The Effect of Moisture and Seed Treatment on the In-Situ and Ex-Situ Regeneration of *Dalbergia Melanoxylon* (African Blackwood) in Pugu Forest Reserve

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Abstract: *A study was conducted to investigate the effect of moisture and seed treatment on the in-situ and ex-situ regeneration of Dalbergia melanoxylon in Pugu forest reserve. Seed germination experiments was conducted for three months between November and December 2005 in the nursery Botany Department of the University of Dare s Salaam while seedling growth rate measurements were conducted for 12 months between January and December 2006 in the Pugu forest reserve. A total of 2 kg of D. melanoxy seeds collected from Pugu forest reserve and some from Tanzania Tree Seed Agency (TTSA) were used for seed germination experiments. A total of 25 seedlings approximately 2 years old were randomly chosen from Pugu forest reserve for seedling growth rate experiments. A split plot experimental design was used for seed germination in the nursery where soaked and non-soaked seeds stood for main plot and different moisture level treatments stood for subplot. Parameters recorded were germination dates, germination percentages, seedling increase in height and seedling increase in girth. Manual computation of data was accomplished according to Zar, 1988 where LSD was used for mean separation to determine statistical differences between treatment means. Tables and Figures were used to facilitate presentation and discussion of results. Data analysis led to the conclusion that, a moderate ex-situ moisture level of 200ml in a 1L pot per day effectively promoted seed germination up to 21% in seeds previously soaked in water for 6 hours compared to high moisture treatment of 300ml in a 1L pot per day which effected seed germination only by 11.6%.. The maximum in-situ growth rate was concluded to be 0.01 cm/month. The study revealed that seeds and young seedlings of D. melanoxylon are vulnerable to high moisture level while older trees are water and light demanding. It was recommended that future researches should endeavour to increase seed viability by harvesting them immediately from their maturity and storage in sealed containers, conducting tissue culture to manipulate seedlings and hybridization experiments with related species to reduce growth period.*

Key words: Regeneration, ebony tree, music plant, Mpingo, Seed germination

INTRODUCTION

The African black wood tree – *Dalbergia melanoxylon* Guillex Perr (Family Leguminoceae, Subfamily Papilionoidea), is also known as the African ebony or *Mpingo* in Kiswahili. *D. melanoxylon* is a small tree that gives small, thin, flattened and indehiscent pods enclosing delicate seeds. It is valuable because it produces the most expensive timber and carvings in the world. *D. melanoxylon* is a small, heavily branched tree, typically 4.5 to 7.5m tall but occasionally reaching 15m. The bark of tree is pale grey to grayish, brown, papery, fairly smooth and flaking in long narrow strips (Bryce, 1967). Their stems are crooked, covered at first with short crisp hairs and are glabrous with pinnately, alternate compound leaves that are 6 to 22cm long. The pods are elliptically oblong or irregularly oblong, bluntly pointed, flat and thin. They range from 3 to 7cm long and 0.8 to 1.4cm wide. They are very light, glabrous and laxly and rather diffusely veined with one or two seeds (Figure 1a-1c).

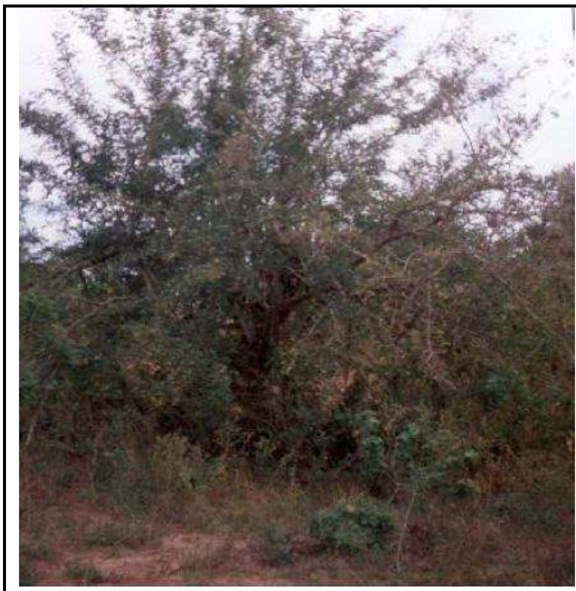
It is estimated that 1kg of *D. melanoxylon* pods contain 67,768 seeds. *Ex-situ* experiments have proved that seed germination under controlled conditions reaches only 50%, germination decreases to 20% in in-situ due to various factors from the environment (Sharman, 1995). Seeds take three months to mature which in Tanzania is from May to July. The trees take more than 70 years to mature to an appreciable harvesting age. Pugu forest reserve is included in T_6 florist region (wet tropical zone) and has reasonable *D. melanoxylon* population since it is protected.

Dalbergia Melanoxylon grows under a wide range of conditions including semiarid, sub-humid and tropical lowland areas (Bryce, 1967). It is often found in dry, rocky sites at elevations from sea level to 1200m but is most frequently found in mixed deciduous forests and savannah of the costal regions (Brenan and Green way, 1949). Pugu forest reserve is one of these costal regions mentioned by Green way, 1949. The minimum temperature in its native range is 18^0C and the maximum is 35^0C. *D. melanoxylon* is not resistant to frost. It prefers an annual rainfall average of 700 to 1200mm, evenly distributed in bimodal pattern of three to six months (Noad and Birnie, 1989). It thrives in soils varying from loamy sands to clayey vertisols "Black cotton soil". The adult trees of this species is water and light demanding, and will not regenerate under heavy vegetation cover. On the other hand, mature tree of *D. Melanoxylon* tolerates fire (Kale, 1984).

Figure 1(a). Pod and seed structure of *Dalbergia melanoxylon*

	Unripe pods are green, changes to pale green when mature and grey when dry. Pods are flat, indehiscent and papery containing 1-3 seeds. Seeds are thin, flat and papery. Seeds are vulnerable to high moisture content, insect and disease attack. Seed viability is very low.

Figure (b). ***D. melanoxylon tree*** **in its native environment. Figure1 (c).** ***D. melanoxylon*** **woods**



In Tanzania, *D. melanoxylon* is distributed in seven floristic zones, which are dominated by miombo woodlands. *D. melanoxylon* is found between 600m to 1200m, having an annual rain between 760mm to 1400mm and temperature of 13^0C. Moist plateau cover Mpanda, Tabora and Kigoma. The dry tropical zone is found between 600m to 1200m, annual rainfall ranges between 760mm and 1200mm and the temperature of 13^OC. Dry tropical zone covers Dodoma, Itigi, Mwanza and Shinyanga. The African black wood is also poorly distributed in the wet tropical zone which in Tanzania covers Rau, Turian, Matombo, Minziro, Korongwe, Muheza, Dar es Salaam, Kibaha, Bagamoyo, Lindi and Mtwara. These areas are also dominated by Miombo woodlands. Wet tropical zone is found in coast areas, around heavy rain mountains, on flat areas at 1140mm and mean temperatures of 13^0C. Wet tropicals extend to Iringa and Njombe on its south west and extend to coast belt southern Tanzania which includes Mtwara and Lindi (Mbuya, Msanga and Tengnas, 1994). Therefore *D. melanoxylon* inhabits both the rainforest environments and the open miombo woodlands which cover about two third of Tanzania. Rainfall is often marginal in the miombo woodlands and long periods of drought are not unusual. Land areas such as these are environmentally fragile, their vitality depend on a delicate balance being maintained between the plants, animal and human population which inhabit them (SOFO, 1999). *D. melanoxylon* has adapted well to this marginal existence, and its long growing period and root suckers germination is an out come of this adaptation to harsh conditions (Read, 1994).

Regeneration is defined as a constant existence of plant individuals of varying sizes from asexual or sexual reproduction. *D. melanoxylon* regeneration can be naturally accomplished in two ways, by seed germination and by root suckers (Mugasha, 1978). The success of natural regeneration is subject to a number of factors, particularly the availability of sufficient, sound, viable seeds and factors affecting their germination ability and their growth rate (Sharman, 1995). Many environmental factors such as temperature, humidity, soil nutrients and moisture level can affect regeneration of plants in natural environments but moisture level are key to *ex-situ* and *in-situ* regeneration (germination, growth and development) of plants as compared to many others (Magingo and Dick, 2001)

Recently, a study conducted by Amri (2002) revealed that *D. melanoxylon* has low seed viability, seed germination and seedling growth rate. Germination of *D. melanoxylon* seeds has been estimated at only 20 – 50% within 8 – 20 days due to factors affecting it is germination in natural environments (Mbuya *et al.*, 1994). It has always been assumed that native trees regenerate sufficiently under natural condition. Various studies reported that mycorrhiza association improves propagation methods of many other plants by enabling re-establishing of plants in disturbed and degraded lands (Munyanziza *et al.*, 1994). Gamborg (2002) reported that tissue culture as a means of mass production of planting materials have been successful in propagating many other plant species that are poorly propagated by natural methods. *D. melanoxylon* seeds are small, flat, thin, papery such that they need a thin layer of soil so that very little energy stored in the seed can easily push the soil for germination. It is possible that most seed are lost in thick cover of soil in *in–situ* regeneration. The temperature requirement for *D. melanoxylon* is 13 – 18^0C and needs moist soil to germinate optimally (Nshubemuki, 1993).

On the other hand, water supply in the field should be sparing so that seeds do not rot. Germination in the field decreases from 50 to 20% if the above factors are not met. Mature seeds remain viable for only few months after maturity so it is advised to plant seeds few days after picking them from the field (Noad, and Birnie, 1989). In addition to use of seeds and root cuttings as means of propagating *D. melanoxylon* species, shoot cuttings, wildlings/saplings and coppices are also methods that can be used to propagate this species (MNRT, 1988). Wildings are seedlings from the natural environment that can be nurtured in nurseries before they can be grown out into the field while coppices are shoot suckers that emerge from pruned/stumped trees. These are devoid of roots and nurturing them in nurseries will allow the shoots to regenerate roots before they can be ready for planting out in the field. The challenges of using root and shoot cuttings for mass/commercial propagation purposes would be destruction of the mature trees in the process of propagation if hard pruning is done. So far, the existence of *D. melanoxylo*n and other endangered plant species in Tanzania are in critical conditions. When conservation strategies are being developed for these endangered species, all available information about propagating *D. melanoxylon* and other endangered species should be taken into consideration (IUCN, 2008).

Crucial to consider are the requirements for successful germination and seedling establishment (Masamba, 1994) which are not much studied and this formed the need of conducting this study i.e. to study the effect of moisture and seed treatments on the *in-situ* and *ex-situ* regeneration of *D. melanoxylon* in Pugu forest reserve. Knowing the type of seed treatment and the exactly moisture level needed for seed germination in our nurseries can improve propagation of the species. Sustainable propagation needs to know the factors affecting the growth rate of the species in the natural environment. Other factor contributing to lack of propagation practices include the lack of scientific knowledge of various species including *D. melanoxylon,* uncontrolled harvesting of the forest products, the change in the land use, rapid urbanization and excessively rising land requirement human settlement (MNRT, 1988). Viable seeds of *D melanoxylon* are difficult to obtain. Difficulty of getting viable seeds is due to the structural morphology of the pods and seeds that are very thin, flat, papery and indehiscent and therefore readily become vulnerable to insect and fungal attack. Alternatively propagation could be conducted by using cuttings, wildings, coppices and root suckers but these methods involve destruction of the adult plants and hence accelerating deforestation. Redhead and Temu (1981) pointed out that advanced plant-production techniques such as tissue culture and mycorrhizal association in *D. melanoxylon* have not been investigated.

MATERIALS AND METHODS

In – situ experiments in Pugu Forest

Pugu forest reserve is located 25km south west of Dar es Salaam and 20km inland from the Indian Ocean covered with Kaolinitic Sand soil. The forest is between the altitude of 100 and 305m, and experiences a yearly rainfall of 760mm and temperature of between 18^0C – 20^0C. (Nshubemuki, 1993). A criteria used to select this area for the research among many others is because the area is protected, does not get animal and human disturbances and therefore records can be taken for a year without interactions. A total of 25 seedlings approximately 2 years old were randomly selected, marked and used in *in-situ* experiment. Growth rates were measured monthly and monitored for a period of 1 year. Meteorological data which can influence and affect plant growth and development which are temperature and rainfall were taken from a nearby weather station at Mwalimu Julius Nyerere International airport, Dar es Salaam. Plant height was measured from ground level to the tip of seedling using a ruler while stem diameter was measured 10cm from the ground using veneer callipers. Seedlings were assessed to determine whether they originated from seeds or root suckers by observing the root structure, normally seedlings from seed have a tap root while those originating from root suckers have adventitious roots.

Ex – situ experiments in UDSM Nursery

In order to compare viability status between *D. melanoxylon* seeds from the forest and that stored at TTSA, a total of 2 kg of seeds from Pugu forest reserve and Tanzania Tree Seed Agency was collected and subjected to different germination treatments. The experiment was arranged in a split plot design with seed soaking and non-soaking treatments as the main plots and moisture level in the potting media as subplots. This was followed by observations on seed germination and seedling growth rates. A total of 360 seeds were soaked in water for six hours before sowing while another total of 360 seeds were sown without prior soaking treatment. Each of these two main treatments was then subdivided into three sub treatments that were assigned to three moisture level treatments namely low, median and high moisture in the potting mixture. The topsoil part of the potting mixture was obtained from Kunduchi quarry so that a recommended ratio of potting media can be made before using them in the pots. The potting mixture comprised of topsoil, rough sand and organic matter in a ratio of 1:1:1 that gave a total porosity of 55%.

Each sub treatment was replicated four times making a total number of 360 seeds for each main treatment making a total of 720 seeds for the whole trial. All seeds for the trial were sown on 19 November, 2005. Moisture treatment levels applied per day in the first month (20 Nov – 17 Dec. 05) were 100ml for low moisture level, 200ml for median level and 300ml for high level. During the subsequent two months moisture treatment was applied as 200ml for low level, 300ml for median level and 400ml for high level per day per pot. For the rest of months water was given as 400ml for low level, 800ml for median level and 1200ml for high level. Number of pots per treatment was 12 pots, and 36 pots made the subplots while the whole treatments comprised of 72 pots. Each pot carried 10 seeds. Sown seeds were inspected every day for germination and germination dates were recorded weekly for a period of three weeks. Seeds were considered germinated when the radical was about 2cm long and cotyledon had emerged from the seed coat. Data on germination percentage obtained were first transformed into arcsine transformation before computation but the original data was used in the summaries of the means (Zar, 1984). Results from each experiment were analyzed separately. Means and Least Significant Difference (LSD) for germination percentages for both seed treatments and moisture levels were computed to get inference.

Parameters recorded

Germination was monitored for one month while subsequent seedling growth rate was monitored for seven month. The following parameters were recoded:

- Germination dates determined as the first day of emergence of the young seedling on the ground.

- Germination percentages as the proportion of germinated seeds every week over total number of sowed seeds.

- Seedling height/month – This was measured in cm from soil level to the tip of seedling using a measuring tape.

The above parameters obtained were subjected to statistical ANOVA.

Data Analysis

Data was analysed according to Zar (1988) and LSD was used to determine statistical differences between treatment means. Data were summarized into relevant tables and figures to give results to facilitate presentation and discussion.

RESULTS AND DISCUSSION

Germination experiments (Ex-situ)

Main effect of seed treatment on seed germination

Germination percentage ranged from 2 to 21% in soaked seeds and 0.5 to15% in non-soaked seeds starting from seven days after sowing to 21days when all viable seeds had germinated. There was no statistically significant difference in seed germination percentage due to seed treatment. (Table 1 & 2). A maximum of 21% of seed germination in this experiment as indicated in table 1&2 is relatively low. These results conform and agree with the results by Mbuya *et al,* (1994) and Amri (2002) in which germination was found to be between 20% to 50% while seed viability and seedling growth were also found to be relatively low.

Table1: Effect of seed treatment and moisture level on seed Germination (*Ex- Situ*)

Seed treatment	Moisture level	Germination %				Mean	LSD
		26 NOV.05	**02 DEC.05**	**10 DEC. 05**	**17 DEC.05**	**Mean**	
Soaking	Low (100ml)	3.8	1.6	13.3	10.8	7.4	15..3
	Mediam (200ml)	2.5	6.6	18.1	21.5	12.2	
	High (300ml)	0.00	0.8	11.6	12.5	6.2	
	Mean Soaked	2.1	3.0	14.4	14.9	8.6	
Non soaking	Low (100ml)	0.8	3.3	15.7	16.5	9.9	14.9
	Mediam (200ml)	0.00	0.8	14.9	19.8	8.9	
	High (300ml)	0.8	1.6	11.7	10.8	6.2	
	Mean Non soaked	0.5	1.9	14.1	15.7	8.4	
LSD	5%	2.99	4.43	5.61	3.65		
	1%	14.99	22.21	28.12	18.28		

Effect of moisture level on seed germination

The effect of moisture level in the potting media on seed germination is as shown in Table 2. Generally germination increased from low to median moisture level. High moisture level was associated with low germination percentages at each date. The median moisture level was the most favourable in effecting seed germination. Here, germination ranged from 1.3 during the first week to 21% three weeks later when all viable seeds had germinated. This is opposed to 0.4 and 11.6% respectively for seeds received high moisture level. Percentage germination due to seed treatment was 15% and percentage germination due to moisture level was 21%. However the differences due to seed treatment and moisture level were not statistically significant. Other literature had reported that in–situ germination of *D. melanoxylon* seeds could decrease from 50% to 20% depending on the available factors affecting germination (Noad and Birnie, 1989) including moisture status of the environment as was observed during conduction of this research.

Table 2: Mean seeds germination in response to different moisture level

Moisture level	26 Nov	02 Dec	10 Dec	17 Dec
Low	2.33	2.28	14.53	13.65
Median	1.25	3.73	16.55	20.68
High	0.41	1.24	11.67	11.64

Germination is the resumption of growth of embryo which begins with imbibition of water followed by activation and mobilization of enzymes in the embryo which breaks down the food reserves in the endosperm and provides the required energy for its development. Embryo growth is accompanied by cell division and expansion resulting into swelling and splitting of seed coat to enable emergence of the radical (Muguga, 1993). Optimum amount of water is necessary for reactivation of enzymes in embryo for cell division, expansion, swelling and splitting of seed coat for germination provided other factors as temperature and aeration are optimum. *D. melanoxylon* seeds have thin seed coat and are fragile such that higher moisture level used in germination during this study could have caused excess moisture in relation to the seed size hence causing hypoxic conditions that affected the metabolic reactions of the endosperm and embryo resulting into seed death. Low moisture level used in this experiment could not have been enough to reactivate the enzymes in embryo and therefore could not activate cell division, expansion, swelling and splitting of seed coat for emergence of radical. The same scenario could be happening in-situ where drought and floods are a possibility such that regeneration of *D. melanoxylon in-situ* is not the rule.

Effect of seed treatment on seedling growth (height)

As indicated in Table 3, the mean height of seedlings from soaked seeds was 48cm while mean height of seedlings from non soaked seeds was 51cm. Non-soaked seed were associated with high seedling growth mean and soaked seed were associated with low mean seedling growth. However the differences observed in seedling growth were not statistically significant.

Table 3: Seedling height during the months of January to July 2005

Treatments		Seedling height (cm)								LSD
	Moisture level	Dates							Mean	
		19/1	19/2	19/3	19/4	19/5	19/6	19/7		
Soaked seeds	Low	4.187	26.43	40.8	55.25	91.00	83.73	84.5	56.55	40.12 5%
	Median	4.23	20.45	31.5	62.75	74.25	58.5	58.5	44.31	37.23 5%
	High	3.36	12.44	22.62	73.5	71.25	48.75	66.25	42.59	35.06 5%
	Soak mean	3.925	19.77	31.47	63.83	78.83	63.66	69.75	48.00	
Non soaked	Low	6.19	25.5	46.15	35.75	80.75	90.5	100.25	55.01	40.78 5%
	Median	3.29	24.82	34	85.25	48.75	66.75	67.5	47.19	39.30 5%
	High	5.19	19.78	35.27	50.0	61.75	76.00	77.25	49.99	38.66 5%
	Non soak mean	4.89	20.10.	38.47	40.33	63.75	77.75	81.66	51.00	
	LSD 5%	9.99	40	23.32	21.75	131.48	333	73		
	1%	50.09	202	116.85	109.06	558.72	1671	368		

Effect of moisture level on seedling growth (height)
As table 4 shows, the highest mean height was observed in seedlings treated with low moisture, and it ranged from 5.2cm in January to 90cm July, 2006. The lowest height was observed in seedlings that were treated with median moisture and it ranged from 3.8cm in January to 63cm July. Seedling growth in high moisture was moderate. From these observations it is evident that low moisture was associated with higher seedling growth while median moisture was associated with lower seedling growth. Moisture levels for irrigation and not seed soaking imparted a significant effect on *D. Melanoxylon* seedling growth rate.

Table 4: **Effect of moisture on seedling growth in height (cm)**

Moisture Level	Dates						
	19/1	19/2	19/3	19/4	19/5	19/6	19/7
Low	5.2	26	44	46	86	87	90
Median	3.8	22.7	32.8	32.8	53	62.2	63
High	4.3	16	29	62	67	62	72

D. melanoxylon growth (in – situ)

Growth in stem height
The actual seedling stem growth in height of *D. melanoxylon* in Pugu forest natural environment during the months of April 2006 to March 2007 is shown in Fig 2a. Seedling growth height ranged from 50.52cm in April 06 to 59.60cm in March 07, giving an average growth height of 57.1cm. The highest growth height was observed between May and April 06 due to higher rainfall in these months in which a growth of 4.09cm was observed. The growth height observed during the rest of the months ranged from 0.5cm to 0.9cm due to drought conditions during these months. The relative growth rate as indicated by Fig 2b ranged from 0.081cm April, 2006 to 0.17973cm on March 2007. The average monthly growth rate during these 12 months was 0.130cm (Figure 2b).

Growth in Stem girth
Fig. 3a depicts growth of *D. melanoxylon* in stem diameter (stem girth) between April 2006 and March 2007. The stem diameter increased from 0.79cm in April 06 to 1.01cm March 07. The average mean in stem diameter growth for these 12 months was 0.00001cm. The highest stem diameter growth of 0.12cm was observed between May and April 2006, stem diameter growth observed between the rest of months ranged between 0.01cm to 0.05cm (Fig 3a). Months with rainfall were associated with increased growth. The incremental growth in stem is as shown in Figure 3b. With the exception of young *D. melanoxylon* seedlings whose survival can be threatened by higher moisture level as reported in *ex–situ* seedling growth during this study, adult *D. melanoxylon* trees are water and light demanding, needs constant supply of water through the time of their existence rather than seasonal moisture available during the rainy season. This result is in agreement with what is reported by Kale (1984).

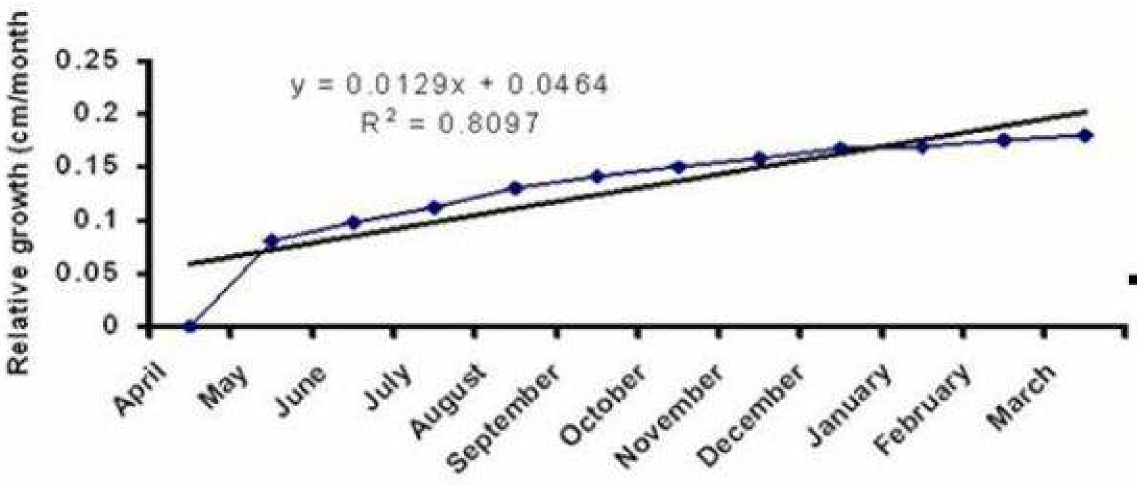


Figure 2b: Incrementa growth (height) of D melanoxylon in Pugu Forest

Among the 25 seedlings investigated in Pugu forest, 14 (56%) were found to germinate from root suckers. These were characterized by the absence of taproot.

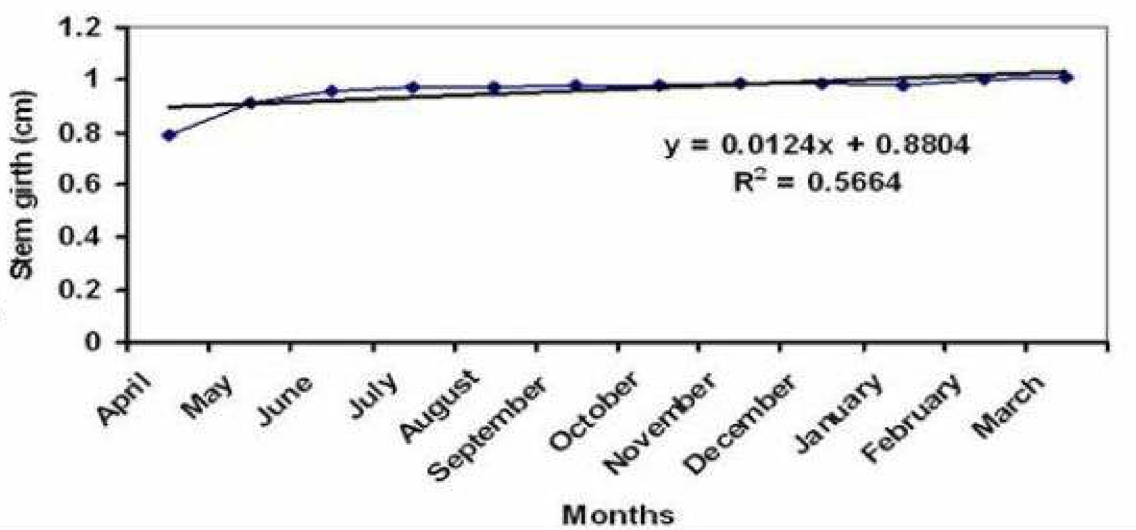


Figure 3a: In-situ seedling growth (girth) in Pugu Forest

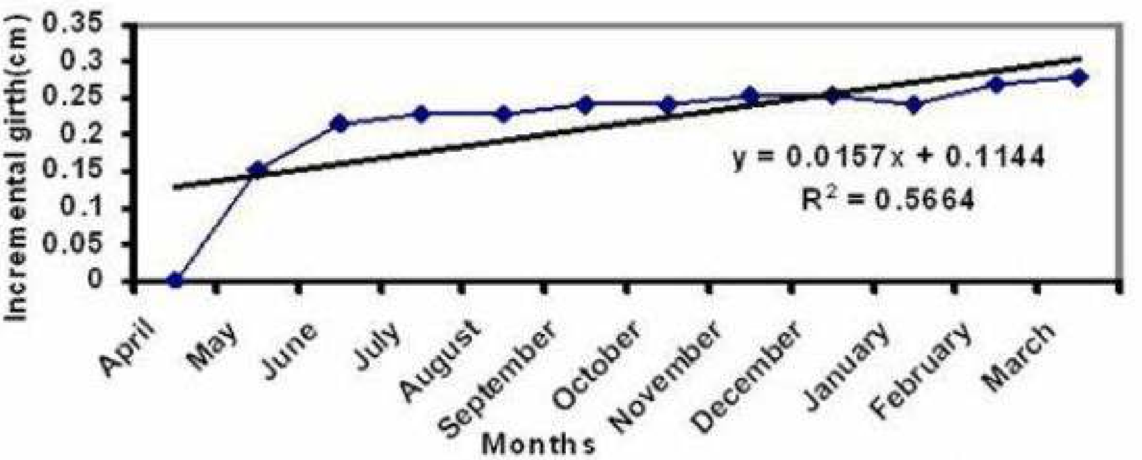


Figure 3b: Increamental stem girth of D. melanoxylon in Pugu Forest

CONCLUSSION AND RECOMMENDATION

It is evident from the study that seeds and young seedlings of *D. melanoxylon* are vulnerable to higher moisture level available while full-grown trees are water and light demanding an observation which is in agreement with findings by Kale (1984). Since seeds are indehiscent, papery, thin and flat, they easily get rotten when irrigated with high moisture. This implies that most seeds produced in natural environment yearly die during the rainy season. This is why 56% of investigated *in-situ* seedlings were found to originate from root suckers.

From this paper it can be concluded that appropriate moisture level is key to *ex – situ* and *in – situ* seed germination and regeneration of *D. melanoxylon* and many other plants as supported by Nshubemuki (1993), Mbuya *et al.* (1994), Msanga (1999) and Magingo *et al.*, (2001). As indicated in table 1&2, the appropriate moisture level for *D. melanoxylon* seed germination and seedling growth in *ex-situ* is 200ml of water in a 1L pot per day using seeds treated by soaking in water for 6 hours. By consideration of findings by Mbuya *et al.* (1994), Msanga (1999) and Amri (2002) the moisture level for better *in-situ* growth rate of the species increases with the age of the plant, that means should increase from the 200ml of this experiment to the above. It is therefore recommended that future researches should endeavour to increase seed viability by harvesting seeds immediately after their maturity and store them in sealed containers. Conduction of seed selection and viability test before sowing of seeds is also recommended so that the number of seeds to be sown for the number of resultant seedlings is adjusted accordingly. Conduction of studies on symbiotic relationships and hybridization with related species such as *D. sissoo*, conducting tissue culture or use of growth hormones to improve growth rate is also recommended. This should include collection and evaluation of germ plasma of *Dalbergia melanoxylon* and related species. Growth rate in-situ was monitored for one year only, extending monitoring period would be appropriate to show that *D.melanoxylon* growth rates are really slow and therefore measures to conserve existing ones should be given priority.

Difficulties in germination and low survival of young seedlings in the natural environment do not match with the speed of harvesting of the mature trees necessitating the need for ex-situ propagation methods for sustainable aforestation and conservation of *D.melanoxylon.* This study stresses a need of propagating *D. melanoxylon* species and planting it as a National program.

The existence of *D. melanoxylon* should not be left to depend on the naturally occurring trees. For a successful propagation of this species and sustainable conservation, the Nation needs to establish nurseries for seedling production that can be used for replanting in selected areas having favourable ecological conditions. *Ex-situ* nurseries are the only places where optimal amount of water for seed germination and survival of young seedling can be controlled.

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