

Effect of Timing of Seed Collection and Provenance on Seed Viability and Germination of *Dalbergia melanoxylon*

^{1,2}E. Amri, ¹H.V.M. Lyaruu, ¹A.S. Nyomora¹ and ¹Z.L. Kanyeka

¹Department of Botany, University of Dar es Salaam, P.O. Box 35060, Dar es Salaam

²Dar es Salaam Institute of Technology, P.O. Box 2958, Dar es Salaam

Abstract: *Dalbergia melanoxylon* is one of the most expensive timbers in the world used for the manufacturing of musical instruments and decorative objects such as carvings. The effect of timing of seed collection and provenance on seed viability and germination were investigated. Percentages of germination capacity and germination energy were significantly higher for seeds collected 12 and 16 weeks after maturity. Seed viability was highest 59.8% at fruit maturity stage and significantly decreased to 25.7% for seeds collected 24 weeks after maturity. Significantly highest germination capacity 25.8% was obtained for seeds collected from Ubena provenance followed 20.5% for seeds collected from Mkundi provenance. Percentage of seed viability collected from Ubena was superior 57.56% to other provenances followed Mkundi provenance, which had 49.42%. Seed collection in *D. melanoxylon* should be done between 8-16 weeks after maturity from superior provenances to obtain seeds with high germination energy and germination capacity.

Key words: *Dalbergia melanoxylon*, germination capacity, germination energy, seed viability, seed collection, provenance

INTRODUCTION

Dalbergia melanoxylon is an economically important tree with high-quality wood and one of the most expensive timbers in the world used for the manufacturing of musical instruments and decorative objects such as carvings (Jenkins *et al.*, 2002). Natural regeneration of *Dalbergia melanoxylon* is limited as the species is usually raised from the seeds with poor germinations (Mbuya *et al.*, 1994). Seeds of *Dalbergia melanoxylon* are papery, pea-like indehiscent pods, which may remain on the trees after maturity for about 7-9 months, however, the seeds remained on the trees are prone to insect infestation (Mugasha *et al.*, 2004). To curb further deterioration of the remaining natural populations, sustain the supply of the raw material and increase the overall productivity, the knowledge of timely seed collection for domestication of *Dalbergia melanoxylon* is considered important.

One of the aspect of quality fruit/seed is that the seed should be collected at the right stage of maturity as early collection may led to collection of immature fruits/seeds while delayed collection leads to loss of seed crop or the seed or fruit may become dormant (Virendra *et al.*, 2005). In some species, seed germination may increase during early stages of collection after full

maturity is achieved at the time of seed fall while other species may be later after maturity (Bhardwaj *et al.*, 2002; Shruti *et al.*, 2006). Seed maturation time is an important factor to consider during collection as harvesting too early may result in losses due to incomplete development while delayed collection may result in reduced viability due to exposure to others factors such as hardening of seed coat, insect-pest and disease damage (Hossain *et al.*, 2005). Seed collection needs to be done at the time that would optimize seed viability, germination and longevity in storage (McCormark, 2004).

Populations of widely distributed species may show significant variation in seed viability and germination responses among seed provenances (Sivakumar *et al.*, 2002; Thomsen and Kjær, 2002; Mkonda *et al.*, 2003; Loha *et al.*, 2006). Thus, a basic knowledge about the nature and extent of seed source variation in relation to seed parameters such as viability and germination characteristics is very useful for the production of quality seedlings of *D. melanoxylon*. Consequently, an attempt has been made in this study to determine the optimum time for seeds collection and the effect of provenance on seed viability and germination characteristics of *D. melanoxylon* for development of strategies for the production of quality seedlings for agroforestry programmes.

MATERIALS AND METHODS

Seed collection: Seeds of *D. melanoxylon* were collected from Mkundi, Mikumi National Park and Ubena provenances in Morogoro region. Other collections were made from Madale and Mbezi provenances in Dar es Salaam region and Kibaha provenance in Coast Region. Selected seed sources ranged from 06°41'43"-07°06'16.1"S latitude and 039°08' 41.6"-037°14'10.3"E longitude and altitude from 104-522 m a.s.l. In each provenance, seeds were collected from 20 healthy trees separated by a distance of 100 m that were randomly selected and marked before fruit maturity.

The observations of flowering up to fruiting were done in all provenances. After on set of fruiting observations were done for pods/fruits development up to maturity stage by observing colour changes of the pods from green as immature tender pods to brown colour pods, finally to permanent grey colour pods, which was an indication of peak of fruit maturity stage. Timing of seed collection started at the peak of fruit maturity at collection date (T_0) followed by other collections T_1 , T_2 , T_3 , T_4 , T_5 and T_6 done after every 4 weeks corresponding to 4, 8, 12, 16, 20 and 24 weeks after peak of fruit maturity.

Experimental design and seed parameters evaluated: The experiment was laid out in spilt plot design, the main plots were the provenances and subplots were timing seed collection dates (T). The following seed parameters were determined: Germination Capacity (GC) and Germination Energy (GE). GC is the proportion of total germinated seeds to that of sown seeds, expressed in percentage. GE, also expressed in percentage, is computed as the percentage of germination when mean daily germination (cumulative germination divided by time elapsed since sowing date) reached its peak. GE is one of the commonly employed indices of speed of germination. Other seed parameters, which were determined are seed viability and seed moisture content expressed in percentages.

Seed germination test: Pods of *D. melanoxylon* collected in each collection date were segmented into parts containing 1 seed in each part and soaked in cold water for 6 h prior to germination test. Four replicates of 25 seeds from each provenance at each collection time were sown on sand medium in plastic trays and then covered with sand at a uniform depth of 0.5 -1.0 cm, which was kept moist by watering at every alternate day. The germination room was maintained with 12 h of daylight and 12 h of darkness, at temperature in range of 25-30°C. The germination process was evaluated daily after commencement of germination and continued till constant

number. A seed was considered germinated when the radicle had emerged above the surface of sowing media indicating that the seedling is likely to become established successfully.

Tetrazolium test for seed viability: Tetrazolium Viability (TZ) test for freshly collected seeds of *D. melanoxylon* was done using four replicates of 25 seeds for each collection date. To prepare the seeds of *D. melanoxylon* for TZ tests, pods were first moistened for 6 h at room temperature then, each segmented pods containing one seed was cut off 1/6 of the pod at the end and imbibed in 1% solution of 2, 3, 5-triphenyl tetrazolium chloride for 24 h in the dark for evaluation of the staining pattern of embryo (Moore, 1985). When staining was complete, seeds were immediately rinsed 2-3 times with distilled water. Seeds were scored in 3 classes: full stain (completely red stain), partial stain (some colour) and no stain. Seeds were considered to be viable only if a completely red stain was observed (Moore, 1985). Viable seeds were expressed as a percentage of the total.

Seed moisture content determination: Seed Moisture Content (MC%), expressed on a Fresh Weight (FW) basis, was also determined for each collection time. Four replicates of 5 g each of *D. melanoxylon* seeds (pods) were first broken down into pieces then dried at 103°C for 17 h and then reweighed. Moisture content was calculated as;

$$MC\% = \frac{FW-DW}{FW} \times 100$$

where:

MC % = Moisture Content expressed in percentage
FW = Fresh Weight
DW = Dry Weight

Data analysis: Data analysis was done using Genstat 5 Release 7.22 DE computer software package. Before analysis, the percentage data in germination, viability and moisture content were arcsine transformed to meet the normality assumption for the analysis of variance. The Analysis of Variance (ANOVA) procedures were used to test for significant effect of treatments. For significant treatments, means were separated by Duncan Multiple Range Test (DMRT) for comparisons of different means.

RESULTS

Flowering and fruit maturity: Variations in dates for flower initiation in *Dalbergia melanoxylon* was observed for the different provenances. On set flowering for Ubena, Mkundi and Mikumi NP provenances were observed in

the 1st week of September and formation of pods were visible the after 7 weeks by the end 3rd week of October. Twenty two weeks since on set of flowering in *D. melanoxyton* in these provenances green pods started turning brown, followed by grey colour, which was a sign of maturity of pods by the end 4th week of March. For Madale, Mbezi and Kibaha provenances on set of flowering were observed in the 1st week of October and formation pods also followed after 7 weeks by the end of 3rd week of November. Pods development and colour changes to brown and finally grey for maturity was marked in the 4th week of April.

Effect of timing seed collection on seed parameters:

Analysis of variance showed that the effect of timing of seed collection had significant ($p < 0.001$) effect on seed viability, moisture content and germination capacity of *D. melanoxyton* (Table 1). Generally for the different timing seed collection dates, seeds germination started between 8-9 days after initiation of the experiment with a rapid germination during 12-15 days, followed by occasional germinations thereafter.

Means separation by Duncan's Multiple Range Test (DMRT) revealed that seed viability was significantly different ($p < 0.05$) between different timing seed collection dates (Fig. 1). Seed viability was highest 59.8% at the peak of fruit maturity in T_0 and continued to decrease for different collection dates up to the lowest 25.7% in T_6 at 24 weeks after the peak of maturity. Seed viability for seeds collected in T_3 and T_4 were not significantly different also for seed collected in T_5 and T_6 were not significantly different from each other.

Seed moisture content was significantly different ($p < 0.05$) between different timing seed collection dates as revealed by DMRT (Fig. 1). Highest mean percentage seed moisture content was 20.2% for seed collected at the maturity stage in T_0 and subsequently decreased sharply to 13.6% in T_2 . Mean separation by DMRT showed that seed moisture content was significantly higher on the first 3 collection dates (T_0 , T_1 , T_2), which correspond to collection at the stage of maturity, 4 and 8 weeks after maturity. However, the difference in moisture content amongst collection dates T_3 - T_6 corresponding to 12, 16, 20 and 24 weeks after maturity was not significant ($p > 0.05$) by DMRT.

Germination capacity significantly differed ($p < 0.05$) between different timing seed collection dates as revealed by DMRT (Fig. 2). The highest mean germination capacity was 27.3% for seeds in collection date T_3 , followed by 25.4% for seeds in collection T_4 . The 2 collection dates T_3 and T_4 corresponding to 12 and 16 weeks after maturity were not significant different in germination capacity. The

Table 1: Analysis of Variance values (mean squares) for the effect of timing seed collection, provenance and their interactions on seed parameters of *D. melanoxyton*

Source of variation	df	Seed parameters		
		Viability (%)	Moisture content (%)	Germination capacity (%)
Replicate	3	344.98 ^{ns}	0.08 ^{ns}	5.87 ^{ns}
PRO	5	3540.90*	6.05 ^{ns}	936.55*
Error (a)	15	116.95 ^{ns}	0.09 ^{ns}	29.42 ^{ns}
TSC	6	4356.41*	277.57*	1870.61*
TSC×PRO	30	71.12 ^{ns}	1.79*	65.30 ^{ns}
Error (b)	108	55.47 ^{ns}	0.16 ^{ns}	40.29 ^{ns}

ns: not significant; *Significant at $p < 0.001$; df: degree of freedom; Rep: Replicate blocks; TSC: Timing Seed Collection; PRO: Provenance

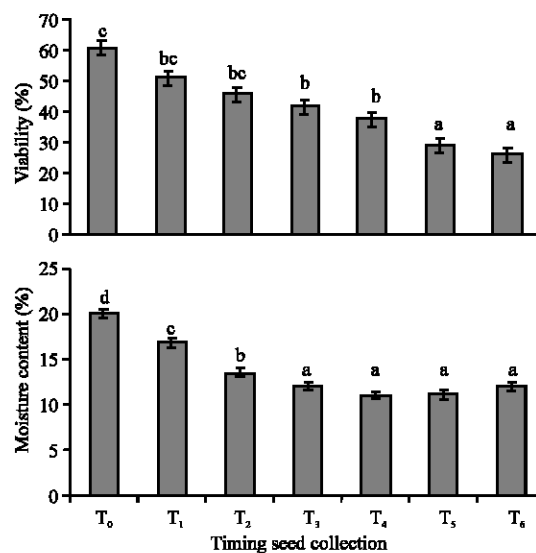


Fig. 1: Effect of timing seed collection dates on percentage seed viability and percentage seed moisture content of *D. melanoxyton* seeds. Timing seed collection dates are denoted as $T_0 = 0$, $T_1 = 4$, $T_2 = 8$, $T_3 = 12$, $T_4 = 16$, $T_5 = 20$ and $T_6 = 24$ weeks after seed maturity

lowest mean germination capacity was 2.2 % for seeds in collection date T_0 at the stage of seed maturity and then followed by significant increase in germination capacity for collection dates T_1 , T_2 and T_3 . Significant reduction in germination capacity was observed in increasing order with advancement of time for timing seed collection from T_4 - T_6 . Generally timing seed collection from T_2 - T_4 corresponding to 8, 12 and 16 weeks after maturity had high germination capacity (Fig. 2).

Results for the determination of speed of germination known as Germination Energy (GE) also significantly differed ($p < 0.05$) between different timing seed collection dates as revealed by DMRT (Fig. 2). Germination energy was low during early collection dates in T_0 - T_2 . The highest germination energy was 16.8% during collection date in T_3 followed 15.3% collection

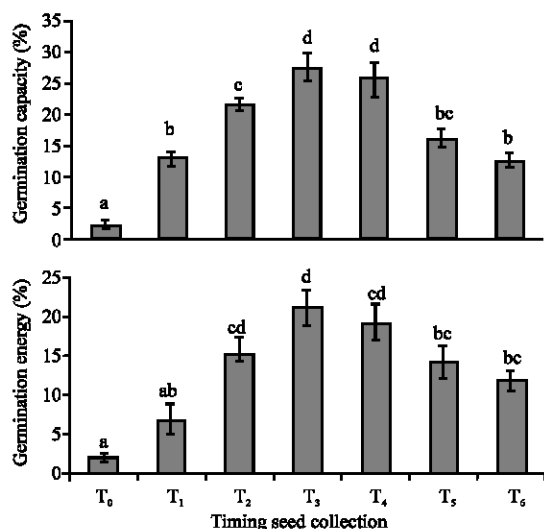


Fig. 2: Effect of timing seed collection dates on percentage germination capacity and percentage germination energy of *D. melanoxylon* seeds. Timing seed collection dates are denoted as T₀ = 0, T₁ = 4, T₂ = 8, T₃ = 12, T₄ = 16, T₅ = 20 and T₆ = 24 weeks after seed maturity

date in T₄ corresponding to 12 and 16 weeks after maturity, respectively (Fig. 2). Germination energy was not significantly different for collection dates in T₅ and T₆. Collection dates with high germination energy corresponded with collection dates which had high germination capacity.

Effect of provenance on seed parameters: Analysis of variance showed that provenance had significant effect ($p < 0.001$) on seed viability and germination capacity (Table 1). The effect of provenance had no significant effect on seed moisture content. Among the seed parameters measured the interactive effect of provenance and timing seed germination was significant for seed moisture content only. Mean percentage seed viability of seeds collected from Ubena was superior 57.56% to other provenances followed by seeds collected from Mkundi which had 49.42%, while the percentage viability of seeds collected from Mikumi NP was extremely low 29.1% (Fig. 3). Means separation by DMRT revealed that seed viability for Mbezi, Kibaha and Mikumi NP provenances were not significantly different ($p > 0.05$). DMRT also revealed that seed moisture were not significant different ($p > 0.05$) among all the provenances in which seeds were collected (Fig. 3).

Significant highest mean germination capacity 25.8% was obtained for seeds collected from Ubena provenance followed 20.5% for seeds collected from Mkundi

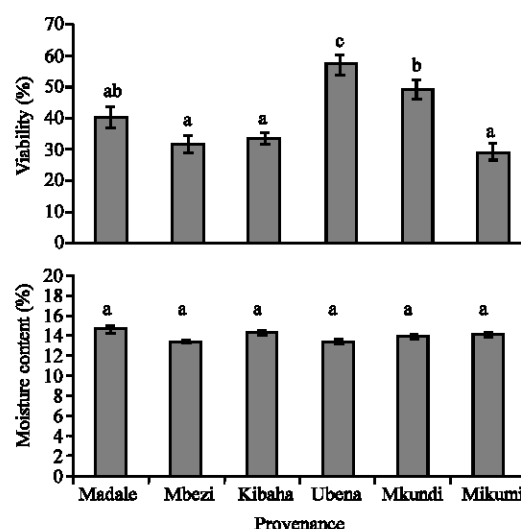


Fig. 3: Effect of provenance on percentage seed viability and percentage seed moisture content of *D. melanoxylon* seeds

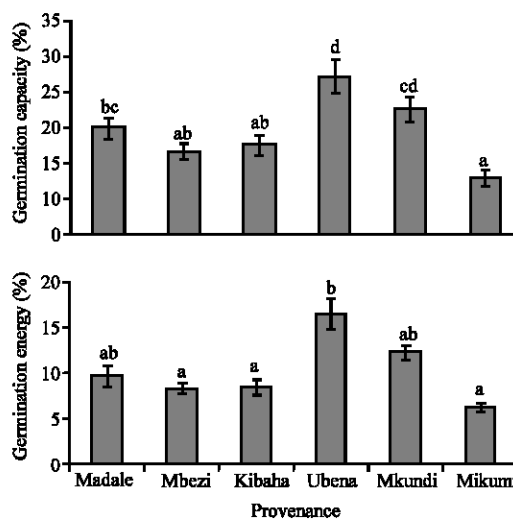


Fig. 4: Effect of timing seed collection dates on percentage germination capacity and percentage germination energy of *D. melanoxylon* seeds

provenance, while germination capacity of seeds collected from Mikumi NP was the extremely low 9.2% (Fig. 4). Mean germination capacity of seeds from Kibaha and Mbezi provenances were not significantly different. The speed of germination, as determined by the germination energy also showed considerable variation among provenances, which was significantly highest 16.6% for seeds collected from Ubena provenance followed by 12.3 and 9.6% for Mkundi and Madale, respectively. Germination energy of seeds from Kibaha, Mbezi and Mikumi NP provenances were not significantly different (Fig. 4).

DISCUSSION

The results of this study demonstrated that germination responses of *D. melanoxylon* seeds were significantly affected by timing seed collection. Low germination capacity in early seed collection dates despite high seed viability might be due to different stages of seed maturity in those collection dates resulting in presence of marginally viable but non germinable embryos. In most of the tree species, seed matures in a phased manner such that few seeds ripen and the number gradually increases till it reaches at the peak of physiological maturity (Shruti *et al.*, 2006). Thus, germination capacity in early collection dates can be poor due physiological immaturity of seed, which is attributed either due to the incomplete development of the embryonic axis and/or to the availability of reserve compounds necessary for the germination and for the initial development of the seedlings (Virendra *et al.*, 2005). High seed moisture content during early collection dates also might have contributed to poor germination capacity in *D. melanoxylon*. Pandit *et al.* (2002) reported that germination of seeds may be inhibited if seed moisture is too high.

High seed germination capacity was achieved for seeds collected from 8-16 weeks after maturity, which seems to be an optimal time for seed collection as well time when most of seeds collected are at physiological maturity period. This is the period of the year between June and August. The range of the seed moisture content between 13.6 and 11.1% coincided with timing seed collection dates, which had high germination capacity and appear to be an indicator of physiological maturity in *D. Melanoxylon* seeds. Seeds collection at an optimal physiological maturity stage is more desirable because at this period seeds are of more uniform quality and higher vigour affecting further the germinability and longevity in storage of seeds (McCormark, 2004).

Low seed germination capacity revealed in late collection dates from 20-24 weeks after maturity could be due to the reason that some seeds exhibited dormancy. Apart from dormancy, low germination capacity could have been attributed to insect infestation to seeds, which were highly observed during seed collections. Harvesting of seeds after the point of physiological maturity have reported in many trees species that can result in reduced viability and germination due to exposure to factors such as insect-pest and disease damage (Hossain *et al.*, 2005), accelerated seed deterioration due to unfavourable environmental conditions (Guterman, 2000) or fruit may become dormant (Virendra *et al.*, 2005).

The findings from this study are in agreement with several workers, who have reported significant effect of seed collection dates on seed germination markedly in different species. For examples Khera *et al.* (2000) in *Azadirachta indica*, Bhardwaj *et al.* (2002) in *Albizia chinensis*, Bhardwaj *et al.* (2003) in *Acacia catechu*, Virendra *et al.* (2005) in *Fraxinus micrantha* and Likoswe *et al.* (2008) in *Terminalia sericea*. One of the major factors influencing vigour and viability is physiological maturity of the seeds at harvest, markedly affected by environmental factors, mainly temperature, humidity and water availability (Górecki *et al.*, 2001). Hung (2003) reported that the response of maturing seeds to environmental changes in temperature was associated to changes in the content and composition of soluble carbohydrates stored in those seeds.

Although, significant improvement in seed germination of *D. melanoxylon* has been revealed in this study for seeds collected between June and August, but the percentage germination being bellow 50% is still low. The causes of such low germination in *D. melanoxylon* have been also reported by several authors. For instance, Msanga (1998) attributed low and sporadic germination in *D. melanoxylon* to fungal infection. Fungal infection was also observed to seeds of some trees marked for this study during timing seed collections. However, Mugasha *et al.* (2004) reported that fungal infection was not only the cause of sporadic germination in *D. melanoxylon*, there are some insects, which infest the fruits before maturity and delayed seed harvesting causing damage to the embryo.

Germination energy was used study to measure speed of germination, since it gives an idea of the vigour of the seed and of the seedling, which it produces (Ginwal and Gera, 2000). Highest germination energy observed in *D. melanoxylon* indicate that seeds collected between 8 and 16 weeks after maturity will result in rapid germination and seedling emergence therefore, establish itself as quickly as possible to take advantage of favorable environmental conditions. The interest in germination energy is based on the theory that only those seeds, which germinate rapidly and vigorously under favourable conditions are likely to be capable of producing vigorous seedling in field conditions, whereas weak or delayed germination is often fatal (Cardoso *et al.*, 2008). Germination energy has been used in other species as an integrated measure of seed quality in *Acacia nilotica* (Ginwal and Gera, 2000) and *Pinus roxburghii* (Roy *et al.*, 2004).

Testing seed viability with 1% tetrazolium chloride revealed high percentage of viable seeds in early seed collection dates. As noted before, the observed high seed

viability in early collection might be due to physiological immaturity of some seeds collected, which may stain normally because they contain live cells, but would give poor results in a germination test. Significant decreases percentage of viable seeds to other collection dates, which followed could be attributed to some empty pods, without seeds or those with seeds were dead as it was revealed during cutting sample pods for tetrazolium test. Proportion of empty seeds in well-developed pods has been reported in other tree species that may either result from post-fertilisation embryo abortion due to lack of water or nutrients, the absence of pollination, or from low pollen vigour (Campbell and Halama, 1993; Teixeira *et al.*, 2001). Loss of viability of seeds depends upon the time-span usually commences at physiological maturity and continues during harvest, processing and storage (McDonald, 1999; Chauhan and Nautiyal, 2007). The Tetrazolium Staining test (TZ) is an established method of assessing seed viability that is widely used for official and nonofficial applications (Sawma and Mohler, 2002; Nurse and DiTommaso, 2005; Cardoso *et al.*, 2008).

Provenances have also displayed significant differences in seed viability, germination capacity and the speed of germination as determined by the germination energy. Similarly, significant provenance variations in seed parameters for other several species have been reported by different researchers such as Gera *et al.* (2000) in *Dalbergia sissoo* Sivakumar *et al.* (2002) in *Tectona grandis*, Thomsen and Kjær (2002) in *Fagus sylvatica*, Mkonda *et al.* (2003) in *Strychnos cocculoides*, Loha *et al.* (2006) in *Cordia africana* and Seltmann *et al.* (2007) in *Polylepis australis*. Causes of such variability might be generally attributed either to genetic characters of source populations (Ginwali *et al.*, 2005), impact of mother plant to environmental factors such as soil, temperature, light quality, water availability and altitude (Guterman, 2000) or controlled by a given microclimate in a given geographical region (Garcia *et al.*, 2000). Also environmental factors interactions with genetic and physiological factors play important role in determination of provenance variation in seed quality (El-Keblawy and Al-Ansari, 2000; Roy *et al.*, 2004).

CONCLUSION

In concluding, the study has revealed that high seed viability and germination capacity and germination energy of *D. melanoxylon* was achieved for timely seed collecting between 8 and 16 weeks after maturity when seeds have moisture between 13.6 and 11.1%. This the period between July and August, thus suggesting seed collection should be done during these months. Further,

the study also has provided evidence that seed viability, germination capacity and germination energy vary considerably among provenances of *D. melanoxylon*. On the basis provenance results, it is advisable that seed collections should be made from Ukena and Mkundi provenances, which had significant high performance of seed parameters to achieve better germination.

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