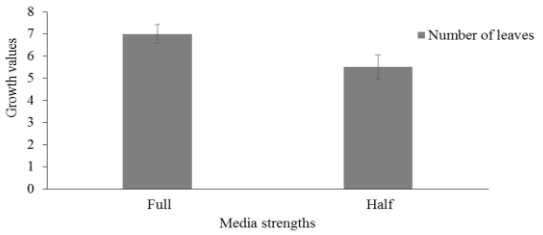

Figure 2: Effect of media strengths on number of leaves of *in vitro* propagated seeds of *Cedrela odorata* at 3 WAI.

Shoot Regeneration

Shoot Length of Regenerated *C. odorata* Plantlets

The effect of media types (MS and WPM) and BAP levels (0.0, 1.0, 2.0 and 3.0 mg/l) was assessed on shoot regeneration of *C. odorata.* Plantlet growth from both media (MS and WPM) were similar in shoot length while BAP levels had comparable effects on the same parameter at 2 WAI (Table 2). The average shoot length as affected by BAP ranged from 1.96 cm to 2.48 cm from media added, 3.0 mg/l and 1.0 mg/l, respectively. The interactive effect of the two factors was not significantly different ($p>0.05$) on the shoot length at same period (Table 2).

Results at 4 WAI indicated that there was significant difference ($p\leq0.05$) in the main effects of the media types and BAP levels but not their interaction on shoot length (Table 2). Shoot length of 2.66 cm from MS medium was significantly higher ($p\leq0.05$) than 2.11 cm obtained from WPM (Figure 3). The average effect of supplementing the media with BAP indicated that shoot length (2.79 cm) from medium added 1.0 mg BAP/l was significantly different from media added 3.0 mg BAP/L and 0.0 mg BAP/l (control) whereas, similar to 2.0 mg BAP/l medium (Figure 4). The obtained average shoot length of 2.39 cm obtained from using 2.0 mg BAP/l was however not significantly different ($p>0.05$) from 2.29 cm and 2.06 cm obtained from 3.0 mg BAP/l and 0.0 mg BAP/l, respectively (Figure 4).

Root Length of Regenerated *C. odorata* Plantlets

The results of root length of the sub-cultured *C. odorata* plantlets as influenced by media types and BAP levels showed that there was no significant difference ($p>0.05$) among the media types as well as interaction between the media types and BAP levels at 2 WAI (Table 2). Conversely, significant difference ($p\leq0.05$) was observed between the mean root lengths as affected by BAP levels at the same period (Table 2). Using the media without BAP (0.0 mg/l) gave significantly higher number of roots compared with other media with varying levels of BAP from which no roots were produced at 2 WAI (Figure 5).

By 4 WAI, mean differences within the factors and between their interactions were observed to be significantly different (Table 2). Longer roots (0.94 cm) were produced using WPM compared with MS (0.44 cm) medium (Figure 3). Similarly, the effects of BAP followed the same pattern as in 2 WAI. Media with no BAP addition produced longer root length compared with others (Figure 5). The interactive effects of the factors on plantlets growth showed that using WPM with no BAP produced significantly longer root (3.75 cm) than MS (1.77 cm) without BAP (Table 2).

Number of Leaves of Regenerated *C. odorata* Plantlets

The effect of media types and BAP on number of leaves of *C. odorata* presented in Table 3 showed that mean differences within the factors and their interaction were significantly different (p≤0.05) at 2 and 4 WAI. Higher number of leaves were produced using MS medium (2.61, 4.29) compared with WPM (1.26, 1.74) at 2 and 4 WAI, respectively (Figure 6)

Considering the addition of BAP, irrespective of the media used, higher average number of leaves (2.61) obtained using 1.0 mg/l BAP was significantly higher (p≤0.05) compared to 1.13 and 1.79 from control (0.0 mg/l BAP) and 3 mg/l BAP at 2 WAI. However, at 4 WAI, the use of 3 mg/l BAP produced more leaves (4.0) than control (1.44) but similar to 3.33 and 3.29 obtained from media added 1.0 and 2.0 mg/l BAP respectively (Figure 7. The interactive effects of media and BAP on number of leaves at 4 WAI indicated that MS medium with 3.0 mg/l BAP produced comparable number of leaves (5.86) to MS medium (5.0 and 4.57) with 1.0 and 2.0 mg/l BAP addition (Table 3)

Table 2: Interactive effects of media types and bap levels on shoot and root length of *C. odorata* at 4 WAI

FACTORS		SHOOT LENGTH (cm)		ROOT LENGTH (cm)	
MEDIA	**Bap (mg/l)**	**2 WAI**	**4 WAI**	**2 WAI**	**4 WAI**
MS					
	0.0	1.9	1.94	0.93	1.77b
	1.0	2.51	3.14	0.00	0.00c
	2.0	2.17	2.83	0.00	0.00c
	3.0	2.13	2.71	0.00	0.00c
WPM					
	0.0	2.07	2.18	1.67	3.75a
	1.0	2.45	2.43	0.00	0.00c
	2.0	1.94	1.96	0.00	0.00c
	3.0	1.79	1.86	0.00	0.00c
L.S.D @ p ≤ 0.05					
Media types		0.31	0.33**	0.33	0.40*
Bap levels		0.43	0.47*	0.47**	0.56**
Media x Bap levels		0.61	0.67	0.67	0.79***

* and ** indicate means difference significant at p≤0.05 and p≤0.01. WAI: Weeks after inoculation.

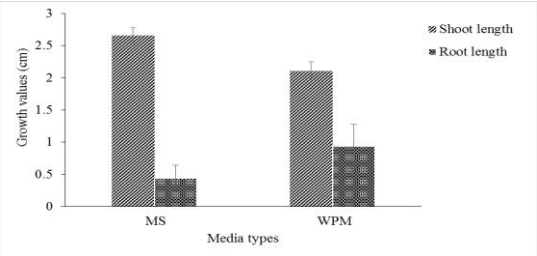

Figure 3: Effect of media types on shoot and root length of sub-cultured plantlets of *C. odorata* at 4 WAI.

Number of Roots of Regenerated *C. odorata* Plantlets

The results of effect of BAP levels and media types as well as their interaction on number of roots of sub-cultured *C. odorata* plantlets are shown in Table 3. It was observed that only mean difference of BAP levels was significant at 2 and 4 WAI. Roots were only formed when the plantlets were inoculated on media with no BAP addition with average of 0.99 and 1.29 roots at 2 and 4 WAI (Figure 8)

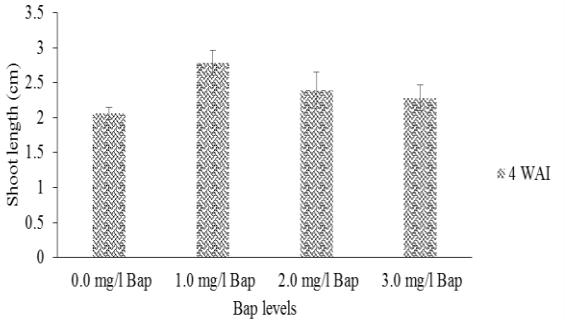

Figure 4: Effect of BAP on shoot length of sub-cultured plantlets of *C. odorata* at 4 WAI

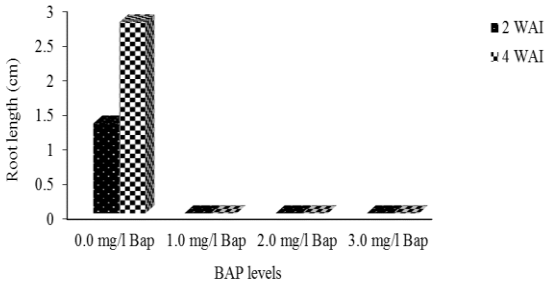

Figure 5: Effect of BAP on root length of sub-cultured plantlets of *C. odorata* at 4 WAI.

Table 3: Interactive effects of media types and bap levels on number of leaves, roots and axillary shoots of *C. odorata* at 4 WAI

FACTORS		NUMBER OF LEAVE		NUMBER OF ROOTS		NUMBER OF AXILLARY SHOOTS
MEDIA	**BAP (mg/l)**	**2 WAI**	**4 WAI**	**2 WAI**	**4 WAI**	**4 WAI**
MS						
	0.0	1.43	1.71b	1.14	1.43	0.00b
	1.0	3.71	5.00a	0.00	0.00	2.00a
	2.0	3.00	4.57a	0.00	0.00	1.71a
	3.0	2.29	5.86a	0.00	0.00	2.14a
WPM						
	0.0	0.83	1.17b	0.83	1.17	0.00b
	1.0	1.50	1.67b	0.00	0.00	0.00b
	2.0	1.43	2.00b	0.00	0.00	0.00b
	3.0	1.29	2.14b	0.00	0.00	0.29b
L.S.D @ p≤0.05						
Media types		0.54**	0.76**	0.22	0.23	0.33**
Bap levels		0.76**	1.07**	0.31**	0.33**	0.47**
Media x Bap levels		1.08	1.51*	0.45	0.46	0.67**

* and ** indicate means difference significant at p≤0.05 and p≤ 0.01; WAI: Weeks after inoculation

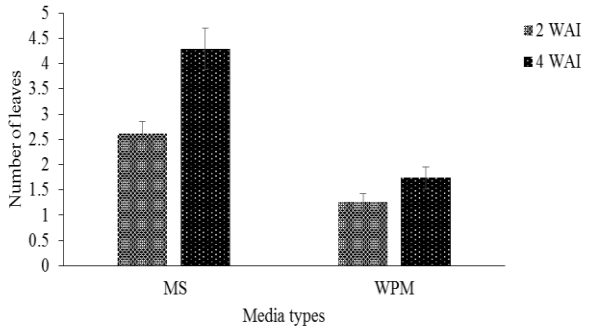

Figure 6: Effect of media types on number of leaves of sub-cultured plantlets of *C. odorata* at 4 WAI.

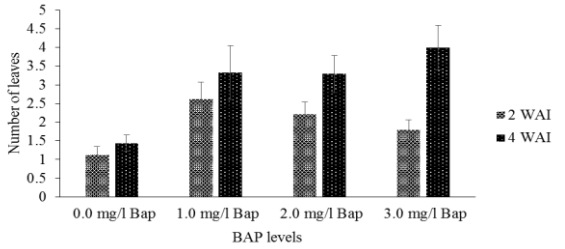

Figure 7: Effect of BAP on number of leaves of sub-cultured plantlets of *C. odorata* at 4 WAI.

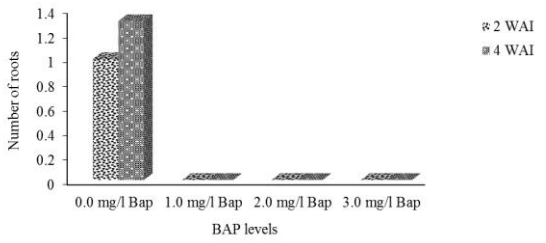

Figure 8: Effect of BAP on number of roots of sub-cultured plantlets of *C. odorata* at 4 WAI.

Number of Axillary Shoots of Regenerated *C. odorata* Plantlets

The results of the axillary shoots produced as influenced by different media and BAP levels are presented in Table 3. Higher axillary shoots (1.46) produced from MS medium was higher than that of WPM (0.071) at 4 WAI (Figure 9).

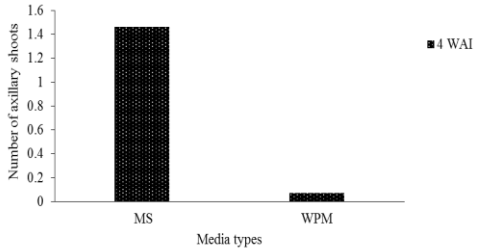

Figure 9: Effect of media types on number of axillary shoot of sub-cultured plantlets of *C. odorata* at 4 WAI

Root induction

The results of effect of different concentration of MS medium with or without 0.5 mg/l NAA is presented in Table 4. It was observed that the effect of MS medium strength was not significantly different ($p>0.05$) on number of roots at 4 and 6 weeks after inoculation (WAI). Similarly, the mean difference of NAA levels and interactive effect of the factors were not significantly different on number of roots and root length at 4, 6 and 8 WAI. However, there was significant different ($p\leq0.05$) in the effect of MS strength on number of roots at 8 WAI and on root length across the successive growth weeks (Table 4 and Plate 1). The average of 3.92 roots obtained from 100% MS medium was higher than 0.33 and 0.33 from 50% and 25% MS medium, respectively (Figure 10 and Plate 1). Through 4, 6 and 8 weeks' period after inoculation, a significant root length of 1.4, 2.65 and 2.93 cm were observed in 100% MS medium higher than 0.13, 0.13 and 0.22 cm from 25% MS medium and 0.07, 0.07 and 0.07 cm from 50% MS medium, respectively (Figure 11 and Plate 1).

Table 4: Effect of media strength and NAA levels on root growth of *C. odorata* at 8 WAI

FACTORS		NUMBER OF ROOTS			ROOT LENGTH (CM)		
MS STRENGTH (%)	NAA (mg/l)	4 WAI	6 WAI	8 WAI	4 WAI	6 WAI	8 WAI
25	0.0	0.17	0.17	0.17	0.25	0.27	0.27
	0.5	0	0	0.5	0.0	0.0	0.17
50	0.0	0	0	0	0.0	0.0	0.0
	0.5	0.5	0.67	0.67	0.13	0.15	0.17
100	0.0	0.83	1	2	1.57	2.97	3.13
	0.5	3.33	4	5.83	1.23	2.33	2.73
L.S.D @ p ≤ 0.05							
Media strength (MST)		2.0	2.31	2.72*	0.99*	1.52*	1.69*
NAA levels (NL)		1.63	1.87	2.22	0.81	1.24	1.38
MST X NL		2.83	3.27	3.84	1.40	2.15	2.39

* indicates means difference significant at p≤0.05 WAI: Weeks after inoculation

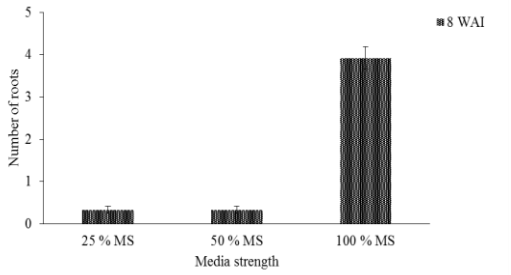

Figure 10: Effect of media strength on number of roots of sub-cultured plantlets of *C. odorata* at 8 WAI.

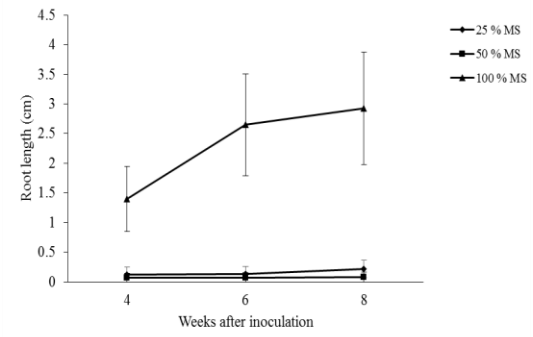

Figure 11: Effect of media strength on root length of sub-cultured plantlets of *C. odorata* at successive growth weeks.

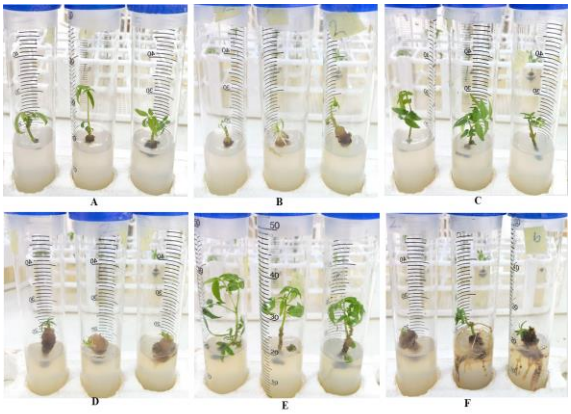

Plate 1: Effect of MS media strength and NAA level on root growth of *C. odorata* at 8 weeks after inoculation

A: MS 25% basal salt/0.0 mg/l NAA; **B:** MS 25% basal salt/0.5 mg/l NAA; **C:** MS 50% basal salt/0.0 mg/l NAA; **D:** MS 50% basal salt/0.5 mg/l NAA**; E:** MS 100% basal salt/0.0 mg/l NAA and **F:** MS 100% basal salt/0.5 mg/l NAA

DISCUSSION

Culture Initiation

Four media (MS, Preece, WPM and DKW) at two strengths (Half and full basal salts) were considered for *in-vitro* seed germination of *C. odorata.* The similarity observed in the effect of media types on shoot and root length and number

of leaves of the seed plantlets (Table 1), indicated that any of the media could be used for the culture initiation of *C. odorata* from seeds. The observed similarity could be attributed to the viability of the seeds which resulted into even germination and growth across the media. It was reported that viability of *C. odorata* seeds drops quickly after about two months of collection (Jones, 1967) while seed viability is a contributor to poor seed germination of many wild fruit tree species (Akinnifesi *et al*., 2007). In this study, freshly collected seeds of *C. odorata* were used hence, the results obtained. This result differs from that of Castillo-Martinez (2017), who observed that MS medium complemented with activated charcoal provided best germination support for *C. odorata* seeds among other media (MS, WPM, Gambor Medium, distilled water, Schenk and Hildebrand) used with or without activated charcoal.

Meanwhile, the significantly higher growth obtained from full strength media (Figure 1 and 2) showed that the plantlet growth required more nutrient than available in half strength media. Seeds can contain sufficient nutrient to allow for germination and considerable increase in plant size (Mcdonald, 1994). However, the small amount of nutrient component of *C. odorata* seed would have been quickly exhausted at the onset of root emergence. Higher root length coupled with the species rapid growth could have resulted into more uptake of nutrients available in the full strength media. Roots are vital organ that supply water and nutrients to growing seedlings (Fageria *et al*., 2014). It was reported that, vigorous root growth ensured efficient acquisition of macro- and micronutrients while high root length to shoot dry matter ratio was stated to favour high macronutrient concentrations in the shoots of spring wheat which was perceived to be important for later plant development (Wang *et al*., 2016).

The obtained results of interaction between media types and media strength in which better support was provided by both WPM and MS each at full strength on all the parameters (Table 1) revealed that both media performed to the same extent in terms of in-vitro seed propagation. This result could be attributed to the variation in the composition of these media when compared with DKW and Preece. Mineral composition of basal medium was stated to be a significant factor in the morphogenic response of *in vitro C. odorata* plants (Perez, 2005)

Shoot Regeneration

MS and WPM supplemented with four BAP levels in a factorial arrangement were considered in this stage. The better results obtained from MS medium in terms of shoot length (Figure 3), Number of leaves (Figure 6) and number of axillary shoots (Figure 9) at 4 WAI indicated that MS medium was better in supporting the shoot growth of *C. odorata* than did by WPM medium. Higher concentration of some essential elements such as nitrogen and presence of some micro elements like cobalt, iron and iodine in MS medium could have accounted for observed shoot growth (Mohammad *et al*., 2014). This result was similar to that of Marzi (2013) who obtained optimal *in vitro* development of date palm (cv. '16-bis') in terms of leaf length and greening, and root number and length when cultured on MS medium compared to WPM and Nitsch media.

The higher shoot length obtained when 1 mg/l BAP was used irrespective of the media at 4 WAI (Figure 4) showed that increasing the BAP concentration from 2 to 3 mg/l might have exerted inhibitory effect on the elongation of the species plantlets *in vitro*. This was evident in the comparable number of leaves and axillary shoot produced even at 3.0 mg/l BAP across the media at 4 WAI (Table 3). This result could be supported by the findings of Schottz *et al*. (2007) that high concentrations of BAP (20 µM) and N6-[2-isopentenyl] adenine (2-iP) (2 µM) caused a significant reduction in plant development and internodal length of *Swietenia macrophylla* which

affected the multiplication rate. The results also corroborated that of Seema & Vijaya, (2015) where BAP applied at lower concentration (0.5 mg/l) gave best shoot bud induction and multiplication on *Clerodendrum serratum* L. whereas, similarity and declining effect was observed at higher concentrations.

The growth of roots from plantlets on media without BAP addition (0.0 mg/l) both in numbers (Figure 8) and lengths (Figure 5) revealed that root induction by *C. odorata* may not require the use of plant growth regulators (PGR). These results correlated with the report of Perez *et al*. (2002) that root induction from node segments derived from *in vitro*-germinated *C. odorata* seeds was inhibited when BAP was added to the basal medium at 2.2 and 6.5 µM. Moreover, it was closely related to that of Olorode *et al*. (2018) who observed that *C. odorata* plantlets developed shoots and roots 13 days after inoculation from media free of Plant Growth Regulator (PGR).

Root induction

The use of MS medium at different strengths with or without NAA had significant effect on root induction of *C. odorata* (Table 4). The obtained highest number of roots (Figure 10) and longest root lengths (Figure 11) from MS medium (100 % basal salts) at 8 WAI showed that high concentration of nutrients in full MS basal salts favoured root induction of the species than in 50% and 25% basal salts. This result could be ascribed to higher nutrient availability and uptake in the MS medium with 100% basal salts. Concentration of salts or other osmotically active compounds that constitute the nutrient medium might have triggered the activation of metabolic pathways that culminated in root induction and development (Elequisandra *et al*., 2017).

The comparable growth of roots from the media irrespective of strength with or without 0.5 mg/l NAA (Table 4) revealed that *C. odorata* root induction was favoured by low concentration of NAA. Auxins like NAA is required by most plant cells for division and root initiation but can suppress morphogenesis at high concentrations. It was clearly shown by the result of this study that plantlets from media with no NAA had better leave formation and overall growth whereas more roots were formed at low NAA (0.5 mg/l) addition (Plate 1). Similar result was reported on *Curculigo latifolia* when highest percentage of root induction and longer roots were obtained from MS devoid of growth regulators while medium supplemented with 0.25 mg/l indole-3-butyric acid (IBA) produced more number of roots (Babaei *et al*., 2014). *Cedrela fissilis* was also reported to have rooted on MS medium with or without 2.5 µM IBA supplement though on half strength (Nunes *et al*., 2002).

CONCLUSION

This study was undertaken in order to develop *in vitro* protocol for mass production of *Cedrela odorata*. From the results, the best supporting medium for culture initiation of *Cedrela odorata* seeds is any of Woody Plant Medium or Murashige and Skoog medium at full strength. The shoot regeneration and rooting of the sub-cultured plantlets were best achieved on 100% MS medium supplemented with 1.0 mg/l BAP with or without 0.5 mg/l NAA. Consequently, the developed protocol is hereby recommended for *in vitro* mass propagation of the species while further study on the area of acclimatization is required.

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