

Full Length Research Paper

***In vitro* propagation of an endangered medicinal timber species *Khaya grandifoliola* C. Dc.**

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The over exploitation of African mahogany in tropical forest has threatened the genetic base of this useful timber and medicinal tree species and as such, an experiment was conducted on the *in vitro* culture of *Khaya grandifoliola*, an endangered tree species commonly found in the high forest zones of West Africa to explore its potential for micropropagation. Embryos excised from matured seeds were cultured on Murashige and Skoog (MS) medium supplemented with naphthalene acetic acid (NAA), benzyl amino purine (BAP) and kinetin (KIN) at different concentrations. The optimum result in relation to shoot length, root length, number of nodes and number of root was obtained on MS medium supplemented with 1.0 mg/L BAP + 0.01 mg/L NAA.

Key words: *Khaya grandifoliola*, African mahogany, *in vitro* culture, medium, embryo, plantlets.

INTRODUCTION

The sub-Saharan Africa is endowed with tropical forest rich in valuable timber tree species which are also of immense benefit to the local people in meeting both their food and medicinal needs. International Union for Conservation of Nature (IUCN, 2006) red list of threatened species listed *Khaya* as one of the endangered valuable timber species. Gbile (1998) listed it also as one of the important medicinal plants species. *Khaya grandifoliola* is a member of *Meliaceae* and it is an important species native to West Africa. *Khaya* wood, African mahogany as it is commercially called is a high priced wood often used for carpentry, joinery, furniture, cabinetry and decorative veneer. It is also suitable for light construction such as staircase banisters, handrails and domestic flooring because the wood usually dries well and rapidly with a beautiful sheen when polished. The water-resistant nature of the timber makes it a very good material for ship-building. The ever-increasing demand for the prepared decoctions and alcoholic products obtained from

the harvested stem bark of *K. grandifoliola* has actually endangered the plant species in Ghana (Ameyaw and Ampaw, 2005). In Nigeria, the upsurge in ethno-botanical studies and scientific research into the use of plant species has further enhanced the pressure on populations of medicinal forest species as more people now use plants' parts for treating various body ailments. Eloff (1998) reported that two out of three people on earth use plants for primary health care. Iwu (1992) showed that more than 70% of the Nigerian population depends on folk medicine for their health.

Most of the *K. grandifoliola* exists in the wild state; the regeneration and long term conservation of these species are at the mercy of the vagaries of nature and the profit driven herb collectors and timber merchants. Gbadamosi (2002) reported that the 'wild syndrome' and common property status of forest resources is responsible for the near extinct exploitation of *Enantia chlorantha* for malaria treatment in Nigeria. *K. grandifoliola* is commonly multiplied by seeds but the number of plants derived through this technique is limited. *In vitro* culture techniques could represent a very useful tool for mass propagation of superior stock plants as well as genetic improvement. Besides, multiplication can continue throughout the year irrespective of the season. Although plant regeneration from tissues cultured *in vitro* has been accomplished in a range of forest species (Thorpe et.al., 1991), there is no

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Abbreviations: BAP, Benzylaminopurine; KIN, kinetin; NAA, naphthalene acetic acid.

Table 1. Effect of plant growth regulators on shoot length, root length, number of nodes, and number of roots regeneration from embryo culture of *K. grandifoliola*

S/N	Media	Shoot length	Root length	Number of nodes	Number of roots
1	0.125 mg/l (BAP) + 0.01 mg/l (NAA)	4.20	1.53	3.00	1.00
2	0.15 mg/l (BAP) + 0.01 mg/l (NAA)	4.67	1.47	2.00	1.00
3	0.05 mg/l (KIN) + 0.01 mg/l (NAA)	3.7	4.53	2.00	1.00
4	0.075 mg/l (KIN) + 0.01 mg/l (NAA)	7.4	4.20	3.00	1.00
5	0.10 mg/l (KIN) + 0.01 mg/l (NAA)	2.7	4.03	2.00	3.00
6	0.125 mg/l (KIN) + 0.01 mg/l (NAA)	4.92	5.53	3.00	1.00
7	1 mg/l (BAP) + 0.1 mg/l (NAA) + 10 mg/l (adenine sulphate)	5.78	3.50	3.00	3.00
8	1 mg/l (KIN) + 0.01mg/L (NAA) + 10 mg/l (adenine sulphate)	4.82	4.50	4.00	1.00
9	1 mg/l (BAP) + 0.1 mg/l (NAA)	5.87	7.53	3.00	2.00
	LSD	0.14	0.13	0.00	0.00

report on successful *in vitro* plant regeneration of *K. grandifoliola*. Meanwhile, there has been success in *Khaya ivorensis*, another member of the *Meliaceae* (Mathias 1988; Newton et al., 1994). Youssef (1994) reported that cytokinin and auxins affect the proliferation and regeneration rate of tree species *in vitro*. *In vitro* propagation of *Paulownia* spp. from leaves and petioles was reported by Bergmann and Moon (1997). Pranati Nayak et al. (2007) in his work on *Aegle marmelos* (L.) obtained highest regenerative response on medium containing 6.6 μM BA + 1.14 μM IAA.

Considering the fact that this forest tree species seeds are recalcitrant in nature and producing adequate number of seedlings for any meaningful plantation establishment programme from seeds stored for long time is very difficult, this present work aims to describe a reliable plant regeneration protocol from matured seed embryo.

MATERIALS AND METHODS

Seeds of *K. grandifoliola* used were collected from the Genebank of National Center for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria (07°23.048'N 003°50.431'E). Ninety (90) seeds used for this experiment were washed with mild liquid detergent (Tween-20) under running tap water for 10 min. This is followed by surface sterilization in 70% ethanol for 5 min and 0.1% mercuric chloride for 10 min followed by 3 rinses in sterile distilled water. The embryos were carefully excised with ease together with some endosperm attached and then cultured on Murashige and Skoog (1962) basal medium supplemented with 3% w/v sucrose, 0.1 g inositol and gelled with 0.7% w/v agar at various concentration of cytokinins and auxin in a 17 ml test tube. The cytokinins used were benzylaminopurine (BAP) and kinetin (KIN), while naphthalene acetic acid (NAA) was the auxin used. All growth regulators were added before autoclaving and pH was adjusted to 5.7 ± 0.2 and autoclaving at 121 °C for 15 min. The cultures were incubated in a growth room at $26 \pm 2^\circ\text{C}$ under a 16 h photoperiod with cool-white fluorescent light. There were nine treatments, and ten explants were cultured per treatment and later arranged randomly on the shelves in the growth room. After four weeks, the cultures were evaluated for shoot length, root length, number of nodes and number of roots. The data taken were subjected to

statistical analysis using SAS/PC version 9.1 (SAS 1999). The observed means of the characters were subjected to Least Significant Difference (LSD) to show the mean separation. The constituents of the media are shown in Table 1.

RESULTS AND DISCUSSION

Data in Table 1 revealed that different concentrations of the cytokinin BAP and the auxin NAA tested in this study had a significant effect on the regeneration of plantlets. The longest shoot length (7.4 mm) was exhibited for explants cultured on MS-medium supplemented with 0.075 mg/L (Kin) + 0.01 mg/L (NAA) and this value is 3 fold higher than that found for embryo cultured on 0.10 mg/L (Kin) + 0.01 mg/L (NAA) whose average shoot length was 2.7 mm. These results showed that the most adequate culture medium for obtaining the longest average root length (7.53cm) per culture after four weeks was MS-medium supplemented with of BAP at 1.0 mg/L plus NAA at 0.1 mg/L, while the shortest root length (1.47 cm) was exhibited by MS-medium supplemented with 0.15 mg/L (BAP) + 0.01 mg/L (NAA), this indicates that increasing the level of auxin (NAA) increases the length of roots and vice-versa. However, the highest number of nodes (4.0) was observed on plantlets cultured on MS-medium supplemented with 1.0 mg/L (KIN) + 0.01 mg/L (NAA).

Figure 4 shows the effect of different levels of kinetin and BAP on the performance of *K. grandifolia*. The highest shoot length was obtained from medium 4 as shown on the radar chart, while the root length performed best on medium 9. The photographs of the cultures are presented in Figures 1, 2 and 3. The emergence of the radicle was observed a week after culture as presented in Figure 1. The plumule started showing two weeks after culture as in Figure 2, while in Figure 3, there was elongation of the roots and the shoots.

These findings are in agreement with those reported by Bustamante and Heras (1990) on *Cacti* (*Pelecypora aselliformis*) and *Nealolydia lophophoroides*; Feng-Feng



Figure 1. The growth stages of *K. grandifoliola* through embryo culture *in vitro* (a week after culture).

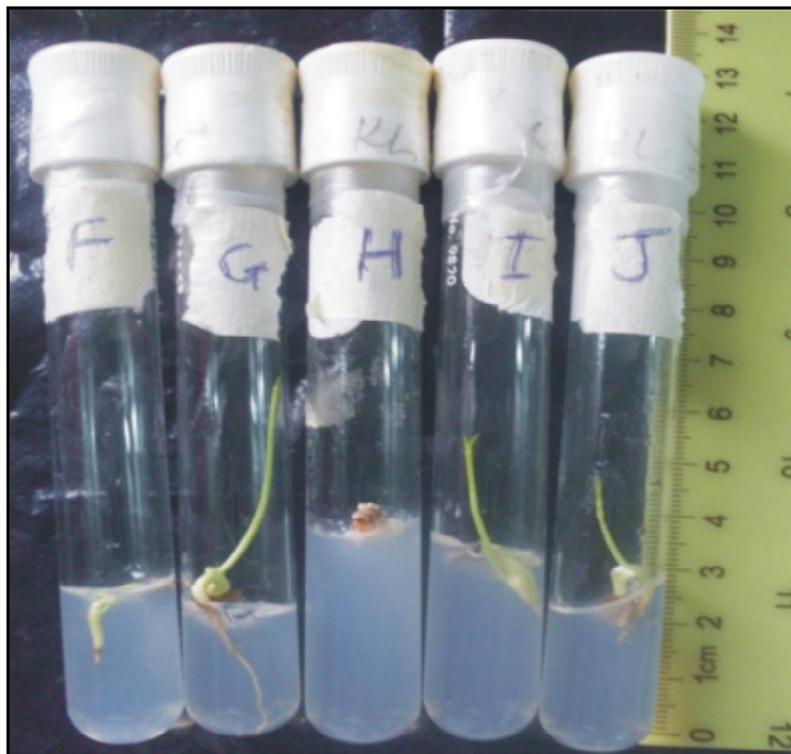


Figure 2. The growth stages of *K. grandifoliola* through embryo culture *in vitro* (two weeks after culture).

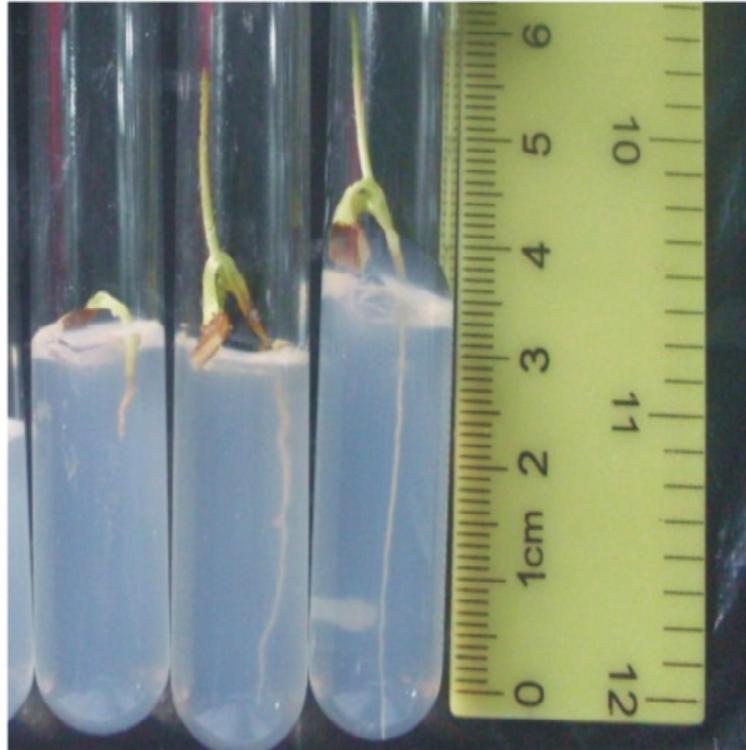


Figure 3. The growth stages of *K. grandifoliola* through embryo culture *in vitro* (four weeks after culture).

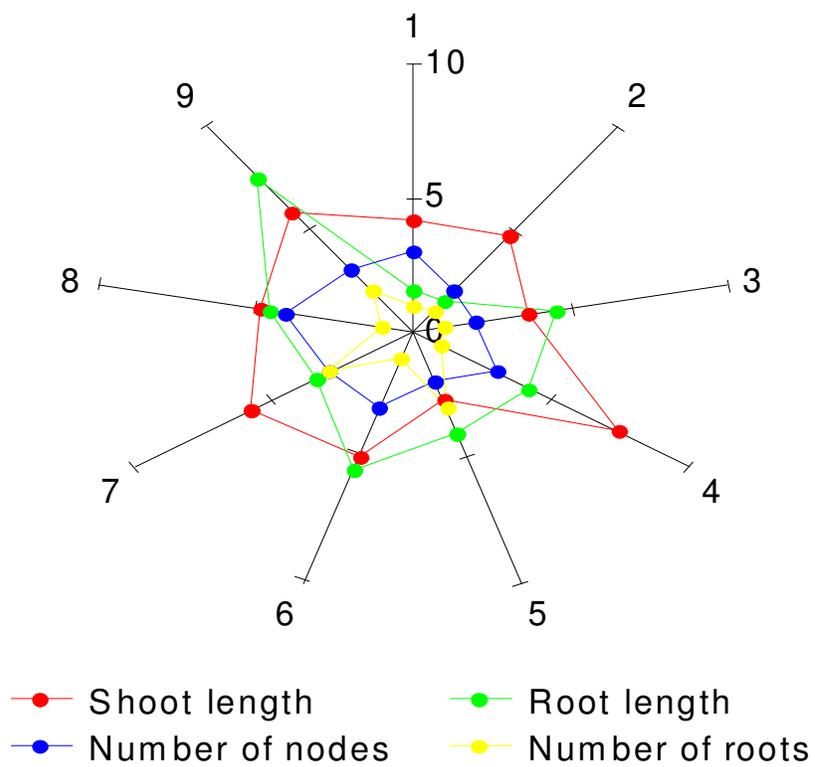


Figure 4. The effect of different levels of kinetin and BAP on the performance of *K. grandifoliola in vitro*

et al. (2000) on *Aloe barbeberis* and Mata-Rosas et al. (2001) on *Turbinicapsus laui* that using a high concentration of BAP and NAA in different concentrations was a limiting factor for shoot formation and increases root formation. The result of this study showed that the optimum medium for regeneration of *K. grandifoliola* is MS-medium supplemented with 1.0 mg/L (BAP) + 0.1 mg/L (NAA) + 10 mg/L adenine sulphate because the values obtained for all the parameter measure was moderately high and optimum. The fact that the number of roots increased to 3 in medium 5 and 7 could be due to the increase in the concentration of NAA to 0.1 mg/L. It has been established that auxins like NAA increases the root formation in the presence of low cytokinins (Youssef, 1994).

Conclusion

The results reported here demonstrate that like *K. ivorensis* (Mathias 1988), *K. grandifoliola* is readily amenable to *in vitro* propagation of the seed embryo and this has set the stage for conservation and micro propagation of the species to meet the sourcing demand for timber in building and construction industries, its medicinal uses in the trado-medical practices and above all to prevent the species from extinction.

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