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PROTOCOL DEVELOPMENT FOR IN-VITRO PROPAGATION OF *Anthocephalus cadamba*

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ABSTRACT

The objective of this study is to establish the most suitable protocol for the micro propagation of Anthocephalus catamba. The seeds of the species were collected from the wild and stored in the Seed bank until time of use. These were subjected to surface-sterilization using systemic fungicide and other disinfectants. Different media strengths of Murashige and Skoog (MS) were used to determine the most efficient nutritional requirement. The media strengths employed in this experiment were ¼, ½ and full. The culture media were supplemented with 6-Benzylamino purine (BAP), Giberrellic acid (GA3) and N-acetic acid (NAA). The generated plantlets had no contamination in the growth room due to the methods employed in the disinfection. In almost all the results obtained, both the ¼ and ½ strength media produced better result than plantlets growing on full strength media. Treatment D (¼ strength, 0.1mg/L BAP, 0.2mg/L GA3 & 0.1mg/L produced the best results in all. From the results obtained from this study, it is recommended that lower basal salts will be required for the in-vitro propagation of A.cadamba.

Keywords: *Anthocephalus cadamba*, growth hormone, media strength, micro-propagation, plantlets.

INTRODUCTION

Anthocephalus cadamba (Roxb) Miq) is a famous tropical large tree with straight cylindrical bole. The tree may reach a height of 45 m with trunk diameters of between 100cm and 160 cm. It has small buttresses and a broad crown. It is generally known as *Kadamb* or *Jabon* in South Asia and Southeast Asia (Asgar and Baharuddin 2017). *Anthocephalus cadamba* is an early-succession species which grows best on deep, moist, alluvial sites. It grows also often in secondary forests along riverbanks and in the transitional zone between swampy, permanently flooded and periodically flooded areas. (Jeyalalitha, 2015).

Anthocephalus cadamba wood is very easy to preserve and it is used for plywood, light construction, pulp and paper, boxes and crates, dug-out canoes, and furniture components. The wood can be easily impregnated with synthetic resins to increase its density and compressive strength (Joshi and Mathur, 2014). Some scientific studies carried out revealed its anti malarial (Slkar et. al., 1996, Sianne and Fanie 2002) and antihepatotoxic activities (Kapil, *et al.,* 1995). *Anthocephalus cadamba* has the prospect in establishment of forest plantations as it is a fast growing species with high production. *Anthocephalus cadamba* being a fast growing species has the prospect of been used in establishment of forest plantations. It can potentially regulate the water system in the soil and Prevent soil erosion (Asgar *and* Baharuddin, 2017).

Despite its usefulness and importance, little work has been done on its cultivation and depletion of natural population. Plantations of this species have not been successfully established due to poor seed germination, seed dormancy and poor efficacy of rooting through conventional method of propagation, making the conservation of *A. cadamba* serious concern (Bose and Choudhary, 1991).

Conventional methods of propagation of *A. cadamba* by seed and cuttings have not been successful this leading to limitation of the distribution of this sacred tree (Anjali and Nishi,

2015). To overcome these challenges and meet up with demand, it is necessary to have this plant in large numbers supported by seed stocks which are superior in both quality and quantity (Asgar and Baharuddin 2017). Tissue culture technique is one of the alternatives that can be used for the supply of seedlings of *A.cadamba* for large-scale propagation and conservation. The aim of this study is to determine the most appropriate media strength and optimum hormonal concentration for the *in-vitro* propagation of *A. cadamba*. Information from the in-vitro propagation of this species will help in mass propagation for possible plantation establishment and sustainable usage.

MATERIALS AND METHODS

Collection of explants: Viable seeds of *A. cadamba* were obtained from the seed bank of the Forestry Research Institute of Nigeria, Ibadan, Nigeria.

Explants disinfection

The seeds were first subjected to antifungal treatment by treating them with 3%w/v systemic fungicide. They were then rinsed using sterile distilled water. The seeds were disinfected by the use of 70% alcohol on the seeds for 5 mins. They were rinsed after with sterile distilled water and 10% hypochloride was added with few drops of Tween20 and allowed to stay for 15mins. These were then rinsed thrice with sterile distilled water in the laminar airflow hood for inoculation.

Culture Media preparation

The culture medium used for this experiment was Murashige and Skoog basal medium. This medium was prepared in quarter, half and full strengths respectively making the sucrose content to be 0.75%, 1.5% and 3% sucrose respectively. All media strength was fortified with different combinations and concentrations of Plant growth regulators (PGRs). These growth regulators included Benzyl amino purine (BAP), N-acetic acid (NAA), and Giberellic acid (G3). They were combined in the concentrations highlighted in Table 1 below.

Table 1: Media Strength and Plant Growth regulators Concentration

Media strength	Treatments	BAP (mg/L)	G3(mg/L)	NAA (mg/L)
Quarter Strength	A	0.0	0.0	0.0
	B	0.1	0.0	0.1
	C	0.2	0.1	0.2
	D	0.1	0.2	0.1
	E	0.0	0.1	0.2
Half strength	F	0.0	0.0	0.0
	G	0.1	0.0	0.1
	H	0.2	0.1	0.2
	I	0.1	0.2	0.1
	J	0.0	0.1	0.2
Full strength	K	0.0	0.0	0.0
	L	0.1	0.0	0.1
	M	0.2	0.1	0.2
	N	0.1	0.2	0.1
	O	0.0	0.1	0.2

A= control for Quarter strength, F= control for Half strength, K= control for full strength media.

This experiment was done to determine the effects different media strengths and different hormonal concentrations on the in-vitro propagation of *Anthocephalus cadamba*. It is a balanced three-factor factorial design (3x2) with ten replicates for each treatment. The comparism test was done using Statistical Product and Service Solution (SPSS) 16.0 software. Analysis of variance was done to determine significant difference.

Shoot initiation:
The seeds of the species were inoculated into culture tubes with the various media strengths under aseptic conditions, sealed with Parafilm and taken to the growth room with 16h photo period and 8 h dark period. Observations were made at intervals of 2 days till the first radical emergence. Subsequent readings were taken at 7 days intervals. The shoot heights, root lengths and number of leaves were taken.

RESULTS
Treatment C (¼ strength, 0.2 mg/L BAP, 0.1 mg/L GA3 and 0.2 mg/L NAA), gave the longest shoot length after 3 weeks of inoculation while treatment O (full strength, 0.1mg/L GA3 and 0.2mg/L NAA), gave the shortest with height of 0.25 cm among the tubes that had growths in them. Treatment C gave the longest shoot length of 4.0cm five weeks after inoculation while treatment M (Full strength, 0.2mg/L BAP, 0.1mg/L GA3 and 0.2 mg/L NAA) gave the shortest shoot length. Treatment D (¼ strength, 0.1mg/L BAP, 0.2mg/L GA3 and 0.1 mg/L) produced the highest shoot at 5.6cm after 7 weeks of inoculation while treatment N (Full strength, 0.1 mg/L BAP, 0.2 mg/L GA3 and 0.1 mg/L NAA) gave the shortest shoot length.

From Table 2, the mean shoot height in plantlets raised in full strength media is lower than those inoculated in both ¼ and ½ strength media. Treatment A (full strength, no PGR) produced the longest root length while treatment O gave the shortest root length (0.25cm) 3 weeks after inoculation. At 5 weeks after inoculation, treatment M produced the highest root length while treatment O gave the shortest (0.7cm). At 7 weeks after inoculation, treatment M gave the longest root length of 5.2, but the shortest root length was produced by treatment O (0.2cm).

Table 2: mean shoot height of *A. cadamba*, 3, 5 and 7 weeks after inoculation.

	Shoot Height		
Treatments	**3 WAI**	**5 WAI**	**7 WAI**
Control	1.23b	1.56a	1.89a
Full Strength	0.27a	1.15a	1.45a
Half Strength	2.06c	2.51b	3.59b
Quarter Strength	1.93c	2.83b	3.83b

P< 0.05, WAI = Week after inoculation.

Table 3: Mean Root length of *A. cadamba*, 3, 5 and 7 weeks after inoculation.

	Root length		
Treatments	**3 WAI**	**5 WAI**	**7 WAI**
Control	1.20b	1.52a	1.79a
Full Strength	0.41a	1.28a	1.91a
Half Strength*	1.31b	1.76a	3.42b
Quarter Strength	1.19b	2.54b	3.54b

P< 0.05,WAI = Week after inoculation.

Table 4: Mean number of leaves of *A. cadamba*, 3, 5 and 7 weeks after inoculation.

	Number Leaves		
Treatments	**3 WAI**	**5 WAI**	**7 WAI**
Control	5.13bc	5.73b	5.67a
Full Strength	1.03a	3.50a	4.75a
Half Strength	4.05b	8.21c	9.38b
Quarter Strength	6.38c	7.05bc	8.75b

P< 0.05, WAI = Week after inoculation.

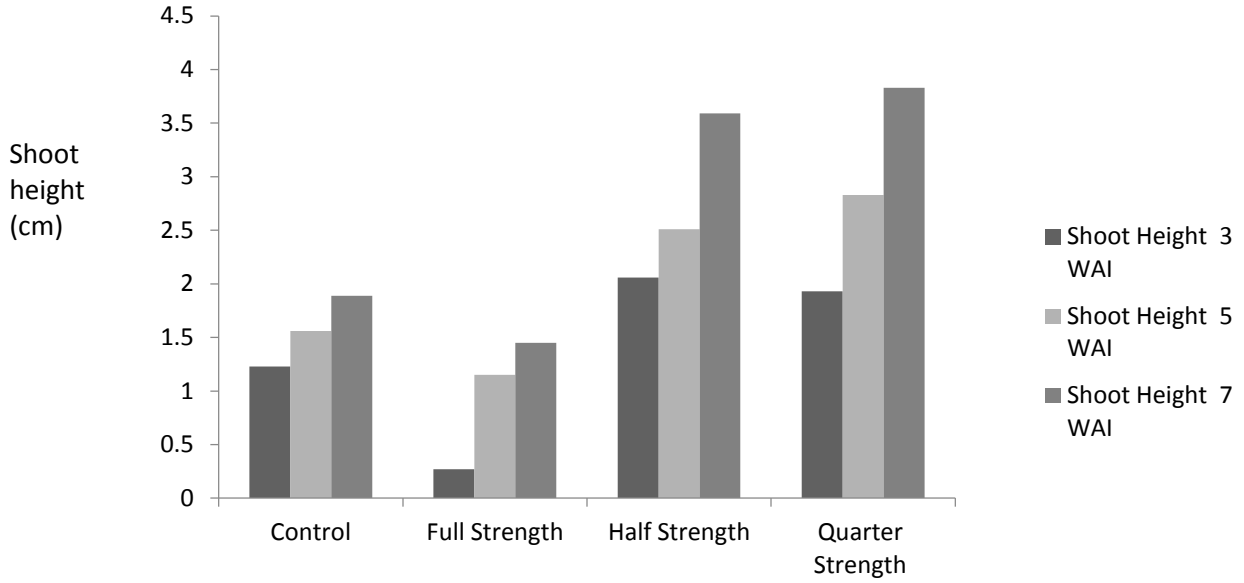

Figure 1: Mean Shoot length, 3 WAI, 5 WAI and 7 WAI.

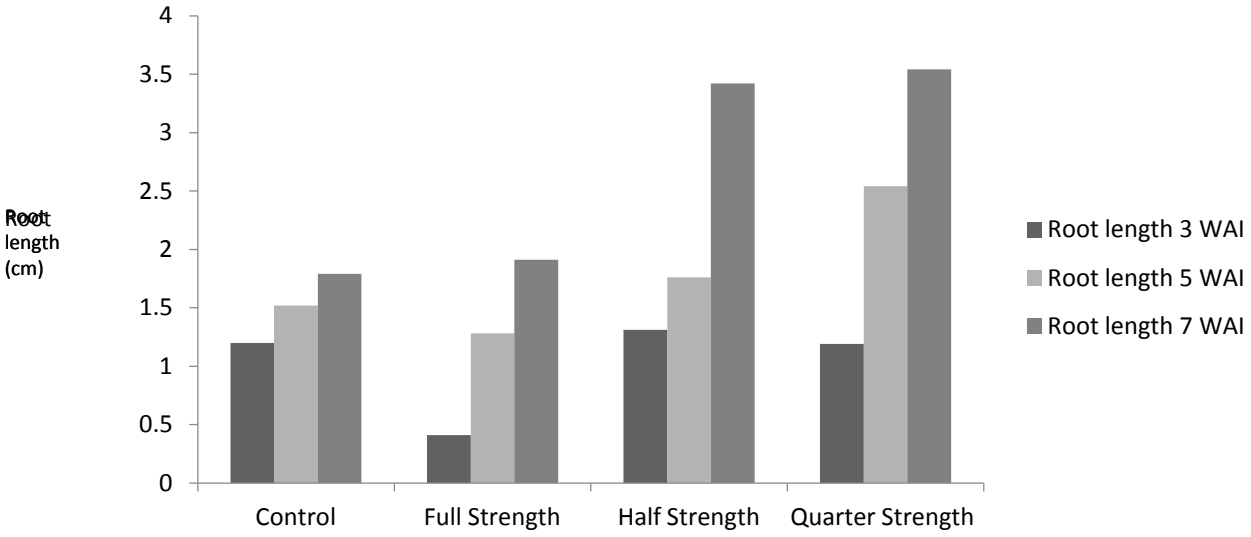

Figure 2: Mean of root length, 3WAI, 5WAI & 7WAI.

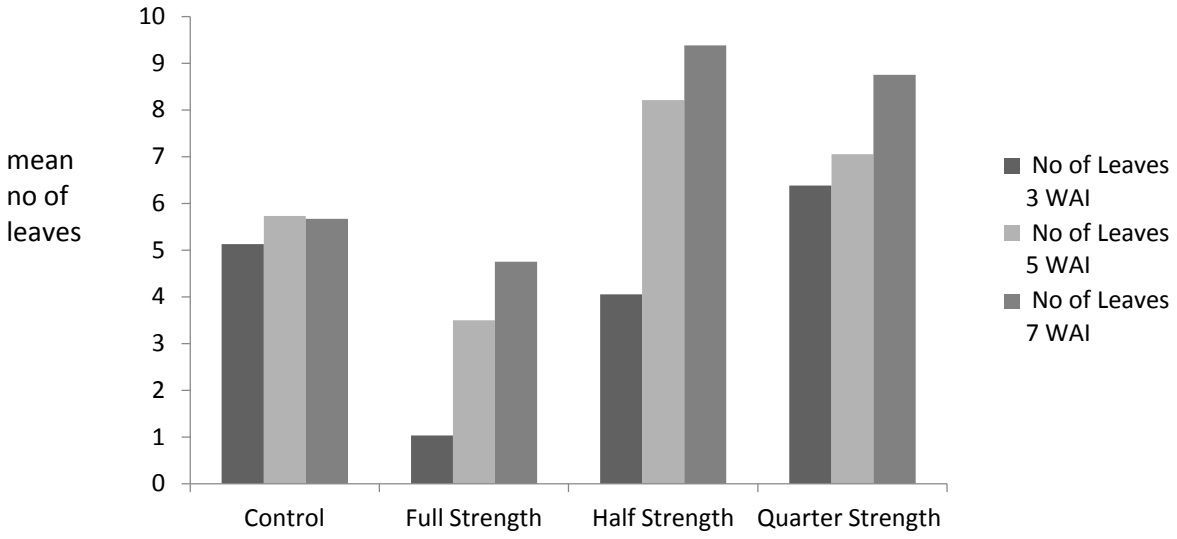

Figure 3: Mean number of leaves

Plate 1: Plantlets of *A.cadamba* in different treatments 3 WAI.

Plate1: Plantlets of *A.cadamba* in different treatments 5WAI.

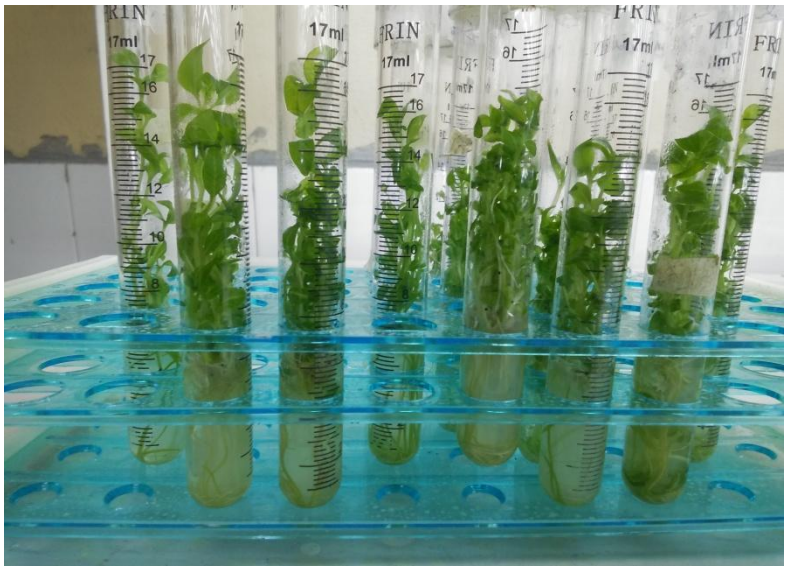

Plate 3: Plantlets of *A .cadamba* in different treatments 7WAI.

DISCUSSION

The mean root length is higher in both ¼ and ½ strength media (Table 3) than the full strength media, this is in line with similar work done by Sarropoulos *et al*. 2015, both ½ and ¼ strengths media gave the optimum results. Treatments N.D and I have the same PGR concentrations but different media strength, ¼ and ½ gave better results than full strength, Figures 1, 2 and 3, this is to validate the claim that lesser basal salts are required for the *in-vitro* propagation of *A. cadamba*. Even without PGRs, ½ strength supported the proliferation of the shoots and roots of the species (Bhalla *et al*.2009).

The full strength and control media which contain maximum basal salt requirement gave the worst results in terms of growth, this is in line with certain study carried out that some plant require lesser concentration of basal salt medium for maximum, growth Fadel *et al*., 2010 – explants inoculated on half strength media had the best results on the organogenesis of *Methana spicata* I.There was no significant difference between the mean shoot lengths of plantlets 3 weeks after inoculation in the media with half and quarter strengths MS media. Plates 1,2, & 3 shows growth of the species at 3WAI, 5WAI and 7WAI. At 5WAI and 7WAI, there was no significant difference in the mean shoot length of the control and full strength media. There was also no significant difference in the mean shoot lengths of the plantlets with quarter and half strengths (Table 2). The mean shoot lengths are higher in both quarter and strength MS media showing that lower nutritional requirement is needed for shoot proliferation of *A. cadamba*, this is in line with the work by Bidarigh and Azarpour, 2013 in which ¼ strength MS media gave one of the best results in shoot and root elongation. Husain *et al*,. 2007, half strength MS media supplemented with growth regulators produced the best results in the *in-vitro* propagation of of Indian Kino (*Pterocarpus marsupium* Roxb.) obtained similar results obtained in this study.

CONCLUSION

According to Okafor *et al*.2010, some tree species like *Treculia Africana* and others will require full strength media for their in-vitro propagation; such cannot be said of other species of all trees especially the Rubiaceae family. It will be therefore recommended that full strength media should not be employed in the *in-vitro* propagation of *Anthocephalus cadamba*.

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