In vitro Propagation of *Dalbergia melanoxylon* Guill. & Perr.: A Multipurpose Tree

Francis Kiondo[1, 2*], Tileye Feyissa[2], Patrick A. Ndakidemi[2] and Miccah Seth[3]

[1]Tanzania Forest Service (TFS) Agency, P.O. Box 40832, Dar es Salaam, Tanzania
[2]School of Life Science and Bio-Engineering, Nelson Mandela African Institution of Science and Technology, P.O. Box 447, Arusha, Tanzania
[3]Mikocheni Agricultural Research Institute, P.O. Box 6226, Dar es Salaam, Tanzania
*Corresponding Author. Email: kiondof@nm-aist.ac.tz

Abstract

Dalbergia melanoxylon (Guill.&Perr.), commonly known as East African Blackwood, is propagated through seeds, but is not successful because of poor seed germination that limits its multiplication. Therefore, the objective of this study was to develop *in vitro* propagation protocol for this species. *In vitro* propagation of *D. melanoxylon* was achieved by culturing nodal explants collected from mature trees on Murashige and Skoog (MS) medium. The influence of various growth regulators on *in vitro* shoots multiplication of *D. melanoxylon* was investigated. The highest number of shoots per explant (5.55 ± 0.18) was obtained on MS medium supplemented with 2.0 mg/l 6-benzylamino-purine (BAP) in combination with 0.5 mg/l α-Naphthalene acetic acid (NAA). NAA was found to be more effective in producing roots than Indole-3-butyric acid (IBA). The best rooting response (86.67%) with highest mean number of roots (3.88 ± 0.17) was obtained on full strength MS medium containing1.0 mg/l NAA after 6 weeks of culture. Plantlets were hardened and transplanted in pots with 66.67% survival rate. This protocol is useful for rapid propagation and conservation of this economically and ecologically important but threatened tree.

***Key words*:** African Blackwood, *In vitro* propagation, Threatened species

{Citation: Francis Kiondo, Tileye Feyissa, Patrick A. Ndakidemi, Miccah Seth. *In vitro* propagation of *Dalbergia melanoxylon* Guill. & Perr.: a multipurpose tree. American Journal of Research Communication, 2014, 2 (11): 181-194} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Dalbergia melanoxylon Guill. & Perr., commonly known as East African Blackwood also known as African ebony, African ironwood or (Mpingo in Kiswahili) is a small tree rarely exceeding 10 m in height (Lovett, 1987). It is a flowering plant native to the seasonally dry regions of Africa (IUCN, 2008). It belongs to family Fabaceae, sub-family Papilionoideae and is an economically and ecologically important tree with first-class wood. *D. melanoxylon* is the national tree of Tanzania and highly valued for its heartwood which is dense and resistant and therefore seen as vital for the production of top quality clarinets which produces a beautiful tone (Arbonnier, 2004).

Genus *Dalbergia* is an important part of many ecosystems, such as the open Miombo woodland that covers two-thirds of Tanzania. *D. melanoxylon* is a nitrogen fixing species that improves soil fertility and helps maintain soil stability so that could therefore be maintained on farms as this helps the plants to get available nitrogen with other crops (Amri *et al.*, 2009) and known to grow in ecosystems that usually has poor soils (Högberg, 1986). Most of Tanzania's stocks are found in open Miombo woodlands but is able to grow in a wide range of conditions including semi-arid, semi-humid and lowland areas (Nshubemuki, 1994) and has no competition conflict with most agricultural crops. It is often found on dry, rocky sites but is most frequent in the mixed deciduous forests and savannahs of the coastal region (Mbuya *et al*., 1994). While it has a much wider distribution, it is only in East Africa that the tree reaches harvestable size in sufficient abundance (Ball, 2004).

The tree gets its name from the fine grained dark-coloured heartwood that can reach densities of 1.1g cm^{-3} (Malimbwi *et al.*, 2000) which is resistant to insect attack and therefore valued for use in musical instruments, principally woodwinds and particularly clarinets but also wooden flutes, oboes and pipes (Arbonnier, 2004) and locally in Tanzania for making woodcarvings (makonde) for tourists (Jenkins *et al.*, 2002). Apart from having excellent timber, different parts of this

species such as leaves, roots and barks have diverse local medicinal uses in Africa including sacred rituals (Thirakul, 1984, Gundidza and Gaza, 1993, Washa *et al.*, 2012). Leaves provide fodder for the migrating herbivores on the east African savannahs and for domestic livestock. Combination made from its roots has been reported to treat abdominal pain, hernia, gonorrhoea as well as complications from abortions (Bryce, 1967). The bark from its stems and roots are used as anti-diarrhoea while smoke from its burning roots is inhaled to treat bronchitis and headache (Bryce, 1967).

The haphazard exploitation of the species for its timber, simultaneously with low percentage of germination and viability, has resulted in a decrease in size of its natural stand, which ultimately has led to its inclusion in the list of International Union for Conservation of Nature (IUCN) Red List as Lower Risk/near threatened species (Schatz, 2009). *D. melanoxylon* is one of the expensive timber species in the world and thus over exploited from its natural stand (Jenkins *et al.*, 2002).

This species has low natural regeneration which is contributed by its slow growth rate, low seed viability and germination (Mbuya *et al.*, 1994); (Washa *et al.*, 2012). Germination rate of *D. melanoxylon* seeds is less than 30% and thus more than 70% are abortive or damaged by insects which increases with time as seeds are still available for insects (Idrees & Mohammed, 2014), and this might be one of the reasons why this species was not having a good natural regeneration. The species is generally raised from seeds with not only poor germinations but also do not retain their viability for more than one year (Mbuya *et al.*, 1994; Msanga, 1998; Washa *et al.*, 2012; Idrees & Mohammed, 2014). Msanga (1998) and Alkhalifa (2006) made efforts to improve seed germination through various pre-sowing treatments but with little success.

D. melanoxylon is a member of leguminous tree species which are not only known for their recalcitrant nature of regeneration (Jha *et al.*, 2004), but also known that the regeneration rate of leguminous trees in natural habitats is relatively low (Dewan *et al.*, 1992) and due to poor germination and deaths of young seedlings of this species under natural conditions. Propagation through seeds with most leguminous trees is variable (Nanda *et al.*, 2004).

In vitro propagation techniques are feasible alternatives to conventional vegetative propagation methods (Hossain, 1997) and many reports have shown the achievement of *in vitro* propagation in many leguminous trees such as *Bauhinia variegata* and *Parkinsonia aculeata* (Mathur &

Mukunthakumar, 1992), *Acacia meansii* (Correia, & Cortezzi, 1995), *Acacia seyal* (Abdelrahman, 2000), *Albizia odoratissima* (Rajeswari and Paliwal, 2006, Borthakur *et al.*, 2011), *Pterocarpus marsupium* (Husain *et al.*, 2007), *Acacia chundra* (Rout, *et al*., 2008), *Albizzia chinensis (Borthakur et al., 2012) Albizzia falcataria* (Ghosh, *et al.,* 2010), *Acacia nilotica* (Abbas *et al*., 2010), , *Albizia amara* (Indravathi, & Pullaiah, 2013) and *Dalbergia sissoo* (Arya *et al.,* 2013).

There are no reports on *in vitro* propagation of this species, but several woody plants have been successfully regenerated through micropropagation techniques including *Allanblackia stulhmannii* (Neondo *et al.*, 2011), *Pterocarpus santalinus* (Chaturani et al., 2006) and *Hagenia abyssinica* (Feyissa *et al.*, 2005). Therefore, the objective of the present study is to develop *in vitro* propagation protocol for *D. melanoxylon* through nodal culture from mature trees.

Materials and Methods

Plant materials and explants sterilization

Nodal segments with lengths of 7 cm were collected from healthy matured mother trees around the Kitulangalo Forest Reserve located at 06°41'S and 37°57'E which is about 50 km east of Morogoro Municipality towards Dar es Salaam and about 150 km west of Dar es Salaam. Samples were collected in early morning and put in plastic bag immediately after collection, sprayed with water and were transported to the tissue culture laboratory. With precautions not to damage the axillary buds, leaves were removed from the nodal and shoot explants. The explants were thoroughly washed with running tap water for 5 min followed by liquid detergent and distilled water for 5 min. The explants were then disinfected in 70% ethanol for 2 min followed by sterilization with 5% (v/v) sodium hypochlorite containing 20 drops per litre of Tween 20 for 20 min. To remove the remaining sterilant on the explants, the explants were washed three times with sterile distilled water for 18 min. The surface sterilised explants were trimmed at the cut ends to 1-1.5 cm prior to culture on culture medium. MS basal medium was used throughout this study.

Shoot initiation

The nodal explants were cultured on growth regulators-free MS basal medium containing 3% (w/v) sucrose for culture initiation. The pH of the medium was adjusted to 5.8 using 1N solution of NaOH before gelling with 0.4% (w/v) agar (Phytagel^{TM}) and autoclaved at 121°C for 20 min. The explants were cultured on the culture medium in 25 x 150 mm test tubes containing 10 ml semi-solid medium and covered with caps and parafilm. One nodal explant was cultured per test tube. All cultures were incubated in a growth room under photoperiod of 16 h at 50 µmol $m^{-2}s^{-1}$ light intensity provided by cool-white fluorescent tubes at 25±2°C. These conditions were also applied for the shoot multiplication and rooting experiments described below.

Shoot multiplication

After four weeks of shoot initiation on growth regulators-free MS medium, shoots were sub-cultured on MS basal medium supplemented with different concentrations of BAP (0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/l) either alone or in combination with NAA (0.0, 0.25, 0.5, and 1.0 mg/l) or IBA (0.0, 0.25, 0.5, and 1.0 mg/l). Number of shoots and length per explant was recorded after four weeks of culture.

***In vitro* rooting and acclimatization**

Shoots from multiplication medium with three or four expanded leaves were cultured on full-strength MS medium containing various concentrations of NAA (0.0, 0.1, 0.3, 0.5, 0.7, 1.0 mg/l) or IBA (0.0, 0.1, 0.3, 0.5, 0.7, 1.0 mg/l) under aseptic conditions for *in vitro* rooting. Number of rooted microshoots, number of roots per shoot and lengths of the roots were recorded after six weeks of culture. The plantlets were washed carefully in running tap water to remove the traces of agar and some were transferred to pots containing autoclaved forest soil:sand (1:1) and others to soilrite. The pots were covered with transparent polythene bags. After two weeks of planting when new leaves started emerging, the polythene bags were completely removed. Successfully acclimatized plantlets under culture room conditions were then transferred to greenhouse. Initially, the plantlets were irrigated with water that contains inorganic salts (polyfeed fertilizer) as starter for growth and later with water containing finisher fertilizer.

Statistical analysis

Experiments were repeated thrice and data represent the mean of three experiments. In our study, results on the percentage of culture response, number of shoots per explant and rooting was observed at regular intervals. Data collected from three independent experiments were subjected to analysis of variance using STATISTICA program using one-way analysis of variance (ANOVA) to analyse the influence of different treatments where means were compared and reported with ± standard errors (SE). Treatment differences were regarded as significant at $P < 0.05$.

Results

Shoot initiation

Plants of *D. melanoxylon* were efficiently initiated from nodal explants. Once nodal explants from the field-grown matured plants of *D. melanoxylon* were cultured on growth regulators-free MS medium, the emergence of shoot buds was observed at 7-9 days after culture. Bud break was achieved in high percentage in all media (MS, MS + BAP and MS + BAP + NAA) within 4-6 days (Table 1). Maximum percentage of bud break (66.67%) was achieved in case of MS + BAP where the medium was supplemented with 2.0 mg/l BAP. Culture of nodes on growth regulators-free MS medium resulted in a single shoot (Figure 1A).

Shoot multiplication

Incorporation of different concentrations of BAP during shoot multiplication promoted multiple shoot proliferation (Figure 1B; 1C). The highest number of shoots per explant (5.55 ± 0.18) was obtained on MS medium containing 2.0 mg/l BAP + 0.5 mg/l NAA whilst highest shoot length (4.13 ± 0.11 cm) was observed on MS medium containing 2.0 mg/l BAP + 1.0mg/l NAA within 30 days of culture (Table 2).

Table 1. Effect of MS media supplemented with BAP showing bud break percentage and number of shoots per explants after 10 days of culture

Days	Growth regulator (mg/l)		Bud break (%)
	BAP	NAA	
1-3	2.0	0.0	25.33
4-6	2.0	0.0	66.67
7-9	2.0	0.0	8
1-3	2.0	0.5	24
4-6	2.0	0.5	64
7-9	2.0	0.5	12

Table 2. Effect of different combinations of growth regulators on shoot multiplication from nodal explants of *Dalbergia melanoxylon* culture on MS medium after 4 weeks

S/N	Growth regulators (mg/l)		% of Responsive explants	No. of shoots/explant	Shoot length/explant (cm)
M0		-	100	1.00 ± 0.00c	1.12 ± 0.09b
M1	BAP	0.5	66.67	1.64 ± 0.06b	1.67 ± 0.09a
M2		1.0	65.33	1.85 ± 0.08d	2.04 ± 0.06d
M3		1.5	60	1.85 ± 0.09d	2.19 ± 0.06d
M4		2.0	86.67	2.37 ± 0.08a	2.50 ± 0.14c
M5		2.5	72	2.01 ± 0.09d	2.29 ± 0.13cd
M6	BAP + NAA	2.0 + 0.25	100	3.61 ± 0.14b	2.76 ± 0.16b
M7		2.0 + 0.50	100	5.55 ± 0.18a	3.13 ± 0.20b
M8		2.0 + 1.0	100	3.40 ± 0.13b	4.13 ± 0.11a
M9	BAP + IBA	2.0 + 0.25	78.67	2.43 ± 0.12b	2.90 ± 0.18a
M10		2.0 + 0.50	72	2.20 ± 0.11b	2.47 ± 0.09b
M11		2.0 + 1.0	92	2.79 ± 0.11a	1.98 ± 0.06c

*Means within a column with the same letter are not significantly different based on Fisher's LSD test at the P = 0.05 level of probability; (-) denotes absence of growth regulator.

Rooting and acclimatization

Our results revealed that auxin plays an important role in the root formation of *D. melanoxylon* (Table 3) as rooting of this species was achieved on MS medium supplemented with either NAA or IBA at various concentrations. Root induction from those *in vitro* raised shoots was carried out in full strength MS medium and those two different kinds of auxins (NAA and IBA) tested for rooting, NAA was more effective than IBA in producing roots. The best rooting response (86.67%) was obtained on full strength MS medium containing1.0 mg/l NAA with 3.88 ± 0.17 roots per shoot (Figure 1D) while highest mean root length of 1.57 ± 0.05 cm was observed in 0.5 mg/l NAA after six weeks of culture. Increasing IBA and NAA in the medium resulted in significantly shorter root length. Well rooted plantlets were transferred to soilrite (Figure 1E) and autoclaved forest soil and kept in growth chamber for 3-4 weeks before transplanted in pots with autoclaved forest soil and kept in the greenhouse (Figure 1F).

Table 3. Effects of IBA and NAA on *in vitro* rooting of *D. melanoxylon*

S/N	Growth regulators (mg/l)		% of Responsive Explants	No. of roots/explant	Root length (cm)
RF0		0	0.0	0.0 ± 0.00d	0.0 ± 0.00d
RF1	MS + IBA	0.1	40	0.56 ± 0.07c	1.35 ± 0.06c
RF2		0.3	52	0.80 ± 0.08c	1.31 ± 0.05c
RF3		0.5	52.67	0.91 ± 0.06bc	1.22 ± 0.06ac
RF4		0.7	57.33	1.19 ± 0.10b	1.14 ± 0.05ab
RF5		1.0	62.67	1.72 ± 0.20a	1.06 ± 0.04b
RF6	MS + NAA	0.1	44	0.63 ± 0.07b	1.54 ± 0.07b
RF7		0.3	68	1.32 ± 0.06d	1.54 ± 0.06b
RF8		0.5	70.67	1.67 ± 0.14cd	1.57 ± 0.05b
RF9		0.7	76	1.95 ± 0.12c	1.56 ± 0.05b
RF10		1.0	86.67	3.88 ± 0.17a	1.22 ±0.03a

*Means within a column with the same letter are not significantly different based on Fisher's LSD test at the P = 0.05 level of probability.

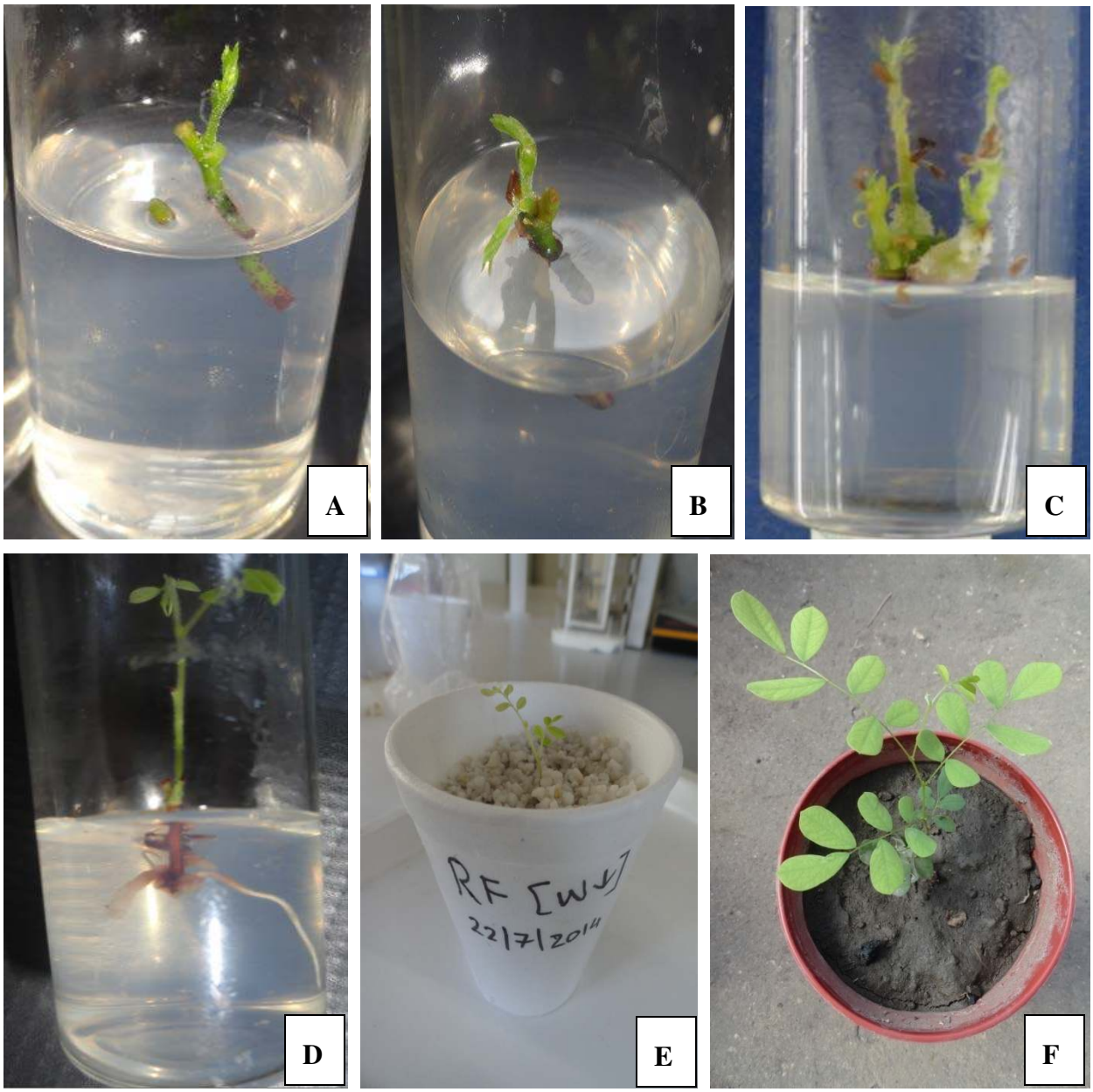

Figure 1: Various growth stages of *D. melanoxylon*: (**A**) Single shoot initiation from a nodal explants on growth regulators-free MS medium; (**B**) Proliferation of multiple shoots on MS + BAP (0.5 mg/l) – 10-day-old culture; (**C**) Elongation and proliferation of multiple shoots on MS + BAP (2.0 mg/l) + NAA (0.5 mg/l) after 2 weeks; (**D**) *In vitro* rooting on MS + NAA (1.0 mg/l); (**E**) *In vitro* rooted plantlet in soilrite and (**F**) An acclimatized plant.

Discussion

The main objective of this study was to determine the optimal conditions and concentrations of PGRs for *in vitro* shoot initiation, multiplication and rooting needed for raising *D. melanoxylon*

that would allow for a mass production of the plant species. The influence of various growth regulators was carried out on *in vitro* derived shoots of *D. melanoxylon*. An assessment of various concentrations of PGRs (BAP, IBA and NAA) on both shoot multiplication and rooting was done to find the optimum PGRs required for raising this species *in vitro*.

Shoots were induced from woody branch explants collected from mature trees on MS medium. In the present study, single shoots were formed when nodes were cultured on growth regulators-free MS medium. Addition of auxins and cytokinins to the medium was essential to induce multiple shoots from the explants (Bari *et al*., 2008). However, all concentrations of BAP promoted development of lateral buds. MS medium supplemented with 2.0 mg/l BAP alone resulted in (2.37 ± 0.08) shoots per explant. This amount was also observed to enhance shoot proliferation by Purohit and Dave (1996) on *Sterculia urens* Roxb. This favorable effectiveness of BAP on plantlets induction and multiplication was also observed by N'doye *et al.* (2012) on *Adansonia digitata*, Rout *et al.* (2008) on *Acacia chundra*, Yadav and Singh, (2010) and Yadav and Singh (2011) on *Spilanthes acmella*, Reddy *et al.* (2012) on *Asclepias curassavica* (L.) Verma *et al.* (2011) on *Stevia rebaudiana*, and Boga *et al*, (2012) on *Dalbergia latifolia*.

The highest number of shoots per explants (5.55 ± 0.18) was obtained on MS medium containing 2.0 mg/l of BAP in combination with 0.5 mg/l NAA (Table 2). Those on growth regulators-free MS medium however, developed shoots but the growth was retarded (less 3.01 cm in height) as compared to those developed on medium supplemented with BAP which grew up to a height of 4.13 ± 0.11 cm (Table 2). There are several reports which revealed the use of BAP and NAA for shoot initiation such as *Dalbergia latifolia* (Swamy *et al.*, 1992; Boga *et al.*, 2012), *Bauhinia variegate* (Singh *et al.*, 2013), *Capparis decidua* Forsk. (Deora and Shekhawat, 1995) and *Dalbergia sissoo* Roxb. (Sahu *et al.*, 2014).

For root induction from shoots, NAA played a significant role. NAA had obvious effects on rooting of *D. melanoxylon* and a well developed root system within 6 weeks of culture. Root induction in MS medium containing NAA gave better results in all parameters of root induction when compared to IBA. It was found that 1.0 mg/l of NAA was most suitable for root induction where high number of roots (3.88 ± 0.17) per explant was observed during *in vitro* rooting (Table 3; Figure 1D). However, sometimes in the medium supplemented with auxin, root growth was

stopped after extending for few mm and the root tips became blackened. However, rooted plantlets were better acclimatized in forest soil:sand (1:1) than soilrite.

Conclusion

For the first time, the present study reports the establishment of an efficient *in vitro* propagation protocol for the mass production of *D. melanoxylon* plant. Nodal explants from mature tree showed good organogenic response and BAP proved to be effective for improving shoot number and shoot length either alone or in combination with NAA or IBA. The optimum concentration of growth regulators that resulted in highest number of shoots per explant was 2 mg/l BAP in combination with 0.5mg/l NAA. NAA was proved to be better auxin than IBA for *in vitro* rooting of *D. melanoxylon*. The present study established an efficient protocol for *in vitro* propagation of *D. melanoxylon* using nodal explants from matured trees. This protocol could thus be useful for conservation and large-scale planting of this economically and ecologically important timber-yielding tree species. Nevertheless, there is possibility in improvement of the technique for better multiplication of shoots and acclimatization of the *in vitro* propagated plantlets.

Acknowledgements

Special thanks go to School of Life Science and Bio-Engineering of Nelson Mandela African Institution of Science and Technology, Tanzania for its incredible efforts and support during the course of this study. We are grateful to the Mikocheni Agricultural Research Institute (MARI) for making lab facilities accessible to this research and for other institutional support. This research is funded by the Commission for Science and Technology (COSTECH) of the government of Tanzania.

References

Amri, E., Kanyeka, Z., Lyaruu, H. and Nyomora, A. (2009). Evaluation of Genetic Diversity in *Dalbergia Melanoxylon* Populations Using Random Amplified Polymorphic DNA Markers. *Research Journal of Cell and Molecular Biology,* 3: 71-79.

Arbonnier, M. (2004). *Trees, Shrubs and Lianas of West African Dry Zones*, Editions Quae.

Ball, S. M. (2004). Stocks and Exploitation of East African Blackwood *Dalbergia Melanoxylon*: A Flagship Species for Tanzania's Miombo Woodlands? *Oryx,* 38: 266-272.

Boga, A., Ram, B. and Reddy, G. R. S. (2012). Effect of Benzyl Amino Purine and Gibberellic Acid on in Vitro Shoot Multiplication and Elongation of *Dalbergia Latifolia* Roxb.: An Important Multipurpose Tree. *Research Article, Biotechnol. Bioinf. Bioeng,* 2: 597-602.

Borthakur, A., Das, S. C., Kalita, M. C. and Sen, P. (2011). *In Vitro* Plant Regeneration from Apical Buds of *Albizzia Odoratissima* (Lf) Benth. *Adv App Sci Res,* 2: 457-464.

Borthakur, A., Das, S. C., Kalita, M. C. and Sen, P. (2012). An *in Vitro* Plant Regeneration System for Conservation of the Leguminous Tree *Albizzia Chinensis* (Osbeck) Merr. *Adv. Applied Sci. Res,* 3: 1727-1732.

Bryce, J. (1967). The Commercial Timbers of Tanzania. *The commercial timbers of Tanzania.*

Chaturani, G., Subasinghe, S., Jayatilleke, M., Mapalana, K. and Lanka, S. (2006). In Vitro Establishment, Germination and Growth Performance of Red Sandal Wood (*Pterocarpus Santalinus* L.). *Tropical Agricultural Research & Extension,* 9: 116-130.

Deora, N. and Shekhawat, N. (1995). Micropropagation of *Capparis Decidua* (Forsk.) Edgew.—a Tree of Arid Horticulture. *Plant cell reports,* 15: 278-281.

Dewan, A., Nanda, K. and Gupta, S. C. (1992). *In Vitro* Micropropagation of *Acacia Nilotica* Subsp. Indica Brenan Via Cotyledonary Nodes. *Plant Cell Reports,* 12: 18-21.

Feyissa, T., Welander, M. and Negash, L. (2005). Micropropagation of *Hagenia Abyssinica*: A Multipurpose Tree. *Plant cell, tissue and organ culture,* 80: 119-127.

Gundidza, M. and Gaza, N. (1993). Antimicrobial Activity of< I> *Dalbergia Melanoxylon*</I> Extracts. *Journal of ethnopharmacology,* 40: 127-130.

Högberg, P. (1986). Soil Nutrient Availability, Root Symbioses and Tree Species Composition in Tropical Africa: A Review. *Journal of Tropical Ecology,* 2: 359-372.

Husain, M., Anis, M. and Shahzad, A. (2007). In Vitro Propagation of Indian Kino (*Pterocarpus Marsupium* Roxb.) Using Thidiazuron. *In Vitro Cellular & Developmental Biology-Plant,* 43: 59-64.

Idrees, B.S & Mohammed, S.M (2014) Physiological Characteristics of *Dalbergia melanoxylon* and *Dalbergia sissoo* seeds. *Journal of Natural Resources & Environment Studies,* 2: 20-28.

Jenkins, M., Oldfield, S. and Aylett, T. (2002). *International Trade in African Blackwood*, Fauna & Flora International.

Jha, A., Prakash, S., Jain, N., Nanda, K. and Gupta, S. (2004). Micropropagation of *Sesbania Rostrata* from the Cotyledonary Node. *Biologia plantarum,* 48: 289-292.

Lovett, J. (1987). Mpingo–the African Blackwood. *Swara,* 10: 27-28.

Malimbwi, R., Luoga, E., Hofstad, O., Mugasha, A. and Valen, J. (2000). Prevalence and Standing Volume of *Dalbergia Melanoxylon* in Coastal and Inland Sites of Southern Tanzania. *Journal of Tropical Forest Science*: 336-347.

Mbuya, L., Msanga, H., Ruffo, C., Birnie, A. and Tengnäs, B. (1994). *Useful Trees and Shrubs for Tanzania: Identification, Propagation and Management for Agricultural and Pastoral Communities*, Regional Soil Conservation Unit, Swedish International Development Authority.

Msanga, H. P. (1998). *Seed Germination of Indigenous Trees in Tanzania, Including Notes on Seed Processing, Storage, and Plant Uses*.

N'doye, A. L., Nahr, S. M. A., Ourèye, S. M. and Of, P. (2012). Propagation of African Baobab (*Adansonia Digitata* L., Bombacoideae, Malvaceae) Germplasm through *in Vitro* Cloning. *Advances in Environmental Biology,* 6: 2749-2757.

Nanda, R. M., Das, P. and Rout, G. R. (2004). In Vitro Clonal Propagation of *Acacia Mangium* Willd. And Its Evaluation of Genetic Stability through Rapd Marker. *Annals of forest science,* 61: 381-386.

Neondo, J., Machua, J., Muigai, A., Nyende, A. B., Munjuga, M., Jamnadass, R. and Muchugi, A. (2011). Micropropagation of Allanblackia Stuhlmannii: Amenability to Tissue Culture Technique. *International Journal for Biotechnology and Molecular Biology Research,* 2: 185-194.

Nshubemuki, L. (1994). Recent Progress in Silviculture Research on *Dalbergia Melanoxylon* in Tanzania. *Nitrogen fixing tree research reports: a publication of the Nitrogen Fixing Tree Association (NFTA)*.

Purohit, S. and Dave, A. (1996). Micropropagation of *Sterculia Urens* Roxb.—an Endangered Tree Species. *Plant cell reports,* 15: 704-706.

Rajeswari, V. and Paliwal, K. (2006). In Vitro Propagation of *Albizia Odoratissima* Lf (Benth.) from Cotyledonary Node and Leaf Nodal Explants. *In Vitro Cellular & Developmental Biology-Plant,* 42: 399-404.

Reddy, S. H., Chakravarthi, M. and Chandrashekara, K. (2012). In Vitro Multiple Shoot Induction through Axillary Bud of *Asclepias Curassavica* L.–a Valuable Medicinal Plant. *International Journal of Scientific and Research Publications,* 2: 1-7.

Rout, G., Senapati, S. and Aparajeta, S. (2008). Micropropagation of *Acacia Chundra* (Roxb.) Dc. *Hort Sci,* 35: 22-26.

Sahu, J., Khan, S., Sahu, R. K. and Roy, A. (2014). Micropropagation of Dalbergia Sissoo Roxb. Through Tissue Culture. *Pakistan Journal of Biological Sciences,* 17: 597-600.

Schatz, G. E. (2009). Plants on the Iucn Red List: Setting Priorities to Inform Conservation. *Trends in plant science,* 14: 638-642.

Singh, B., Wawrosch, C., Joshi, S. and Kopp, B. (2013). Micropropagation of *Bauhinia Variegata* L. From Tissue Culture. *Nepal Journal of Science and Technology,* 13: 39-41.

Swamy, B. R., Himabindu, K. and Sita, G. L. (1992). In Vitro Micropropagation of Elite Rosewood (*Dalbergia Latifolia* Roxb.). *Plant cell reports,* 11: 126-131.

Thirakul, S. (1984). Manual of Dendrology, Bahr El Ghazal and Central Regions. *Groupe Poulin, Theriault Ltee Consultants. Quebec*.

Verma, S., Yadav, K. and Singh, N. (2011). Optimization of the Protocols for Surface Sterilization, Regeneration and Acclimatization of *Stevia Rebaudiana* Bertoni. *Am Eurasian J Agric Environ Sci,* 11: 221-227.

Washa, W., Nyomora, A. and Lyaruu, H. (2012). Improving Propagation Success of *D. Melanoxylon* (African Blackwood) in Tanzania (Ii): Rooting Ability of Stem and Root Cuttings of Dalbergia Melanoxylon (African Blackwood) in Response to Rooting Media Sterilization in Tanzania. *Tanzania Journal of Science,* 38: 43-53.

Yadav, K. and Singh, N. (2010). Micropropagation of *Spilanthes Acmella* Murr.–an Important Medicinal Plant. *Nature and Science,* 8: 5-11.

Yadav, K. and Singh, N. (2011). In Vitro Flowering of Shoots Regenerated from Cultured Nodal Explants of *Spilanthes Acmella* Murr.—an Ornamental Cum Medicinal Herb. *An Univ Oradea Fasc Biol,* 18: 66-70.