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Micropropagation of baobab, an economic plant

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Abstract: *Adansonia digitata* L. has been repeatedly reported to have ethno-medicinal uses such as food and fodder, fibre in clothing and ropes, ethno-veterinary medicine and other herbal applications. Currently, it is widely used in the many traditional systems of medicine and yet detailed report of its mass propagation is lacking. This study was therefore conducted to develop multiple formation of shoot and buds of *Adansonia digitata* using embryos as a starting material. Matured embryos of baobab plant were inoculated on two basal media namely Murashige and Skoog (MS) and Woody Plant Medium (WPM). Nodal explants excised from the regenerated plantlets were cultured on MS basal medium supplemented with various concentrations of cytokinin such as Benzylaminpurine (BAP) and Kinetin (KIN), auxin - Naphthalene Acetic Acid (NAA) and an additive (coconut water). The effects of growth hormones and additive (coconut water) on *in-vitro* propagated plantlets were evaluated. In embryo regeneration both basal media could be used, though MS basal medium proved to be better than WPM basal medium with the highest number of nodes coupled with the maximum length of roots and shoots. However in shoot multiplication MS basal medium with or without KIN and NAA performed better than BAP and NAA. The sufficiently rooted plants were then transferred for acclimatization and later taken to screen house for hardening.
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Introduction

Adansonia digitata Linn. commonly called baobab belongs to the family Bombacaceae. It is important for the livelihood of the people in the arid zones (Becker, 1983). Monkey bread is one of the common English names derived from the fact that monkeys eat the baobab's fruit (Rashford, 1994). Baobabs are often the most prominent tree species wherever they occur because of their great size and bizarre shapes. They are widespread throughout the hot and the drier regions of tropical Africa. It has an extensive root system, a high water holding capacity and is resistant to fire. This adaptation allows it to grow in zones with 100mm-1000mm annual rainfall. Baobab was found to be among the effective tropic plants at controlling its water loss (Abdalla *et al.* 2010).

Among many plant species that have been reported to have ethno-medicinal uses, baobab has been widely used in the traditional systems of medicine. According to the United Nations (2005), the fruit pulp of *A. digitata* is traditionally used for the treatment of fever, diarrhoea, dysentery, haemoptysis and small pox in humans. Leaf infusions are used as treatment for diarrhoea, fever, kidney and bladder diseases, blood cleansing and asthma in humans. The bark is used for treatment of fever caused by malaria. As far as ethno-veterinary medicine is concerned, reports indicated that bark of *A. digitata* is used for the treatment of diarrhoea in poultry (Guèye, 1999). Fruits are used for treatment of Newcastle diseases in poultry (Gebauer *et al.* 2002; Wynn and Fougère, 2006).

The fibrous bark of *A. digitata* is used to make ropes, mats, fishing nets, fishing lines, sacks as well as clothings. The tree provides food, shelter and material for hunting and fishing (Venter and Venter, 1996). Literature review showed the just only work published on micropropagation of *Adansonia digitata* was by Katsuaki and Sie (2007) where four different basal media were used for its regeneration. The four basal media were half strength of Quoirin and Lepoivre (LP), half strength of Murashige and skoog (MS), half strength of Gamborg's (B5) and half strength of Woody Plant Medium. These four basal media were used at different stages of propagation of the plant. In any case, this study is the first in which a single basal medium was used to complete the different stages of the propagation.

Considering the above mentioned facts, this study was conducted to standardize the *in-vitro* protocol for regeneration of *Adansonia digitata*. This was done to evaluate a reliable and prolific shoot multiplication procedure using mature embryo explants as starting material. Moreoso, this work aimed at evaluating and comparing the effect (s) of

cytokinin, auxin and coconut water on *in-vitro* propagated plantlets.

Materials and Methods

Matured fruits of *Adansonia digitata* were collected from fruiting stand in the nursery located at the Department of Botany, University of Ibadan, Ibadan. Seeds were collected from dry fruits by cracking the fruit open and washing away the dry, powdery coating. The dark brown to black, kidney-shaped seeds were soaked in a container of hot water and allowed to cool after soaking for 24 hrs. The seeds were sterilized by immersing in 70% ethanol for 5 minutes, decanted off and added 10 % v/v Sodium hypochlorite with 2 drops of between 20 for 20 minutes, also decanted off and added 5 % v/v Sodium hypochlorite with 2 drops of between 20 for 10 minutes. The embryo was excised out and inoculated on prepared media.

The experiment was conducted at the Biotechnology laboratory of the National Centre for Genetic Resources and Biotechnology (NACGRAB) Moor plantation, Ibadan, Oyo state.

Media for Micropropagation

MS (Murashige & Skoog, 1962) and Woody Plant Medium (Lloyd and McCown, 1981) media supplemented with different concentrations and combinations of growth regulator and additive (coconut water) were prepared as media for micropropagation. For embryo regeneration, embryos were cultured on MS and WPM basal media supplemented along with various concentrations of cytokinins (BAP and KIN) ranging from 0.01 - 0.05 mgl^{-1} and 0.01 - 0.5 mgl^{-1} respectively, with 0.01mgl^{-1} of auxin (NAA). For shoot proliferation, nodal explants were cultured on MS basal medium supplemented with various concentrations of cytokinin (BAP and KIN), auxin (NAA) and additives (coconut water). The BAP concentrations used were 0.02 and 0.1 mgl^{-1} while the KIN concentrations used were 0.02 - 0.03 mlg^{-1} and 0.1 mlg^{-1} with 0.01 and 0.02 mlg^{-1} NAA and 0.01 mgl^{-1} coconut water. The media were dissolved in oven and dispensed into clean test tubes (5ml each), covered and sterilized at 121ºC of a pressure of 15 psi for 15 minutes. Each treatment consisted of 10 test tubes. A period of 7-12 weeks was set aside for shoot proliferation.

Transfer to the soil

Rooted plantlets of subculture nodal explants were taken out from culture tubes and washed thoroughly. The washed plantlets were planted in sterilized soil in small perforated polythene bags of about 5 to 7cm long and 3 to 5cm wide arranged in tray and placed in a transparent perforated polythene bag. After 7 days the plantlets were taken from the perforated polythene bags to screen house for hardening.

Data collection and statistical analysis.

Weekly Visual observation of culture was made and frequency of culture showing plantlet, shoot and root formation and multiplication was recorded. Data was subjected to analysis of variance (ANOVA) and mean separation were also carried out using the Duncan multiple range test. The level of significance was determined at 5%.

Results and Discussion

An efficient and reliable system for *in-vitro* propagation of *Adansonia digitata* has been optimized. Two basal salt mixtures, Woody Plant Media (Lloyd & McCown, 1980) and MS Media (Murashige & Skoog, 1962) were used initially to study the effect of basal media on embryo regeneration. In the light of the study, MS medium proved to be better than WPM medium since they showed highest number of nodes, maximum length of shoots and roots with or without growth regulators (Tables 1 and 2). The woody plant medium was basically designed to overcome the chloride ion susceptibility of the woody plants (George, 1993).

The results on regeneration showed that excised embryo of *Adansonia digitata* culture on treatment composition of 0.02mg/l BAP and 0.03mg/l Kin promoted more nodal growth than the control and other ranges of cytokinin. Treatments containing 0.04mg/l BAP gave the highest shoot length while treatments with 0.03mg/l Kin gave the highest root length as shown in Table 1. The different concentrations of BAP and KIN used proved successful for the germination of embryos in *Adansonia digitata* and this is in accordance with Sebastian *et al.* (2005) who reported a satisfactory germination of embryos of *Phyllanthus emblica* L. and *Hevea brasiliensis* on the same medium. Benzyl amino purine (BAP) in combination with NAA gave the optimal result in embryo culture of *Adansonia digitata.* Though MS without growth regulator regenerated the culture embryo of *Adansonia digitata, it was* however observed that KIN encourage root formation.

Table 1: Effect of BAP, KIN and 0.01mg/l NAA on the growth of the embryo of *Adansonia digitata* on MS medium after 3 weeks

Code	Concentration (mg/l)	Number of nodes	Shoot length (cm)	Root length (cm)
A	0.00	3.33 ± 0.33^{a}	3.87 ± 0.54^{a}	6.23 ± 2.38^{a}
B1	0.01 BAP	3.00 ± 0.00^{a}	6.20 ± 1.60^{a}	14.50 ± 2.00^{b}
B2	0.02,,	4.00 ± 0.00^{a}	5.67 ± 1.28^{a}	11.23 ± 2.41^{b}
B3	0.03,,	3.67 ± 0.33^{a}	5.57 ± 0.92^{a}	6.43 ± 3.48^{a}
B4	0.04,,	3.67 ± 0.88^{a}	8.07 ± 2.53^{a}	14.07 ± 2.70^{b}
B5	0.05,,	3.00 ± 0.58^{a}	5.07 ± 0.27^{a}	8.33 ± 0.90^{a}
K1	0.01 KIN	3.67 ± 0.33^{a}	7.03 ± 1.24^{a}	13.63 ± 1.73^{b}
K2	0.02,,	3.00 ± 0.00^{a}	4.87 ± 1.37^{a}	5.90 ± 4.31^{a}
K3	0.03,,	4.00 ± 0.00^{a}	6.00 ± 0.70^{a}	14.63 ± 2.82^{b}
K4	0.04,,	2.67 ± 0.33^{a}	5.37 ± 1.32^{a}	4.93 ± 2.66^{a}
K5	0.05,,	3.33 ± 0.88^{a}	6.07 ± 1.65^{a}	10.03 ± 3.41^{b}

Values are mean of three determinations. Data with the same letter along each column are not significantly different from each other according to Duncan's multiple range test ($p < 0.05$).

Key

A: Control Experiment
B: 6- Benzyl-amino purine (BAP)
K: 6- Furfuryl amino purine (KIN)
NAA: Naphthalene acetic acid
MS: Murashige and Skoog media (1962)

Table 2: Effect of BAP, KIN and 0.01mg/l NAA on the growth of the embryo of *Adansonia digitata* on WPM after 3 weeks

Code	Concentration (mg/l)	Number of nodes	Shoot length (cm)	Root length (cm)
A	0.00	3.00 ± 0.00^{a}	4.50 ± 0.25^{a}	8.07 ± 1.21^{a}
B1	0.01 BAP	3.67 ± 0.33^{a}	5.70 ± 0.78^{a}	11.13 ± 0.68^{b}
B2	0.02,,	3.33 ± 0.33^{a}	6.90 ± 0.86^{a}	13.33 ± 0.68^{b}
B3	0.03,,	3.00 ± 0.00^{a}	4.93 ± 0.56^{a}	9.80 ± 1.83^{a}
B4	0.04,,	3.67 ± 1.20^{a}	4.37 ± 0.43^{a}	7.37 ± 0.70^{a}
B5	0.05,,	4.00 ± 0.00^{a}	5.30 ± 0.51^{a}	10.53 ± 1.45^{b}
K1	0.01 KIN	3.33 ± 0.33^{a}	4.97 ± 0.46^{a}	7.90 ± 0.65^{a}
K2	0.02,,	2.00 ± 0.00^{a}	4.13 ± 4.13^{a}	5.90 ± 1.46^{a}
K3	0.03,,	2.67 ± 0.33^{a}	5.33 ± 1.05^{a}	8.20 ± 1.86^{a}
K4	0.04,,	3.67 ± 0.33^{a}	6.10 ± 0.31^{a}	11.23 ± 0.83^{b}
K5	0.05,,	3.33 ± 0.33^{a}	5.97 ± 1.23^{a}	7.60 ± 2.43^{a}

Values are mean of three determinations. Data with the same letter along each column are not significantly different from each other according to Duncan's multiple range test ($p < 0.05$).

Key

A: Control Experiment
B: 6- Benzyl-amino purine (BAP)
K: 6- Furfuryl amino purine (KIN)
NAA: Naphthalene acetic acid
WPM: Woody Plant Medium

Table 3: The growth of sub-cultured micro shoots of *Adansonia digitata*

Code	Concentration (mg/l)	Number of nodes	Shoot length (cm)	Root length (cm)
A	0.00	3.33 ± 1.86[a]	0.93 ± 0.74[a]	--
B	0.02 KIN + 0.01 NAA	2.33 ± 0.33[a]	0.57 ± 0.13[a]	--
C	0.02 KIN + 0.02 NAA	3.00 ± 1.00[a]	0.73 ± 034[a]	1.87 ± 1.87[a]
D	0.03 KIN + 0.02 NAA	1.33 ± 0.33[a]	0.20 ± 0.10[a]	3.47 ± 1.87[a]
E	0.02 KIN + 0.01 IAA	1.33 ± 0.33[a]	0.13 ± 0.03[a]	--
F	0.02 KIN + 0.02 IAA	2.67 ± 1.20[a]	0.93 ± 0.74[a]	1.53 ± 1.53[a]
G	0.01 Coconut	0.67 ± 0.07[a]	0.40 ± 0.00[a]	1.60 ± 0.60[a]
H	0.02 BAP + 0.01 NAA	2.67 ± 1.20[a]	0.60 ± 0.45[a]	2.13 ± 2.13[a]
I	0.02BAP+0.01NAA+C.NUT	0.67 ± 0.33[a]	0.10 ± 0.06[a]	--
J	0.1 BAP	1.33 ± 1.33[a]	1.10 ± 0.00[a]	1.53 ± 1.53[a]
K	0.1 KIN	0.67 ± 0.07[a]	0.20 ± 0.00[a]	--

Values are mean of three determinations. Data with the same letter along each column are not significantly different from each other according to Duncan's multiple range test ($p < 0.05$).

Key

A: Control Experiment
B-F: MS + KIN + NAA
G: MS + Coconut water
H: MS + BAP + NAA
I: MS + BAP + NAA + Coconut water
J: MS + BAP
K: MS + KIN
KIN: 6- Furfuryl amino purine
NAA: Naphthalene acetic acid
BAP: 6- Benzyl-amino purine
MS: Murashige and Skoog media

Shoot multiplication was achieved through plantlet generated from the embryo of *Adansonia digitata*. Nodes excised from the regenerated plantlets were cultured on MS basal medium supplemented with various concentrations of cytokinin (BAP and KIN), auxin (NAA) and additives (coconut water). The result showed that micro shoots (single nodes) excised from *in vitro* grown seedlings of *Adansonia digitata* sub-cultured, induced shoot regeneration from the axillary buds which is in accordance with McCartan and Crouch (1998), who developed a micro propagation protocol for *Mondia whitei using* single-node explants from *in vitro* grown seedlings. Shoot regeneration was obtained on all the concentrations of cytokinins (BAP and KIN) and coconut water used in combination with a different auxin (NAA and IAA) as shown in Table 3.

The optimal growth of the micro shoots was on MS only, which gave the optimal shooting, number of nodes without root initiation. BAP gave the best result on shoot length while the optimum root length was obtained from Kin with increased NAA. This disagrees with the report of Tetyana and van Staden (2001) on the *in vitro* culture of *Cussonia paniculata* that showed the best growth regulator for shoot initiation and number of shoots with kinetin. It is important to note from Table 3 that low concentrations of auxin (NAA and IAA) could not initiate root formation. Coconut water also initiated shooting and rooting which is in conformity with several reports on the beneficial effects of coconut water for micro-propagation (Brain and Richard, 1993; Sajina *et al*., 1997; Wondyifraw and Surawit, 2004). Plantlets with well developed root systems were transferred for acclimatization (Plates 1 & 2).

A

B

Plate 1: (A) Showing *in vitro* regenerated plantlets of *Adansonia digitata* in MS at 3 weeks
(B) Showing *in vitro* regenerated *Adansonia digitata* plantlet in WPM at 3 weeks

A

B

Plate 2 (A) Showing subcultured micro shoots of *Adansonia digitata*
(B) Showing acclamatized plantlet of *Adansonia digitata*

Conclusion

On the basis of this experiment, mature embryo is a good starting material for micro propagation of *Adansonia digitata.* In the light of the findings, it is established that enhanced shoots and buds formation can be achieved by using MS and WPM for the embryo growth. It was however observed that MS performed better than woody plant media and thus recommends that MS basal medium should be used as it gave a better result. Though for shoot multiplication in *A. digitata*, MS in combination with cytokinins, auxins and additives (coconut water) is greatly advantageous. The protocol reported in this study could be used for the conservation of this valuable medicinal and economic tree plant.

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References

1. Abdalla AA, Mohammed MA, Mudawi, HA. Producton and quality assessment of instant Baobab (Adansonia digitata L.) *Advanced*

Journal of Food Science and Technology 2010. 2 (2): 125-133.
2. Becker B. The contribution of wild plants to human nutrition in the Ferlo (Northern Senegal). *Agrofor. Syst*. 1983 1: 252–267.
3. Brain KW, Richard AC. Clonal propagation of pink ginger *in vitro*. *Hort. Science* 1993. 28:1203.
4. Duncan B. Multiple range and Multiple F test. *Biometrics* 1955.11; 1- 42.
5. Gebauer J, El-Siddg K, Ebert G. Boabab (Adansonia digitata L.): A review on a multipurpose tree with promising future in the Sudan. Gartenbauwssenschaft, 2002. 67 (4) 155 - 160.
6. George EF. Plant propagation by tissue Culture. Part 1, The Technology, Exegenetics Limited, Edington, England. 1993. Vol.1.
7. Guèye EE. Ethnoveterinary medicine against poultry diseases in African villages. *World's Poultry Sci. J*. 1999. 55: 187-198.
8. Ishii K, Kambou S. *In vitro* culture of an African multipurpose tree species: A*dansonia digitata. Propagation of ornamental plants* 2007. **7**.2: 62-67.
9. Lloyd G, McCown B. Commercially-feasible micropropagation of Mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. *International Plant Propagators' Society Proceeding,* 1981. 30: 421-427.
10. McCartan SA, Crouch, NR. *In vitro* culture of *Mondia whitei (*Periplocaceae), a threatened Zulu medicinal plant. *South Afr. J. Bot*. 1998. 64.5: 313-314.
11. Murashige T, Skoog FA. Revised medium for a rapid growth and bio assays With tobacco tissues cultures. *Physiologia plantarum* 1962. 5: 473-497.
12. Rashford J. Africa's Baobab tree: Why monkey names? *J. Ethnob*. 1994. 14: 173–183.
13. Sajina A, Mini PM, John CZ, Babu KN, Ravindran PN, Peter KV. Micropropagation of large cardamom (Amomum subulatum Roxb.). *J. Spices Aromatic Crops* 1997.6:145–148.
14. Sebastian R, Karamthanan PV, Mathew MM. Somatic embryogenesis and plant regeneration in *Phyllanthus emblica* L. *Plant Cell Biotechnology and Molecular Biology*. 2005.6(3/4):155-58.
15. Tetyana P, Van Staden J. Micropropagation of *Cussonia paniculata*- a medicinal plant with horticultural potentials. *South Afr. J. Bot*. 2001. 67.2: 367-370.
16. United Nations. Market Brief in the European Union for Selected natural ingredients derived from native species *Adansonia digitata L.* (Baobab). 2005. [http://www.unctad.org/biotrade/docs/biotradebriefbaobab.pdf].
17. Venter F, Venter JA. Marking the Most of Indigenous Trees. Briza Publications, Singapore, 1996. ISBN 1875093052.
18. Wondyifraw T, Surawit W. Micropropagation of Krawan (Amomum krervanh Pierre ex Gagnep). *Sci. Asia* 2004. 30: 9 - 150.
19. Wynn SG, Fougère B. Veterinary herbal medicine. Elservier Health Services. 2006. 23pp [http://books.google.co.ke/books?id=iLbZDzumqt0C & pg=PA23 & vq=Adansonia digitata & source=gbs_sea].

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