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Research article

Seedling Production Techniques in *D. melanoxylon*

Washa B. Washa.

Mkwawa University College of Education

P.O.BOX. Private Bag, Iringa

E-mail: wbugalama@yahoo.com

0752356709

ABSTRACT

A study was conducted in *D. melanoxylon* comparing germination in soil to that of the Murashge and Skoog medium. An overharvested species *D. melanoxylon* have a highly valued wood but not propagated. This is why in recent years there have been some efforts to conduct investigations which can improve seedling production. About 2 kilograms of seeds were purchased from TTSA for experiment on 2010 at Botany Department University of Dar es Salaam. Three treatments were employed for soil germination (Low, Median and High moisture level) while two treatments were employed for MS germination (Half strength and Full strength). Sterilizing reagents were 35%, 70% alcohol and 2.6% sodium hypochlorite. Sterilizing duration were 10, 20 and 30 minutes. Data recorded include germination percentage, moisture level, MS concentration, sterilizing reagent concentrations and time used to sterilize the seeds. Standard procedures were used to analyze and compare germination Data as described by Zar, (2010). Highest germination in the soil was 21% while that of the MS was 19.8%. The two germination mediums did not differ significantly. More investigations especially genetic transformation of the species for easy access in tissue culture is needed to improve seedling production of *D. melanoxylon* for propagation of the species.

Key words: *Dalbergia melanoxylon*, Murashige and Skoog, Germination, moisture level.

INTRODUCTION

Dalbergia melanoxylon plant

Dalbergia melanoxylon Guill & Perr is a flowering plant that belongs to Phylum Tracheophyta, class Magnoliopsida, Order Fabalies, Family Leguminoseae and sub-family Papilionoidea. It is also known as African Blackwood and Zebrawood (in English), African ebony (in Mozambique) and Mpingo (in Swahili) (IUCN, 2008). *Dalbergia melanoxylon* wood is highly valued because it is used in wood wind instruments, clarinets, oboes, and pipes. *Dalbergia melanoxylon* is a native to dry regions of Africa. The Genus *Dalbergia* yields other famous timbers such as rosewood, tulipwood and cocobolo (Bekele, 2007). As a member of Papilionoidea species *D. melanoxylon* seeds are usually hard and with a complex hilar valve and enclosed in pods (legumes),

plants are shrubs or trees (habits)**,** leaves are usually compound and alternate, flowers are usually bilaterally symmetrical (Zygomorphic), Perfect or hermaphroditic (bisexual), entermophilous (insect pollinated) and white (Arbonnier, 2004).

Status of *Dalbergia melanoxylon* in Tanzania

The IUCN Red List (IUCN, 2008) uses three categories to evaluate conservation status of plant species (Lower Risk, Threatened and Extinct category). Lower Risk spp may be the Least concern (Lc), near threatened (nt) and Conservation dependent (cd). Threatened spp may be vulnerable species (Vu), endangered species (EN) and critically endangered (CR) and the extinct spp may either be Extinct in the wild (EW) or Total Extinct (EX). *Dalbergia melanoxylon* in Tanzania is classified as Lower Risk / near threatened meaning that it is neither endangered nor of least concern. The current conservation status of *Dalbergia melanoxylon* shows that is threatened in Kenya, extinct in Burkina Faso and therefore need conservation attention in Tanzania (Arbonnier, 2004).

Geographic distribution and Natural habitat of *Dalbergia melanoxylon*

The species is wide spread in tropical Africa from Senegal and Cote d'Ivore in the West, to Kenya and Ethiopia in the East, and extending south to South Africa. It is found in at least 26 sub-Saharan countries (Nshubemuki, 1993). Apart from Africa, *Dalbergia melanoxylon* is also found in India around Pune City (Lamrood *et al*., 2001). In Tanzania, *Dalbergia melanoxylon* is abundantly found in T_4, T_5, T_6 and T_8 floristic regions and less abudant in T_1, T_2, T_3 and T_7 floristic regions (Redhead and Temu, 1981). *Dalbergia melanoxylon* inhabit rainforest and open miombo woodlands of Tanzania in which rainfall is often marginal with long drought periods. Such areas are environmentally fragile and species vitality depends on a delicate balance between populations within them. *Dalbergia melanoxylon* has adapted well to this marginal habitat and its long growing period is an outcome of this adaptation to harsh conditions (Arbonnier, 2004). *D. melanoxylon* grows under a wide range of conditions including semi-arid, sub-humid and tropical lowland areas. It is often found on dry, rocky sites but is most frequent in the mixed deciduous forests and savannahs of the coastal region. This species demands water and light and therefore will not regenerate under heavy cover. Mature trees are fire tolerant (Arbonnier, 2004).

Growth characteristics and biophysical limitations of *Dalbergia melanoxylon*

Dalbergia melanoxylon growth is naturally slow (Arbonnier, 2004). It thrives well from sea level to 1200m altitude with an average temperature of between 18^{o}C and 35^{o}C and 700mm to 1200mm of rainfall. The species grows in soils varying from loamy sand to vertisols "black cotton soil" (Nshubemuki, 1993). The species is able to withstand regular fires and hence mature trees can be damaged but usually not killed by bush fires (Nshubemuki, 1993).

Propagation efforts of *D. melanoxylon* in Tanzania

As an effort towards propagation of *D. melanoxylon* in Tanzania, the African Blackwood Conservation Project (ABCP) was established in 1996 by James Harris, a woodworker from Texas, USA, and Sebastian Chuwa, a botanist from Tanzania (Msanga, 1999). Its purpose was to replenish the population of the African Blackwood, to sensitize the community on economic and ecological importance of the species, to sensitize the community on seedling production for propagation of the species, to conduct research and keep records of their findings. Efforts by ABCP used natural regenerative method (seed germination in soil) which is associated with low regenerative ability and therefore efforts were not successful. Because of the extensive use of this wood for carvings, instrument and woodworking trades, its supply have been decreasing due to exploitation at an unsustainable rate (Msanga, 1999). Although the tree grows in other parts of Africa, the major supply is from Tanzania and Mozambique, countries which have ideal climatic conditions and a characteristic suitable to the specific uses of the wood materials for instrument and carving trades. Trees from other areas lack some of the special qualities (eg tissue packing, color and density) that makes the wood materials not ideal for commercial enterprises (Msanga, 1999). Because of over exploitation from its natural habitat, the tree is now commercially depleted in Kenya and in some areas of Tanzania. If present exploitation continued with no attempts to replant the tree, geographic areas that are practically devoid of the tree will increase, thus adversely increasing conservation concern in the East African ecosystem in which it grows and affecting numerous commercial enterprises throughout the world. (Msanga, 1999). The ABCP propagation efforts were not based on advanced

seedling production techniques but were based on seed germination which was limited by low seed viability and germinability. *D. melanoxylon* has not been cultivated extensively.

Previous and recent propagation studies on *D. melanoxylon* in Tanzania

The IUCN, (2008) reported that *D. melanoxylon* on one side have low regeneration ability while in the other hand is over harvested such that it is threatened in African countries like Kenya and categorized as Low/Risk near threatened in Tanzania. Previously, studies by Redhead and Temu (1981), Nshubemuki (1993), Sharman (1995), TTSA (1995) and Msanga, (1999) reported low seed viability of less than 30% in *D. melanoxylon* which is also associated with low seed germination and that the growth rate of the species is 70 to 100 years to attain a harvestable age. These reports are supported by recent studies conducted by Amri, (2008), Amri, (2010). A study conducted by Amri, (2008) reported that *Dalbergia melanoxylon* has low seed viability, seed germination and seedling growth rate. In a related study seed viability varied with different time of seed harvesting, low seedling growth rate was also observed On the other hand, Amri, (2010) reported an increased rooting ability of 70% stem cuttings using root promoting hormones (IBA). All these studies did not employ the advanced plant-production techniques such as tissue culture in *Dalbergia melanoxylon* as already pointed out by Redhead and Temu, (1981). Although micropropagation has not been conducted on *D. melanoxylon*, investigations have been done in Tanzania and other countries to improve cassava, sisal, cashew nuts, banana and other crops by micropropagati This study intended to test if *D. melanoxylon* seed germination in tissue culture (MS) can exceed that natural germination in the soil.

Objective of the research

To assess the effective means of seedling production in the African Blackwood) *Dalbergia melanoxylon* between soil and Murashige and Skoog (MS) seed germination.

MATERIAL AND METHODS

D. melanoxylon seed germination in MS

A total of 2 kilograms of *D. melanoxylon* seeds were purchased from TTSA on January 2010 for MS and soil germination experiments. These seeds were 62% tested viable by tetrazolium test. A total of 500 seeds were used in this experiment in 10 replicates having 50 seeds each replicate. The purpose was to compare full and half strength Murashige and Skoog (MS) in effecting seed germination. About 150 seeds were sterilized in 70% ethanol for 30 minutes and 2.6% sodium hypochlorite for 30 minutes and inoculated in full strength MS at pH 5.8. Another 100 seeds were sterilized in 35% ethanol for 20 minutes and 2.6% sodium hypochlorite for 20 minutes and inoculated in half strength MS at 5.8. Other 150 seeds were sterilized in 70% ethanol for 10 minutes and 2.6% sodium hypochlorite for 10 minutes and inoculated in full strength MS at pH 5.8 while the last 100 seeds were sterilized in 35% ethanol for 10 minutes and 2.6% sodium hypochlorite for 10 minutes and inoculated in half strength MS at 5.8 pH. The cultures were incubated in a culture room at 28^{o}C under a photoperiod of 16 hours at 3000 lux light intensity provided by cool white fluorescent tubes. Germination was monitored for 4 months between January to April 2010.

D. melanoxylon seed germination in the soil

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. A total of 360 seeds were soaked in water for 6 hours before sowing while another total of 360 seeds were sown without prior soaking treatment. Each of these 2 main treatments was then subdivided into three sub treatments that were assigned to 3 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. Moisture treatment levels applied per day in the first month (January 2010) 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 12pots, and 36pots 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 4 weeks per replicate. 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, 2010). 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.

RESULTS

D. melanoxylon seed germination in MS media

A total of 19.8% of seeds inoculated in half strength of MS medium germinated while only 6.8% of seeds inoculated in full strength of MS medium germinated (Tables 1, 2 and Plates 1, 2). This germination was at sterilization of 20 minutes in 35% ethanol and 20 minutes in 2.6% sodium hypochlorite at pH 5.8 and culture room condition of 28^{o}C under a photoperiod of 16 hours at 3000 lux light intensity provided by cool white fluorescent tubes.

D. melanoxylon seed germination in the soil

Germination percentage ranged from 2 to 21% in soaked seeds and 0.5 – 15% in non-soaked seeds starting from 7 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 3 and plate 3). A maximum of 21% of seed germination in this experiment as indicated in table 3 is relatively low. These results conform and agree with the results by Amri (2008) in which germination was found to be between 20% to 50% while seed viability and seedling growth were also found to be relatively low.

Effect of moisture level on seed germination in the soil

The effect of moisture level in the potting media on seed germination is as shown in Table 4. 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 favorable 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 (Nshubemuki, 1993) including moisture status of the environment as was observed during conduction of this research.

DISCUSSION

When Murashige and Skoog medium is used for seed germination, callus production, shoot or root initiation what can affect the intended result is viability of the inoculated ex-plant (seed, root tip, shoot tip, leaf bud, embryo), concentration of the MS medium (Full strength or Half strength), concentration of the sterilizing reagents (e.g. 35% alcohol or 70% alcohol) and duration used to sterilize the ex-plants. If viability could be the only factor to determine germination in the MS in this experiment, it was likely possible to get more than 50% germination as supported by viability test results. The co-factors are source of 19.8% germination in this experiment. For example when seeds were sterilized in 35% ethanol for 30 minutes and 2.6% sodium hypochlorite for 30 minutes, most of cultures remained uncontaminated but did not germinate. Three interpretation can be given to these results: one is successful sterilization but which killed the ex-plants, second is successful sterilization but there was no viable ex-plant in the trial, lastly is that some other necessary conditions was not attained such as light intensity or temperature. Other sterilization trials were subjected to 70% ethanol for 30 minutes and 2.6% sodium hypochlorite for 30 minutes. In this trial inoculums also did not get contaminated but also did not germinate the seed meaning that sterilization was successful but killed the ex-plants or sterilization was successful but there was no viable ex-plant in the trial or any other necessary condition was not attained. When only10 minutes was used to sterilize *D. melanoxylon*, ex-plants in either 35% ethanol and 2.6% hypochlorite or 70% ethanol and 2.6% sodium hypochlorite all inoculums gate contaminated

meaning that this duration does not keep the ex-plants in aseptic conditions. When 20 minutes was used to sterilize *D. melanoxylon* ex-plants in either 35% ethanol and 2.6% hypochlorite or 70% ethanol and 2.6% sodium hypochlorite at least 19.8% of inoculated ex-plants germinated meaning that this duration did not kill cells or embryo of all ex-plants but also sterilized successful most of the viable ex-plants. This range of concentrations and duration for sterilizing *D. melanoxylon* ex-plants was in agreement with Gamborg, (2002) who reported that "some plant species can respond to many media combinations, others can respond to only one media combination while others do not respond to any media combination". It is evident from the study that seeds of *D. melanoxylon* are vulnerable to higher moisture level available which is in agreement with findings by Msanga, 1999. This was also pointed out by Arbonnier (2004) that, *D. melanoxylon* seeds are orthodox (Moisture intorelant) needs 9-12% moisture content and 3^{o}C to stay viable for a long period. 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 the *in-situ* seedlings are reported to originate from root suckers (Washa, 2008).

Most domesticated plant species have been subjected to genetic transformation such as chromosomal and DNA recombination in such a way that they can be easily assessed and sterilized for tissue culture (Kumar *et al*., 2010). *D. melanoxylon* is one of the forest plant which is neither genetically transformed nor domesticated and therefore is colonized by various microorganisms hence is difficult to keep in aseptic conditions for tissue culture. For such forest plants it needs so many trials of sterilizing materials. Some treatment combinations of *D. melanoxylon* ex-plants in this study did not generate seed germination in the media because there were either no viable seed, sterilization was not enough or sterilization killed the seed embryos because the extent of sterilizing and ratios for the hormonal combination for *D. melanoxylon* was not known. For plant which is subjected to tissue culture for the first time such as *D. melanoxylon*, the conditions and parameters needs many trials to be known including hours of light exposure of inoculated materials, hours in darkness, quantity and quality of light intensity, temperature and type of ex-plant which can respond easily. All these have lead to difficulties for *D. melanoxylon* seed germination in the MS.

CONCLUSSION AND RECOMMENDATION

Dalbergia melaanoxylon low seed viability seems to be a strongest hindering factor of seedling production in both the soil and the MS medium since 21% germination in the soil and 19.8% of the MS does not show a statistically significant difference but soil germination is still have high percentage. Since the low seed viability have been reported from Redhead and Temu (1981), Nshubemuki (1993), Sharman (1995), TTSA (1995), Msanga (1999), Amri (2008) and Amri (2010), two suggestions are given from this research, one is to genetically transform the species so that can be easily assessed to germinate and produce good number of seedlings, second is to use the few occurring seedlings from soil and MS germination in tissue culture for multiple production of seedlings through callus.

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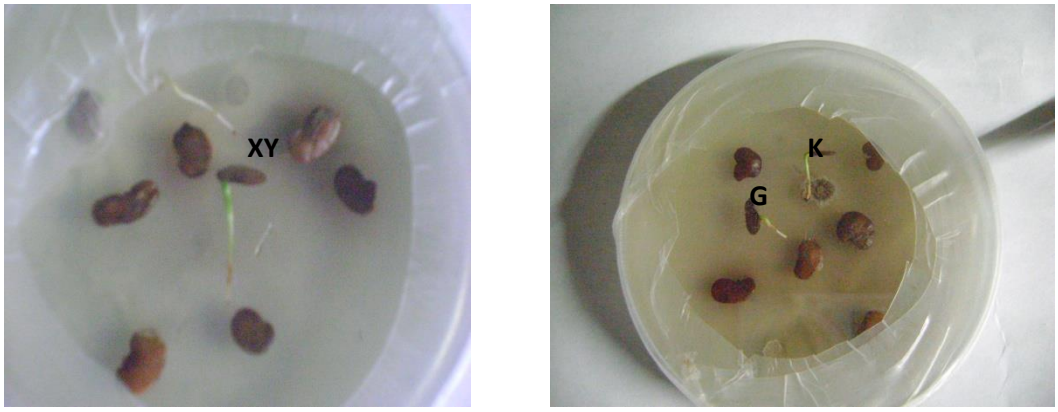
Table 1: *D. melanoxylon* seed germination in MS medium January-April 2010

Replica	#seed inoculated	#seed germinated	MS status	Sterilization time
1	50	0	1	1
2	50	3	1	1
3	50	2	1	3
4	50	5	2	3
5	50	3	2	3
6	50	0	1	3
7	50	10	2	2
8	50	2	2	2
9	50	0	1	1
10	50	0	1	3

NB: Full strength MS= 4.310 g/l, Half strength MS=2.155 g/l, MS stands for Murashige and Skoog (1962), MS status 1= Full strength, 2= Half strength, Sterilization status 1= 30 minutes, 2= 20 minutes, 3= 10 minutes

Table 2: One-Sample Statistics

	N	Mean germination	Std. Error Mean
Half Strength germination	10	10.40 ±2.716	.859
Full Strength germination	10	3.40 ±2.066	.653



Plates 1 and 2: Germinated seeds in MS media (Seed XY, G and K)

Table 3: Main effect of seed treatment on seed germination

Seed treatment	Moisture level	Germination %				Mean	LSD
Soaking		January 2010	February 2010	March 2010	April 2010	Mean	
	Low (100ml)	3.8	1.6	13.3	10.8	7.4	
	Mediam (200ml)	2.5	6.6	18.1	21.5	12.2	
	High (300ml)	0.00	0.8	11.6	12.5	6.2	15..3
	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	
	Mediam (200ml)	0.00	0.8	14.9	19.8	8.9	
	High (300ml)	0.8	1.6	11.7	10.8	6.2	14.9
	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		

Table 4: Mean seeds germination in response to different moisture level

Moisture level	January 2010	February 2010	March 2010	April 2010
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

Plate 3: Sample of germinated *D. melanoxylon* seed in the soil