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GROWTH EVALUATION OF IN-VITRO PROPAGATED SEEDS AND SHOOT TIPS OF *Mansonia altissima* (A Chev.) A Chev

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ABSTRACT

This research work aimed at developing protocol for in-vitro propagation of Mansonia altissima. Culture-initiation experiment involved four treatments (Control (distilled water), 25 %, 50 % and 100 % Murashige and Skoog (MS) basal medium) with ten replications. The shoot regeneration involved 2 x 3 x 2 factorial treatments with five replications. Factors were two MS media strengths (Half and Full), three Benzyl Amino Purine (BAP) levels (0, 1.0 and 2.0 mg/L) and two explant types (shoot tips and lower stem). Root induction experiment consisted four treatments (0, 1.0, 2.0 and 3.0 mg/L Naphthalene Acetic Acid) with five replications in MS medium. All treatments were laid out in completely randomised design. The results showed that 100 % seed germination was obtained in distilled water only and 100 % MS basal medium at 2 weeks after inoculation (WAI). However, 25 % MS medium gave highest support for shoot growth of the seed plantlets in terms of shoot length (6.22 cm) and adventitious roots (33.5) at 3 WAI. The explants were best regenerated using full strength MS medium, 1.0 mg BAP/L and shoot tips with highest average number of leaves (3.2) at 8 WAI. None of the rooting treatments induced any root on the plantlets at 12 WAI. It could be inferred that culture of M. altissima could be initiated in-vitro using seeds on sterile distilled water or 25 % MS basal medium while its shoot-tips could be best regenerated when sub-cultured on 100 % MS basal medium supplemented with 0.1 BAP mg/L.

Keywords: Culture-initiation, Protocol, Root induction and Shoot regeneration

INTRODUCTION

Mansonia altissima of the family Sterculiaceae is commonly found in West Tropical Africa countries like Benin, Cameroon, Cote d'Ivoire, Ghana and Nigeria (Maku *et al.*, 2014). It is a deciduous forest species growing up to 37 m in height and girth of 2.5 m, bearing a dense canopy in dry season (Maku *et al*., 2014). The species is classified as a non – pioneer light demander with high economic importance (Gyimah *et al.*, 2003; Beet, 1989). Its wood is of medium weight, moderately hard, very durable and resistant to fungi, borers and termites. These qualities made its wood useful for different purposes including general and high-class joinery, cabinet work, furniture, turnery, decorative veneer and handicrafts (Ken, 2019). Because of its usefulness, the species is over exploited, making it vulnerable and facing the dangers of extinction. (Myers, 2000; IUCN, 2008). Hence, the urgent needs for its conservation and reforestation. However, *M. altissima* like other several candidate species for domestication have some challenges ranging from short periods of seed viability, damage from pests and pathogens to irregular flowering (Bonner, 1990). This in turn results into unavailability of materials for both commercial forestry and provenance testing. According to Osunlaja *et al*., (2017), researchers over the years have exploited various means of determining the best factors that will support the early growth of *M. altissima* and its plantation establishment. The existing challenge of lack of dependable supply of planting material can be overcome through micro-propagation techniques. This entails rapid vegetative propagation of plants under in vitro conditions of high light intensity, controlled temperature and a defined nutrient medium.

Consequently, the aims of this study was to evaluate the growth of in-vitro propagated seeds and shoots tips of *M. altissima* with a view to evolve methods required for provision of elite clones and mass multiplication of the species.

MATERIALS AND METHODS

Study Area

The investigation was carried out in the Biotechnology section of Department of Bioscience, Forestry Research Institute of Nigeria. The Institute is located on the longitude 07°23'18'' to 07°23'43''N and latitude 03°51'20'' to 03°23'43''E (FRIN, 2018).

Treatment and Experimental design

The culture initiation experiment consist of four treatments with ten replications. The treatment were control (distilled water) and three concentrations (25 %, 50 % and 100 %) of Murashige and Skoog (MS) medium basal salts. The shoot regeneration experiment involves three factors. They were two media strengths (Half and Full Murashige and Skoog (MS) basal salts), three levels of Benzyl Amino Purine (BAP) (0, 1.0 and 2.0 mg/l) and two explant types (shoot tips and basal stem). These were combined in a factorial arrangement making up twelve treatments with five replications. Rooting experiment was designed with four treatments which were MS medium supplemented with four levels of Naphthalene Acetic Acid (NAA); 0, 1.0, 2.0 and 3.0 mg/l with five replications**.** All treatments were laid out in completely randomised design (CRD).

Media preparation and Explant sterilization

The various MS media enumerated above were prepared following standard procedures **(**Murashige and Skoog, 1962). The media for experiment 1 was basally supplemented with 0. 3% Activated charcoal. All the media pH were adjusted to 5.8 and gelled with 8.5g/l of agar (Sigma Aldrich, Lot 83112). 20 ml was dispensed per tube, cocked and sterilized at 121 °C and 15 *psi* for 15 minutes in the autoclave.

Freshly collected seeds of *M. altissima* were obtained from the Seed Section of Sustainable Forest Management Department in FRIN. The seeds were de-coated and surface sterilized as follows. The seeds were dipped in the combination of antibiotics (5 g/l Z-force + 5 g/l Cibaplus + 0.4 g/l Ciproxamed) under sterilized air laminar hood for 60 minutes, Then dipped in 70 % ethanol for 5 minutes, and 10 % hypochlorite solution + 2 drops of Tween 20 for 15 minutes. Each steps was preceded with 3 times rinse using sterile distilled water while it was finally rinsed four times. The tubes were inoculated at one seed each positioned flat at the media surface. Four Weeks after Inoculation (WAI), the plantlets were sub-cultured into freshly prepared MS media specified for shoot regeneration (Experiment 2) above. All inoculated tubes were kept at 18 ± 2 °C and 16/8 hours light/dark photoperiods in the growth room.

Data collection and analysis

Germination attributes which include percentage germination, radicle and shoot emergence were collected at 2 WAI while shoot and root lengths, and number of adventitious roots were collated at 3 WAI on seed growth. Shoot lengths and number of leaves were collected on the sub-cultured plantlets at 4 weeks interval, starting from 4 WAI. The quantitative data were analysed descriptively while others were subjected to analysis of variance using GenStat 4th edition. Significantly different means were separated at $P \leq 0.05$ with Duncan multiple Range Test.

RESULTS

Seed germination attributes

Figure 1 showed the growth attributes of in-vitro propagated seeds of *M. altissima* on MS media of varying concentration of basal salts at 2 WAI. It was observed that 100 % of the seeds inoculated on distilled water only and full strength media (0 % and 100 % MS basal salts) germinated while 85.7 and 57.1 % were obtained from half and quarter (50 % and 25 % MS basal salts) strength media. Similarly, 71.4 % radicle emergence was obtained from both distilled water only and full strength media whereas, 42.9 and 14.3 % were obtained from half and quarter strength media. Conversely, shoot emergence of 42.9 % was similar in both half and quarter strength media but higher than 28.6 % obtained from only distilled water and full strength media at 2 WAI.

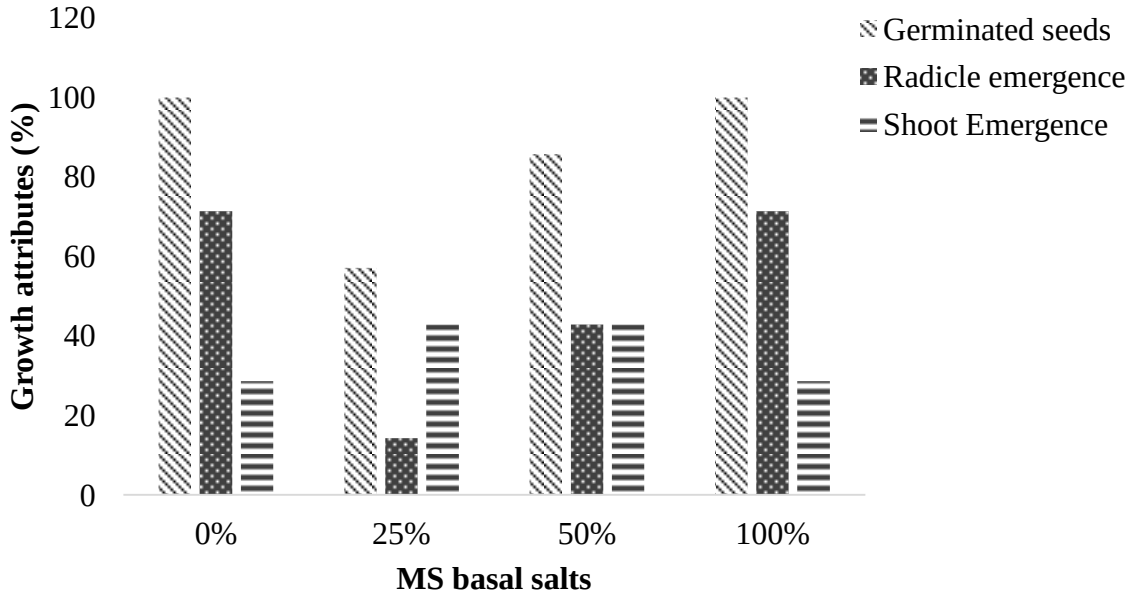


Figure1. Growth attributes of in-vitro grown seeds of *Mansonia altissima* at 2 Weeks after inoculation

Seed Shoot and Root lengths (cm), and Number of adventitious roots

The results of the growth attributes of the in-vitro inoculated seeds of *M. altissima* at 3 WAI showed that there was no significant difference ($p > 0.05$) among the average shoot lengths of the plantlets (Table 1 and Plate 1 A-D). The shoot length ranges from the highest (6.22 cm) in medium B (25 % MS basal salts) to the lowest (4.0 cm) in control (distilled water only).

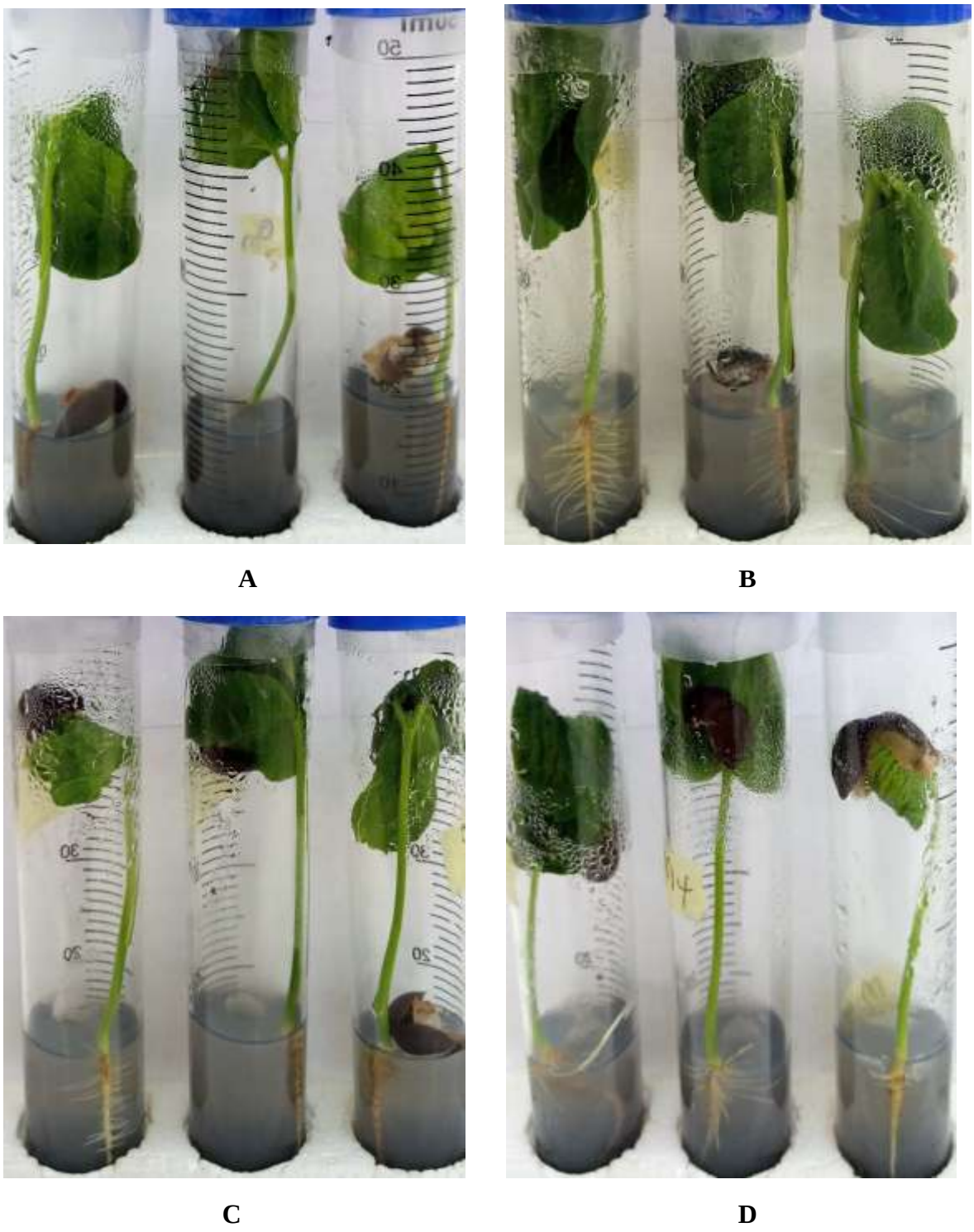
Analysis of variance performed on the root lengths of the plantlets indicated that there was significant difference ($p \leq 0.05$) between the treatments. Root lengths of plantlets obtained from media C, B and D (50, 25 and 100 % MS basal salts respectively) were significantly higher than those of medium A (Distilled water only). Meanwhile those of medium C was higher than medium D but similar in values to medium B (Table 1 and Plate 1 A-D).

Similar results were obtained in the number of adventitious root of the species at 3 WAI. There was significant difference among the average number of adventitious roots of the plantlets as supported by the growth media. Media B (25 %) and C (50 %) were higher than Medium A. Moreover, medium B was higher than C and D (100 %). Whereas, C was not different from D. Similarity was observed between number of adventitious root from Media D and A (Table 1 and Plate 1 A-D).

Table 1. Growth response of in-vitro germinated *Mansonia altissima* seeds at 3 WAI

Treatment Code/ MS basal salts (%)	Growth parameters			
	Shoot length (cm)	Root length (cm)	Number of leaves	Number of adventitious roots
A/0	4.0	2.84	2	4.9
B/25	6.22	5.55	2	33.5
C/50	5.91	5.63	2	18
D/100	5.17	4.47	2	13.9
L.S.D@P≤0.05	ns	1.14	ns	12.47

ns: means difference not significant at P ≤ 0.05.



A **B**

C **D**

Plate 1. Growth of in-vitro germinated seeds of *Mansonia altissima* at 3 WAI
A: 0 % MS basal salts; **B**: 25 % MS basal salts; **C**: 50 % MS basal salts and **D**: 100 % MS basal salts

Shoot regeneration of *Mansonia altissima*

In-vitro shoot regeneration of *Mansonia altissima* was examined using two media strengths (Half and full MS basal salts), three BAP levels (0.0, 1.0 and 2.0 mg/l) and two explant types (shoot tip and lower-stem).

Shoot length (cm)

The results of the analysis of variance performed on shoot length data showed that there was significant difference ($p \leq 0.05$) between the explant types whereas other treatment factors and all interactions did not indicate any significant difference ($p > 0.05$) at 4 Weeks after inoculation (WAI) (Table 2). The inoculated shoot tips responded better to treatments than the use of lower-stem. The average shoot length of 1.62 cm

obtained using shoot tips was higher than non-responsive lower-stems at 4 WAI.

At 8 WAI, there was significant different in the effect of media strengths (MDS), explant types and their interaction on shoot length while BAP levels and other interactions did not show significant difference (Table 2). Average shoot length of 0.97 cm obtained from MS medium with full basal salts was higher compared with 0.84 cm obtained from half strength MS medium. In relation to results at 4 WAI, average shoot length (1.81 cm) from shoot tip explants was higher than that of lower stem at 8 WAI.

The interaction between media strengths and explant types revealed that inoculation of shoot tips in full strength MS medium gave higher shoot length (1.94 cm) compared with when inoculated on half strength MS medium (1.68 cm). Inoculation of lower stem either in full or half strength medium did not show any shoot induction and growth at 8 WAI (Figure 2 and Plate 2 G).

Number of leaves

The results of number of leaves of sub-cultured *M. altissima* plantlets followed similar trend at both period of observations. There was significant difference ($p \leq 0.05$) in the effect of the factors and their interactions except media strengths and MDS by explant type interaction (Table 2). In terms of BAP levels, supplementing the media with 2.0 mg/l BAP gave the most significant effect on number of leaves with the value of 0.65 similar to 0.50 obtained from 1.0 mg/l BAP and higher than 0.35 from control media (0.0 mg/l BAP) at 4 WAI. However at 8 WAI, higher number of leaves (1.25) was obtained from media added 1.0 mg/l BAP, comparable to 1.15 from 2.0 mg/l BAP media while 0.0 mg/l BAP media gave the least (0.75) (Table 2). Considering the explant types, average number of leaves produced by shoot tips explant (1.0, 2.10) was higher and better than nonresponsive lower-stem at 4 and 8 WAI respectively (Table 2 and Plate 2).

Table 2. Effect of Media strengths, BAP levels and Explant types on growth of sub-cultured *Mansonia altissima.*

Factors	**Shoot length (cm)**		**Number of leaves**	
	4 WAI	**8 WAI**	**4 WAI**	**8 WAI**
MS media strengths (MDS)				
Half	0.77	0.84	0.47	0.93
Full	0.85	0.97	0.53	1.17
BAP levels (BAPL) (mg/l)				
0.0	0.74	0.81	0.35	0.75
1.0	0.84	0.99	0.50	1.25
2.0	0.85	0.92	0.65	1.15
Explant types (EXPT)				
Shoot tip	1.62	1.81	1.0	2.10
Lower stem	0.0	0.0	0.0	0.0
LSD @ $P \leq 0.05$				
Media strengths (MDS)	0.10	0.13*	0.19	0.32
BAP levels (BAPL) (mg/l)	0.13	0.16	0.23*	0.39*
Explant types (EXPT)	0.10*	0.13**	0.19**	0.32**
MDS x BAPL	0.18	0.22	0.33*	0.56**
MDS x EXPT	0.14	0.18*	0.27	0.46
BAPL x EXPT	0.18	0.22	0.33*	0.56*
MDS x BAPL x EXPL	0.25	0.32	0.47*	0.79**

* and ** indicates means difference significant at $P \leq 0.05$ and $P \leq 0.01$

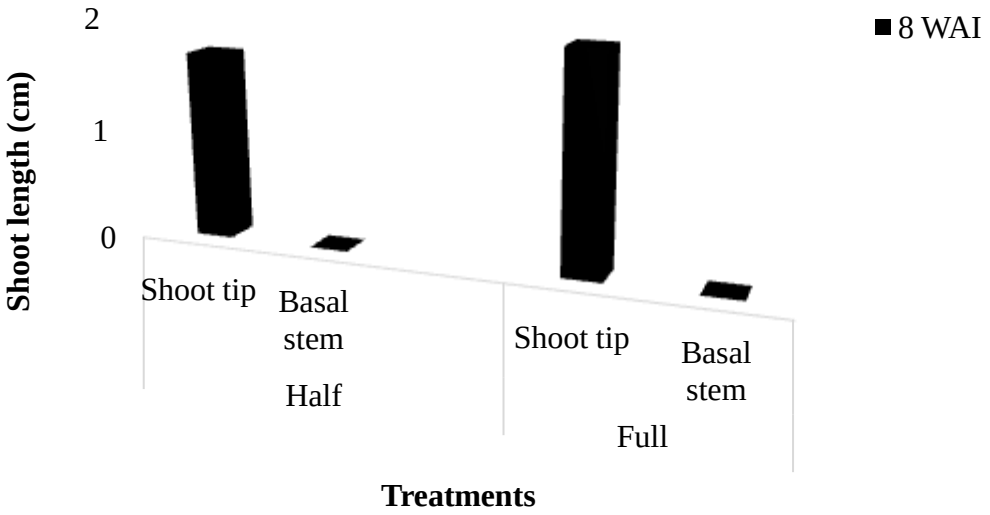


Figure 2. Interactive effect of media strengths and Explant types on shoot length of sub cultured *Mansonia altissima*

Interactions

Media strengths x BAP levels

The results of interaction between medium strengths and BAP levels on number of leaves showed that higher average number of leaves (1.6) was produced from full strength MS medium without 1.0 mg/l BAP addition at 8 WAI (Table 3). This value was significantly higher than what was obtained from half strength media with or without BAP supplements in-between which there were similarities at 8 WAI). The result was also higher than 0.5 from full strength MS medium without BAP but comparable to 1.4 of the same medium with 2.0 mg/l BAP addition (Table 3).

Table 3. Interactive effect of media strengths and BAP levels on number of leaves of sub cultured *Mansonia altissima.*

	Factors	Number of leaves	
MS media strength	**BAP levels (mg/l)**	**4 WAI**	**8 WAI**
Half	0.0	0.49abc	1.0bc
	1.0	0.4bc	0.9bc
	2.0	0.5abc	0.9bc
Full	0.0	0.2c	0.5c
	1.0	0.6ab	1.6a
	2.0	0.8a	1.4ab

BAP levels x Explant types

The results of BAP levels by explant type interactions showed that shoot tips inoculated on media with 1.0 mg/l BAP addition gave highest number of leaves (2.5) at 8 WAI. This value was comparable to 2.3 from media added 2.0 mg/l BAP while both values were higher than others which were similar in values at same period (Table 4).

Table 4. Interactive effect of BAP levels and Explant types on number of leaves of sub cultured *Mansonia altissima*

	Factors	Number of leaves	
BAP levels (mg/l)	**Explant types**	**4 WAI**	**8 WAI**
0.0	Shoot tip	0.7b	1.5b
	lower stem	0.0c	0.0c
1.0	Shoot tip	1.0ab	2.5a
	lower stem	0.0c	0.0c
2.0	Shoot tip	1.3a	2.3a
	lower stem	0.0c	0.0c

Media strengths x BAP levels x Explant types

The results of the interactive effect of the three factors was presented in Table 5. It showed that highest average number of leaves (3.2) was obtained from the shoot tips explant inoculated on full strength MS medium with the addition of 1.0 mg/l BAP at 8 WAI. The value was similar to 2.8 from shoot tips inoculated on full strength MS medium with 2.0 mg/l BAP addition and significantly higher than others (Plate 2). The number of leaves (2.0) from shoot tips inoculated on half strength MS media without BAP addition was related to that (1.8) obtained from shoot tip inoculated on half strength MS medium with 1.0 or 2.0 mg/l BAP addition at 8 WAI. Irrespective of the media strength and BAP levels, no number of leaves was produced by lower-stem explants at 8 WAI (Table 5).

Table 5. Interactive effect of Media strengths, BAP levels and Explant types on number of leaves of sub cultured *Mansonia altissima*

	Factors		Number of leaves	
MS media strength	**BAP levels (mg/l)**	**Explant types**	**4 WAI**	**8 WAI**
Half	0.0	Shoot tip	1.0b	2.0b
		Basal stem	0.0d	0.0d
	1.0	Shoot tip	0.8bc	1.8bc
		Basal stem	0.0d	0.0d
	2.0	Shoot tip	1.0b	1.8bc
		Basal stem	0.0d	0.0d
Full	0.0	Shoot tip	0.4cd	1.0c
		Basal stem	0.0d	0.0d
	1.0	Shoot tip	1.2ab	3.2a
		Basal stem	0.0d	0.0d
	2.0	Shoot tip	1.6a	2.8a
		Basal stem	0.0d	0.0d

Root induction

The experiment on root induction was conducted using MS medium supplemented with 0.0, 1.0, 2.0 and 3.0 mg/L NAA. Result showed that none of the treatments induced any root on the plantlets at 12 WAI.

A

B

C

D

E

F

G

Plate 2: Growth of sub-cultured *Mansonia altissima* shoot tips and lower-stem at 8 WAI

A: Half strength MS/0.0 mg/l BAP/Shoot tip, **B**: Half strength MS/1.0 mg/l BAP Shoot tip, **C**: Half strength MS/2.0 mg/l BAP Shoot tip, **D**: Full strength MS/0.0 mg/l BAP/Shoot tip, **E**: Full strength MS/1.0 mg/l BAP Shoot tip, **F**: Full strength MS/2.0 mg/l BAP Shoot tip and **G**: Full strength MS/2.0 mg/l BAP lower-stem.

DISCUSSIONS

Seed germination

In-vitro propagation of *Mansonia altissima* was assessed. The similarity observed in the number of germinated seeds, radicle and shoot emergence from distilled water medium and 100 % Murashige and Skoog (MS) medium (Figure 1) is an indication that distilled water can be used for culture initiation of *M. altissima* instead of the nutrient MS medium. Seeds germination is subject to both internal and external factors. External factors affecting seed germination include water, Temperature, Aeration and Light (Rakesh, 2011). While the last three factors were assumed to be constant in this experiment, the result could then be attributed to the availability of more water in the distilled water medium compared with the nutrient media. The results also underscored the importance of endosperm in seed germination which might have supported the early growth of the seeds until exhausted. Availability of nutrients might have been the reason for higher growth observed from the MS media compared with distilled water medium at 3 WAI (Table 1). Higher number of adventitious roots in MS 25% basal salts couple with better root length might have resulted into highest shoot length obtained from the medium plantlets (Figure 1). Whereas, lack of nutrient and exhaustion of endosperm might have given rise to lower shoot and root growth from distilled water medium plantlets even though radicle and shoot emergence was better in the medium from the onset. Germination of seeds and growth of *Mansonia altissima* seedling was once recorded to respond well to 0.03g/l IAA and 0.005g/l IBA in the natural media (Maku *et al*., 2014) however, the result of this study showed that the species can be germinated in-vitro without addition of such growth regulators.

Shoot regeneration

The result showed that inoculated shoot tips of the *M. altissima* responded better than lower stem explants in shoot length and number of leaves at 8 WAI (Table 2). This depicts that shoot tips of the species can only be used for its in-vitro shoot regeneration and multiplication. The results could be attributed to the availability of actively dividing regions called meristem in the shoot tips. On the other hand, the non-responsiveness of the lower stem explants could be due to the concentration of auxin in the stem of the plantlets sub-cultured at that young stage. The Auxin might have inhibited the induction of axillary bud that would have given rise to new shoot growth (Susan, 2002). This result corresponded to that of

Emam, (2006) who observed that shoot tips of *Pyrus communis* rootstock successfully developed when cultured on MS medium compared with one-nodal cutting.

In relation to Media strengths, plantlet growth was better in MS 100 % basal salts medium compared with MS 50 % basal salts. This showed that the species required more nutrients for in-vitro shoot growth. Higher nutrient concentrations in the MS 100 % basal salts might have caused increased uptake through osmosis which culminated into higher mean shoot length and number of leaves (Table 2 and Figure 2) from the medium. This result corresponds to that of Elequisandra *et al*., (2017) who did similar study on *Dipteryx alata*. They reported that *D. alata* seedlings in the MS medium (100% original concentration) developed better than those in other concentrations at 120 days.

The use of BAP at 1.0 and 2.0 mg/L positively affected the growth of *M. altissima* plantlets compared with control at 8 WAI. The observed better growth of plantlets in MS medium supplemented with 1.0 mg/L BAP (Table 2) showed that it was the optimum BAP level required for in-vitro shoot regeneration and development of sub-cultured *M. altissima* plantlets. Addition of BAP at high concentration such as 2 mg/L in this study might have triggered inhibitory effect on shoot growth. This result corroborated the finding of Mohammad *et al*., (2014) who obtained 8.5 new micro shoots/explant of almond x peach hybrid from MS medium supplemented with 1 mg/L BAP.

Considering the interaction of the three factors, the observed highest average shoot length and number of leaves from shoot tips inoculated on MS 100 % basal salts medium supplemented with 1.0 mg/l BAP revealed that in-vitro regeneration of sub-cultured *M. altissima* cuttings could be achieved using these components.

CONCLUSION

Mansonia altissima is a threatened indigenous tree species in Nigeria. Successful protocol establishment for in-vitro propagation of the species will ensure its cryopreservation and mass production of it elite clones for plantation purposes. The present research work has shown that the seed of *M. altissima* can be germinated in-vitro using sterile distilled water and 25 % MS basal medium. Similarly, its shoot tip explant can be best regenerated when sub-cultured on 100 % basal salt MS medium supplemented with 0.1 BAP mg/L. These conditions are therefore recommended for culture initiation and shoot regeneration of the species in-vitro. Efforts to root the species plantlets did not yield any positive results. Hence, in-vitro root induction of the species is hereby suggested for further research.

REFERENCES

Beet W.C. (1989). The potential role of agro forestry in ACP countries. Technical Centre of Agriculture and Rural Cooperation (ACP-SEC IOME CONVENTION) Netherland.

Bonner, F.T. (1990). Storage of seeds: potential and limitations for germplasm conservation. *Forest Ecology and Management* 35:35-43.

Elequisandra da Costa Araruna, João Paulo Ribeiro-Oliveira, Vanderley José Pereira, Simone Abreu Asmar and Berildo de Melo. (2017). Salt concentrations in culture media for the development of *Dipteryx alata* in vitro. *Pesquisa Agropecuária Brasileira.* 52 (12). http://dx.doi.org/10.1590/s0100-204x2017001200020.

Emam, H.E. (2006). Physiological studies on in vitro propagation of *Pyrus communis* root stock. Ph.D. Thesis, Cairo University, Egypt.

Forestry Research Institute of Nigeria (FRIN) (2018). Annual Meteorological Report 2017-18. Nigeria. Unpublished report.

Gyimah, R. and T. Nakao (2003). Effect of light intensity and nutrient on growth and electron transport of tropical tree found in Ghana, Faculty of Agriculture, Miyazaki University, Japan. 40(1-2) pp 69-78.

IUCN (2008). International Union for Conservation of Nature and Natural Resources Red list of threatened species.

Ken Fern (2019). Tropical Plants Database, tropical.theferns.info. Online resources. Available at; <tropical.theferns.info/viewtropical.php?id=Mansonia+altissima> Date assessed, 27/06/2019.

Maku J. O., Gbadamosi A. E., Fadoju O. A. (2014). Germination and Seedling Growth

of *Mansonia altissima* (A. Chev) in Response to Hormonal Treatment International *Journal of Agriculture and Forestry* 4(4): 269-274 DOI: 10.5923/j.ijaf.20140404.02.

Mohammad M. Arab, Abbas Yadollahi, Abdolali Shojaeiyan, Saber Shokri, Shoresh Maleki Ghojah (2014). Effects of nutrient media, different cytokinin types and their concentrations on in vitro multiplication of G x N15 (hybrid of almond x peach) vegetative rootstock. *Journal of Genetic Engineering and Biotechnology*. 12: 81–87.

Murashige T and Skoog E. (1962). A revised medium for rapid growth and bioassays with Tobacco tissue cultures. *Physiol. Plant* 15: 473-497.

Myers, N. (2000). Deforestation Rates in Tropical Forests and their Climatic implications. Friends of the Earth, London U.K., 116 pp. 4. WF Climate Change Campaign. 2000

Osunlaja, O.A., Olomola,D.B, Nwogwugwu, J.O., Afolabi, J.O. and Oloyede, E.O. Effects of Media Composition on Shoot Initiation of *Mansonia altissima* A. Chev. Using Nodal Culture Technique *Journal of Forestry Research and Management. Vol. 14 (1), 103-109; 2017, ISSN 0189-8418*.

Rakesh kumar Nama (2011). Seed Physiology. Available at slideshare.net. Date assessed, 15/7/2019.

Susan T. Rouse (2002). Shoots-Biology Encyclopedia-cells, plant body, function, System. Retrieved July 17, 2019 from www. biology reference.com.