

11

SHOOT REGENERATION FROM NODAL SEGMENT OF ACACIA SENEGAL IN BORNO STATE OF NIGERIA

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ABSTRACT

Purpose: Acacia senegal is a valuable leguminous tree species of the Sudano/Sahelian region sought after for its economic and ecological importance. Developing *in vitro* propagation protocol for this tree in Borno State of Nigeria will provide a sustainable means of re-foresting and improving the nutrients of the degraded soil of the Sahel environment of Nigeria.

Design/Methodology/Approach: Nodal segments derived from 6-month old seedlings growing on the experimental farm of the Biotechnology Centre, University of Maiduguri, were cultured on Murashige and Skoog (MS) medium, supplemented with 0.025–1.5mg/l of 6-benzylaminopurine (BAP), alone and in combination with 0.02mg/l α-naphthalene acetic acid (NAA) and 0.2–1.6mg/l of kinetin (KN), alone or in combination with 0.2mg/l α-naphthalene acetic acid (NAA).

Findings/Results: The maximum number of shoots per explants (2.31 \pm 1.24) and longest shoots (2.59 \pm 1.38cm) were obtained from MS medium supplemented with 1.0mg/l and 1.5mg/l BAP respectively, while 1.16 \pm 0.71 shoots with maximum 2.34 \pm 1.35cm length were found in medium

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containing 1.2mg/l kinetin after four weeks of culture. Inclusion of NAA (0.02mg/1) with BAP at the various concentrations in the culture medium was not effective in enhancing shoot proliferation. However, obtained results indicate that both shoot number (1.70 \pm 0.95cm) and length (2.33 \pm 0.92cm) were enhanced by adding 0.2mg/l NAA to 0.4mg/KN.

Originality and value: This work would be the first attempt to propagate Acacia senegal by in-vitro method in Borno State of Nigeria, with the view of generating reproducible protocol for future mass propagation of the tree crop in the State.

Keywords: Acacia senegal; in vitro micro-propagation; gum arabic; induction; Murashige and Skoog (MS); Kinetin (KN); 6-Benzylaminopurine (BAP); α-naphthalene acetic acid (NAA); indole-3-acetic acid (IAA); indole-3-butyric acid (IBA)

INTRODUCTION

The plant *Acacia senegal*, commonly known as Hashab (Hausa), Kolkol (Kanuri) is a widespread leguminous tree of the Sudano-Sahel zone of Africa from Senegal to Sudan (Raddad, 2006). It is a medium height tree of about 5m and survives in the most adverse conditions - hot wind, sand storm, poorest soils and sand, in slightly acid to moderately alkaline soils. It grows naturally in areas of 200–800mm rainfall with 7–11 dry months per year and requires free drainage. The tree crop does not tolerate water logging.

Over 300 species of the *Acacia* family exist (FAO, 1995), but more attention has been given to *Acacia senegal* because of its significant contribution towards environmental protection and economic development in the Sudano-Sahelian region.

Sudan is known to be the major producer of gum arabic in the world (Beshai, 1984), Nigeria being the second largest producer, covering an area of about 250,000sq km in the Sahel region of the country (Plate 1). Nigeria has three grades of gum arabic produced by *Acacia senegal* (grade 1), *Acacia seyal* (grade 2), and *Acacia combretum* (grade 3).

Being a drought resistant tree, *Acacia senegal* helps to ameliorate the Sahelian ecosystem, which is losing about 350,000m² of land mass to desert conditions, with increased movement southward at a rate of 0.6km per annum (Gadzama, 1995). It is planted for sand dune fixation, wind erosion control, nitrogen fixation, and to provide vegetative cover for the degraded soils in the Sahel of Africa.

Gum arabic has huge foreign exchange potential. In 2008 alone, Nigeria exported a total of 20,000 metric tonnes of gum Arabic, estimated at US\$43.55m (N6.5325 billion) (Commodity Network Ltd, 2008).

Beverage and Pharmaceutics require as polyvalent additive, protective colloid, oxidative inhibitor and emulsifier, and as a food adhesive agent. The food industry

utilises 60–75% of world production of gum arabic as stabilising, encapsulating agents and other purposes.

Because of the importance of this tree crop, the Federal Government of Nigeria has encouraged R&D that would enhance improved livelihoods of the rural communities in gum arabic sustainable businesses.

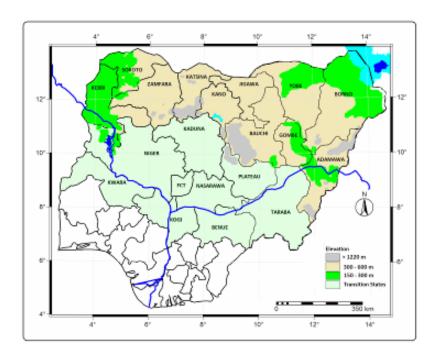


Plate 1 Nigeria's Eleven Sahelian States with A. Senegal Distributions; Best Gum Arabic Produced in Borno, Yobe and Jigawa

Source: Gadzama and Ayuba (2014)

MATERIALS AND METHODS

Establishment of Aseptic Seedlings

Seeds of *A. senegal* were acquired from a gum arabic tree plantation grown in Gubio Local Government Area of Borno State, Nigeria. Seedlings were raised on the experimental site of the Biotechnology Centre, University of Maiduguri.

Nodal segment explants with one axillary bud were excised from 6-month old seedlings growing in the nursery. The explants were washed under running tap water for 30 minutes to remove surface dust, and then soaked in a solution mixture of 100mg/l ascorbic acid and 150mg/l citric acid for 10 minutes. The explants were

then immersed in 70% ethanol for 30 seconds, washed by several changes of sterilised distilled water; they were then immersed in 100ml Clorox solution of 10% and 15% mixed with two drops of Tween 20 (detergent) for 10 minutes each, with continuous shaking. Explants were rinsed several times with sterile distilled water under laminar airflow cabinet. Sterilised explants were cultured in culture bottles containing Murashige and Skoog (MS) (1962) basal medium.

Shoot Proliferation

MS medium was supplemented with various concentrations of 6-benzylaminopurine (BAP) at 0.025-1.5mg/l, alone and in combination with α -naphthalene acetic acid (NAA) (0.02mg/l) or kinetin (KN) (0.2-1.6mg/l), alone and in combination with NAA at 0.2mg/l for shoot induction and proliferation.

Culture Media and Conditions for in vitro Growth

Murashige and Skoog (1962) basal media, cited above, was used as basal culture medium for shoot induction, supplemented with 3% sucrose, solidified by 0.7% agar and adjusted to pH5.7 \pm 1 by drops of 1 N NaOH or 1 HCl, then dispensed in 20ml into culture vessels and sterilised by autoclaving at 121 $^{\circ}$ C and 1.06 bars for 15 minutes. All cultures were incubated in the culture room at 25 $^{\circ}$ C \pm 1 and under photoperiod of 16/8 light and dark hours daily with exposure to 1,000 lux, provided by LED lamps.

STATISTICAL ANALYSIS

For the above experiments, 10 explants were used for each treatment and each experiment was repeated thrice. In the shoot proliferation experiment, shoots parameters per explants were determined after four weeks of culture. The results are expressed as mean \pm S.D. of the three experiments. The data were analysed statistically using Statistic 9.5 and the significance of differences among mean values was carried out using Duncan's Multiple Range Test (DMRT) and paired sample T-test at p<0.05.

RESULTS/FINDINGS

Shoot Multiplication

MS medium was supplemented with various concentrations of cytokinin BAP (0.025, 0.05, 0.5, 1.0 and 1.5) mg/l, alone and in combination with 0.02 mg/l NAA; KN (0.2, 0.4, 0.8, 1.2 and 1.6) mg/l, alone and in combination with 0.2 mg/l NAA.

Results obtained after four weeks of culture indicate the superiority of BAP over kinetin in initiating shoot bud formation (Tables 1 and 3). The addition of NAA to BAP does not seem to be essential in promoting shoot proliferation in the nodal explants of *Acacia senegal*.

Effect of BAP and KN on Shoot Proliferation

Several workers have used various explants and culture media to introduce tissue culture methods for the regeneration of *Acacia senegal*. Among the various explants, the nodal explants are most commonly used (Khalisi and Al-Joboury, 2012). Nodal explants showed their first response by enlarging and bursting within two weeks of culture (Figure 1A). New shoot development was observed within three weeks of culture, and more shoots were found to develop during subcultures (Figures 1B, 1C, 1D, 1E and 1F).

Cotyledonary nodal explants cultured on MS medium devoid of growth regulator produced shoots that may be due to the presence of endogenous cytokinin in the nodal segments (Rajeswari and Paliwal, 2008). However, the addition of exogenous cytokinin to MS medium induced a shoot multiplication rate indicating the requirement of exogenous cytokinin supply in the medium for better axillary shoot proliferation. Out of the two different cytokinins used in this experiment, BAP and KN, the best shoot proliferation was observed on medium containing BAP, with the highest number of shoots (2.31 \pm 1.24) and longest shoots (2.59 \pm 1.38cm) formed on 1.0mg/l and 1.5mg/l BAP respectively (Table 1; Figures 1B and 1C), while 1.16 \pm 0.71 shoots with maximum 2.34 \pm 1.35cm length were found in medium containing 1.2mg/l KN (Table 3; Figure 1D) after four weeks of culture.

Table 1 Effect of Various Concentrations of BAP on Shoot Induction from Nodal Segments of Acacia senegal on MS Medium after Four Weeks Culture Period

Hormone Concentrations BAP (mg/l)	Response (%)	Average No. of shoots	Average Length of shoots (cm)	Mean no. of leaves	No. of nodes
Control	70	0.70 ± 0.48bc	0.33 ± 0.18 ^c	0.70 ± 0.42°	0.70 ± 0.63°
0.025	80	1.10 ± 0.74^{abc}	0.83 ± 0.51^{bc}	1.00 ± 0.67^{bc}	0.90 ± 0.57^{bc}
0.05	70	0.70 ± 0.67^c	1.60 ± 1.41^{abc}	1.55 ± 1.38^{abc}	1.40 ± 1.26^{abc}
0.50	80	1.58 ± 0.93abc	1.70 ± 0.92^{abc}	1.62 ± 0.95^{abc}	1.40 ± 0.84^{abc}
1.00	80	2.31 ± 1.24^{a}	2.19 ± 1.26^{ab}	2.41 ± 1.37^{ab}	2.00 ± 1.15^{ab}
1.50	80	2.16 ± 1.32ab	2.59 ± 1.38^a	2.50 ± 1.43^{a}	2.20 ± 1.32^a

Note: Different letters in each column show significant differences (p<0.05)

Source: devised by authors

Table 2 Effect of Various Concentrations of BAP and NAA on Shoot Induction from Nodal Segments of *Acacia senegal* on MS Medium after Four Weeks Culture Period

Hormone Concentrations BA + NAA(mg/l)	Response (%)	Average No. of shoots	Average Length of shoots (cm)	Mean no. of leaves	No. of nodes
Control	70	0.70 ± 0.48^{a}	0.68 ± 0.53^{b}	1.07 ± 0.98^a	1.00 ± 0.94 ^a
0.025 + 0.02	60	0.60 ± 0.52^a	$0.35\pm0.31^{\text{b}}$	1.20 ± 1.13^a	0.90 ± 0.99^a
0.05 + 0.02	70	0.80 ± 0.63^a	1.71 ± 1.24^a	1.60 ± 1.35^a	1.50 ± 1.27^a
0.50 + 0.02	70	0.70 ± 0.48^a	0.35 ± 0.37^{b}	0.80 ± 0.63^a	$0.80\pm0.63^{\text{a}}$
1.00 + 0.02	50	0.50 ± 0.53^a	0.59 ± 0.64^b	0.60 ± 0.69^a	0.50 ± 0.53^a
1.50 + 0.02	60	0.60 ± 0.52^a	0.54 ± 0.48^{b}	0.70 ± 0.67^{a}	0.60 ± 0.52^{a}

Note: Different letters in each column show significant differences (p<0.05)

Source: devised by authors

Table 3 Effect of Various Concentrations of KN on Shoot Induction from Nodal Segments of Acacia senegal on MS Medium after Four weeks Culture Period

Hormone Concentrations KN (mg/l)	Response (%)	Average No. of shoots	Average Length of shoots (cm)	Mean no. of leaves	No. of nodes
Control	80	0.80 ± 0.42^{a}	1.09. ± 0.62bc	1.09 ± 0.70 ^b	0.80 ± 0.42 ^b
0.20	90	0.90 ± 0.32^a	0.82 ± 0.42^{bc}	$0.90\pm0.32^{\scriptscriptstyle b}$	$0.90\pm0.32^{\scriptscriptstyle b}$
0.40	60	$0.60\pm0.52^{\scriptscriptstyle a}$	0.18 ± 0.19^{bc}	1.02 ± 0.97^{b}	1.00 ± 0.94^{b}
0.80	50	$0.50\pm0.52^{\text{a}}$	0.14 ± 0.17^{c}	0.80 ± 0.92^{b}	$0.50\pm0.52^{\scriptscriptstyle b}$
1.20	80	1.16 ± 0.71^a	2.34 ± 1.35^a	2.80 ± 1.75^a	2.60 ± 1.71^a
1.60	70	$0.70\pm0.48^{\mathrm{a}}$	1.13 ± 0.86 ^b	1.60 ± 1.17^{ab}	1.20 ± 0.92 ^b

Note: Different letters in each column show significant differences (p<0.05)

Source: devised by authors

Table 4 Effect of Various Concentrations of KN and NAA on Shoot Induction from Nodal Segments of Acacia senegal on MS Medium after Four weeks Culture Period

Hormone concentrations KN + NAA (mg/l)	Response (%)	Average No. of shoots	Average Length of shoots (cm)	Mean no. of leaves	No. of nodes
Control	70	$0.70\pm0.48^{\scriptscriptstyle b}$	0.42 ± 0.38^c	$1.00\pm0.82^{\scriptscriptstyle b}$	$0.80\pm0.62^{\scriptscriptstyle b}$
0.20 + 0.20	70	0.90 ± 0.74^{ab}	1.42 ± 1.03^{ab}	1.82 ± 1.50^{ab}	1.60 ± 1.43^{ab}
0.40 + 0.20	90	1.70 ± 0.95^a	2.33 ± 0.92^a	2.32 ± 1.05^a	2.10 ± 0.99^a
0.80 + 0.20	80	$0.80\pm0.42^{\scriptscriptstyle b}$	0.60 ± 0.39^{bc}	$1.30+0.82^{ab}$	1.10 ± 0.74^{ab}
1.20 + 0.20	90	0.90 ± 0.32^{ab}	0.38 ± 0.28^c	1.50 ± 0.71^{ab}	1.50 ± 0.71^{ab}
1.60 + 0.20	70	0.85 ± 0.67^{b}	1.13 ± 0.81bc	1.40 ± 1.08ab	1.20 ± 1.03^{ab}

Note: Different letters in each column show significant differences (p<0.05)

Source: devised by authors

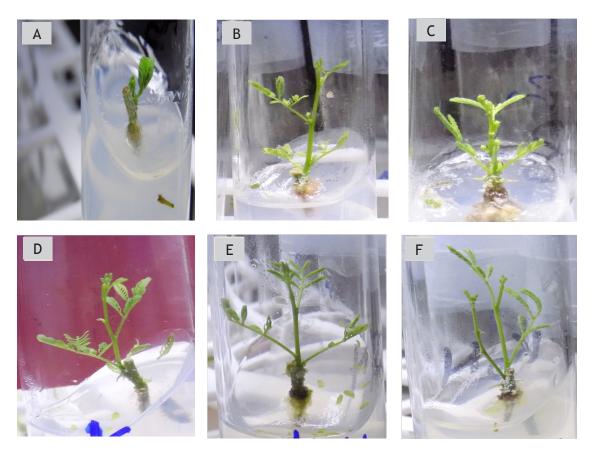


Figure 1 In vitro propagation of A. senegal Willd. from nodal explants. A. Two-week old in vitro shoot on MS basal medium; B. Multiple shoot formation from nodal explants after four weeks of culture on MS + 1.0mg/l BAP; C. In vitro proliferated shoots from nodal explants after four weeks of culture on MS + 1.5mg/l BAP; D. In vitro shoot on MS + 1.2mg/l kinetin; E. in vitro shoot on MS + 0.2mg/l kinetin and 0.2mg/l NAA; F. In vitro shoot on MS + 0.4mg/l kinetin and 0.2mg/l NAA

Source: produced by authors

DISCUSSION AND CONCLUSIONS

The results indicated that BAP was superior to KN in enhancing shoot proliferation. BAP has been the most popular and widely used cytokinin for stimulating shoot multiplication in a broad range of species (Gaspar et al., 1996). The superiority of BAP over KN has also been reported in *in vitro* propagation of other species of *Acacia* (Shahinozzaman et al., 2013; Badji et al., 1993; Beck et al., 1998; Dewan et al., 1992; Galiana et al., 1991; Junior et al., 2004; Khalafalla and Daffalla, 2008; Mittal et al., 1989; Nandwani, 1995; Rout et al., 2008; Singh et al., 1993; Vengadesan et al., 2002). The proliferation of shoots in nodal explants of *Acacia senegal* at a concentration of 1.0mg/l BAP was also reported by Khalaffalla and Daffalla (2008). Khalisi and Al-Joboury (2012), obtained similar results with *Acacia farnesiana*.

This study revealed that the addition of exogenous auxin (NAA) to cytokinin (BAP) was not essential to initiate shoot bud formation (Table 2). The results might indicate the antagonistic effect of NAA with BAP in *in vitro* shoot proliferation of A. senegal. Vengadesan et al. (2002) also observed that auxins (NAA, IBA and IAA), together with BAP, were not effective for shoot proliferation from cotyledonary nodes of Acacia sinuata. In Acacia senegal, similar results were reported by Khalafalla and Daffalla (2008). This finding is also in agreement with the work of Mallikarjuna and Rajendrudu (2009) on Holarrhena antidysenterica, and Hussain et al. (2007) on Sterculiaurens. Garland and Stoltz (1981) demonstrated that in a number of cases, cytokinins alone are enough for optimal shoot multiplication, as also supported by the works of Amin and Jaiswal (1993) and Ndiaye et al. (2006). However, shoots were more elongated in the combination, 0.05 mg/l BAP + 0.02 mg/l NAA (Table 2). This is consistent with the observations of many researchers who suggested that incorporation of low level auxin with BAP promoted shoot induction in different tree species, including Acacia catechu (Kaur et al., 1998), Acacia seyal (Al-Wasel, 2000), Acacia tortilis (Nandwani, 1995), Aegle marmelos (Nayak et al., 2007), Colute aistria (Hegazi and Gabr, 2010), Nyctanthes arbor-tristis (Siddique et al., 2006), Pterocarpus marsupium (Husain et al., 2008) and Terminalia belerica (Rathore et al., 2008).

Obtained results also indicate that both shoot number (1.70 ± 0.95) and length $(2.33 \pm 0.92 \text{cm})$ were enhanced by adding 0.2 mg/l NAA to 0.2 mg/lKN and 0.4 mg/l KN respectively (Table 4; Figures 1E and 1F). This result agrees with the work of Khalisi and Al-Joboury (2012). Similar results were reported in other legume trees, *Acacia mearnsii*, *Albizia odoratissima* and *Acacia nilotica* (Mittal et al., 1989; Jones et al., 1990).

CONCLUSIONS

In conclusion, this is the first report of an *in vitro* propagation of *A. senegal* in Borno State, Nigeria. We were able to induce shoots from nodal explants excised from 6-month old seedlings in a nursery that were successfully germinated from seeds obtained from Gum Arabic plantation in Gubio Local Government Area of the State. Although similar work had been done by others, this protocol is the first using the indigenous tree species. The number and length of shoots were maximum in the culture medium optimised with the combination of 1.0mg/l and 1.5mg/l BAP. However, the combinations of kinetin with NAA resulted in the improvement of shoot number and shoot length. Although BAP showed superiority over kinetin in terms of axillary shoot bud stimulation, kinetin alone (1.2mg/l) significantly increased shoot length. Therefore, for the up-scaling of this work, further experiments are needed to achieve a higher percentage of shots and subsequent root induction (Kaldapa and Gadzama, 2018).

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BIOGRAPHY

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