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Variation in Pre-Dispersal Seed Predation and Seed Traits among Provenances of *Dalbergia Melanoxylon* (Guill. & Perr.)

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Abstract

Dalbergia melanoxylon is an important timber species used for the manufacturing of musical instruments and decorative objects such as carvings. The extent of pre-dispersal seed predation variation among *D. melanoxylon* provenances for seed harvested after maturity at different collection dates was investigated. Provenance variation in seed morphological traits in terms of seed length, width, number of seeds/pod and 100 seed weight were also examined. The effect of provenance and seed collection date was significant ($p<0.001$) for seed predation and seed viability. Madale provenance had the highest seed predation of 42.52% while Mkundi provenance had significant lowest seed predation of 31.54% which was marked with significant high seed viability and germination 71.92% and 36.62% respectively. Optimal seed germination 43.14% was achieved for seeds collected 8 weeks after peak maturity which had 37.93% of seed predation. The highest seed predation was 47.13% for seeds collected 20 weeks after peak of fruit maturity. Provenance variation in seed morphological traits also revealed that Mkundi provenance had significant high pod length 4.53 cm, pod width 1.47cm and 100 seed weight 64.25g. Correlation analysis revealed highly significant positive correlation between pod width with seed viability and seed weight with seed germination. Provenance with low seed predation and good morphological traits should be used as seed source and seed collection date should be between 4 to 12 weeks after peak maturity to obtain the desired quality seeds of *D. melanoxylon*.

Keywords

Dalbergia melanoxylon; Seed predation; Seed morphological traits; Seed collection dates; Provenance

Introduction

Dalbergia melanoxylon (Guill. & Perr.) known as African blackwood is an economically important tree with high-quality wood and one of the most expensive timbers in the world mainly used for the manufacturing of musical instruments and decorative objects such as carvings [1,2]. As a result of the valuable products derived from *D. melanoxylon*, high exploitation pressure has been exerted to the extent of threatening future existence in its natural habitats. Natural regeneration of *D. melanoxylon* is limited due to poor seed germination while its genetic diversity in its native range currently reported as threatened [3,4]. *D. melanoxylon* is categorized as lower risk/near threatened in the International Union for Conservation of nature (IUCN) red list of threatened species [5]. To curb further deterioration of the remaining natural populations of *D. melanoxylon*, improving its propagation and in order to attain sustainable exploitation, understanding of seed predation in *D. melanoxylon* is important for timely collection of good quality seeds for domestication of this species.

Seeds of *D. melanoxylon* are papery, pea-like indehiscent pods which may remain on the trees after maturity for about 7 to 9 months, however, the seeds remained on the trees are prone to insect predation [3]. Seed predation by insect can cause damage to plants by insects directly infesting the reproductive structures such as flowers, fruits and seeds. Seed predators have direct and evident effects on plant health and frequently affect the patterns of plant recruitment for individual species [6]. Seed predation is considered as selection pressure affecting a wide range of host plant traits including morphology, life history and mating system which is very important for maintaining genetic diversity, composition and dynamics and even community-level diversity [7,8]. Changes in seed predation have also been shown to interact with other species interactions to the extent of radically altering distance and frequency-dependent recruitment in tropical trees, being a central mechanism maintaining diversity in tropical forests [9,10].

Quality seed has been considered as an important input in domestication of economically important plants. Seed traits polymorphism and seed source variation have been found to play a great role in seed germination, survival and seedling growth [11,12]. One of the aspects of quality fruit/ seed is that the seed should be collected at the right stage of maturity stage to avoid seed predation due to delayed collection after maturity. Seed germination may increase during early stages of collection after full maturity [13,14].

Many seed predators attack seeds pre-dispersal or while they are still attached to the plant, thus delayed seed collection may result in reduced viability due to exposure to others factors such as hardening of seed coat, insect-pest and disease damage [15]. Pre-dispersal insect seed predators frequently kill >90% of developing seeds [6]. Although a trial on provenance variation in seed germination has earlier been reported [16], currently scant information is available on seed predation in *D. melanoxylon*. The species has received relatively little attention despite the known low natural regeneration in the wild. Consequently, an attempt has been made in this study to determine whether there is a significant variation in pre-dispersal seed predation and seed traits among provenances of *D. melanoxylon*. The study also was carried out to determine whether there is a significant variation in seed predation, viability and germination for the different seed collection dates of *D. melanoxylon* after fruit maturity. This study was as an effort for development of strategies for obtaining quality seed for the production of quality seedlings of *D. melanoxylon*.

Materials and Methods

Seed collection

Seeds of *D. melanoxylon* were collected from Madale, Ubena and

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Mkundi provenances in Dar es Salaam and Morogoro region from the Eastern part of Tanzania. Selected seed sources (Table 1) ranged from S06°4I' 43.5" to S06° 40' 03.8" latitude and E039°08' 41.6" to E037°39' 26.0"longitude and altitude from 104 to 475 m a.s.l. In each provenance, seeds were collected from 20 healthy trees separated by a distance of 100 m apart from each other that were randomly selected to avoid narrowing down genetic base due to relatedness or inbreeding and marked before fruit maturity.

The observations from onset 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 young and soft immature tender pods to hardened brown color pods, which later on turned to permanent grey color pods which was an indication of peak of fruit maturity stage. Timing of seed collection dates for investigation of pre-dispersal seed predation started at the peak of pods/fruits maturity at collection date (T0) followed by other collections T1, T2, T3, T4, and T5 done after every 4 weeks corresponding to 4, 8, 12, 16 and 20 weeks after peak of pods maturity. Collections of matured pods of *D. melanoxylon* were done from marked parent trees from the selected provenances.

Experimental design, seed parameters and seed morphological traits evaluated

The experiment was laid out in Spilt plot design for seed parameters namely percentages infested seeds, viable seeds and germinated seeds. The main plots were assigned to provenances with three treatment levels (the selected three provenances) while the subplots allocated to the timing seed collection dates after maturity which had six treatment levels (T0-T5). Percentage infested seeds was used for investigation of seed predation where five replicates with 20 pods from each provenance at each collection date were used. Seed predation was assessed by visual examination of the fruits (pods) exterior and cutting test to examine seed predation inside the pods. Seed viability percentage and seed germination percentage were also done using five replicates each with 20 pods from each provenance at each collection date.

Experimental design for evaluation of seed morphological traits was laid out in One way design (in Randomized Blocks). Seed morphological traits evaluated were pod length, pod width, number of seeds/pod and seed weight. Sample collection for seed morphological traits was only done at once from each provenance at the first collection date after maturity (T0). Five replicates with 20 pods each was used for measurements pod length, pod width, number of seeds/pod. Seed weight measured in five replicates was for each replicate with 100 seeds as per International Seed Testing Association [17].

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 hours prior to germination test. Seed were then 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 hours of daylight and 12 hours of darkness, at temperature in range of 25 to 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.

Viability test

Seed viability was determined using 2,3,5-triphenyl-tetrazolium chloride (TZ). Fleshly collected pods of *D. melanoxylon* were 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 hours in the dark for evaluation of the staining pattern of embryo [17]. When staining was complete, seeds were immediately rinsed two to three times with distilled water. Seeds were scored in three 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 [17]. Viable seeds were expressed as a percentage of the total.

Data analysis

Data analysis was done using GenStat Release 10.3DE Edition 4 computer software package. The analysis of variance (ANOVA) procedures were used to test for significant effect of treatments. Before analysis in order to improve assumptions of normality, data in terms of percentages were converted by arc-sine transformation, whereas in terms of numbers were converted by square root transformation. For the significant effect on treatments, means were separated by Duncan Multiple Range Test (DMRT) for comparisons of different means. Correlation coefficients (Pearson) were also determined in order to know the strength of linear relationship among the parameters as dependent variables.

Results

Twenty four weeks since onset of flowering in *D. melanoxylon* from respective provenances green pods started turning brown, then was followed by permanent grey colour which was a sign of maturity of pods in these provenances. Madale provenance had set on flowerers two weeks earlier than Ubena and Mkundi provenances which on set of flowering were observed in the third week of October compared to other provenances which occurred on the third week of November.

The effect of provenance

Table 1: Seed sources location of *D. melanoxylon* from different provenances.

Provenance	Code	Latitude	Longitude	Altitude (m.a.s.l)
Madale	MDL	S 06°4I' 43.5"	E 039°08' 41.6"	104
Ubena	UBN	S 06°36' 03.6"	E 038°09' 24.0"	350
Mkundi	MND	S 06°40' 03.8"	E 037°39' 26.0"	475

Table 2: Analysis of variance for the effect of provenance and seed collection date on seed parameters of *D. melanoxylon*.

Source of variation	df	Mean squares		
		Seeds infested (%)	Seed viability (%)	Seed germination (%)
Rep	4	46.40^{ns}	392.00^{ns}	27.57^{ns}
Provenance	2	730.25*	1912.67*	896.79*
Collection date	5	595.99*	1905.07*	1447.06*
Provenance X Collection date	10	138.09^{ns}	26.53^{ns}	143.93^{ns}
Residual	51	86.93^{ns}	57.41^{ns}	25.39^{ns}

Analysis of variance for seed parameters evaluated showed that the effect of provenance was significant ($p<0.001$) for percentage seeds infested, percentage seed viability and seed germination percentages (Table 2). Analysis of variance for seed morphological parameters evaluated revealed that the effect of provenance was significant ($p<0.001$) for pod length, pod width, number of seeds/pod and seed weight (g/100 seeds) as indicated (Table 3).

Means separation by Duncan's Multiple Range Test (DMRT) for the effect of provenance on seed parameters revealed that seed predation was significantly different ($p<0.05$) among provenances (Table 4). Madale provenance had the significant highest percentage of infested seeds 42.52% and the provenance with the lowest infested seeds was Mkundi 31.54%. DMRT also revealed significant difference ($p<0.05$) among provenances (Table 4) for percentage seed viability and germination where Mkundi provenance had 71.92% and 36.62% as significant highest percentage of seed viability and seed germination respectively. Madale provenance had significant lowest 53.86% seed viability and 22.29% seed germination.

Means separation by DMRT for seed morphological traits was significant ($p<0.05$) among provenances (Table 4). Seed pod length traits for Madale and Ubena provenances were not significantly different ($p>0.05$) while Mkundi provenance had significant 4.53 cm high pod length compared to other provenances. Pod width varied from 1.16 cm to 1.47cm for Madale provenance and number of seeds per pod varied from 1.13 to 1.41 for Madale provenance and Mkundi provenance respectively (Table 4). The lowest and highest average 100 seed weight was 51.74g and 64.25g observed in Madale provenance and Mkundi provenance, respectively (Table 4).

Effect of collection date

Analysis of variance revealed that the effect of collection date was significant ($p<0.001$) for percentage seeds infested, percentage seed viability and percentage seed germination (Table 2). The interactive effect of provenance and seed collection date was insignificant for all seed parameters evaluated (Table 2).

DMRT for the effect of collection date on seeds parameters of *D. melanoxylon* was significant ($p<0.05$) for the different seed collection dates (Table 5). Percentage of seeds infested was significantly different ($p<0.05$) between different seed collection dates as revealed by DMRT (Table 5). Significant and highest mean percentage of seeds infested was 47.13 % for seed collected at T5 corresponding to 20 weeks after fruit maturity while the lowest percentage seed infested was 28.16% for seed collection date at T0 corresponding to the first collection date after maturity. Mean separation by DMRT showed that high percentage seed viability was significant ($p<0.05$) on the first four collection dates (T0, T1, T2 and T3) which correspond to seed collection at peak maturity, 4 weeks, 8 weeks and 12 weeks after maturity (Table 5).

Seed germination percentage also significantly ($p<0.05$) among seed collection dates as revealed by DMRT (Table 5). Significant highest seed germination was 43.14 % for seeds in collection date T2, followed by 41.97 % for seeds in collection date T3 corresponding to 8 and 12 weeks after peak of fruit maturity. Other collection dates T4 and T5 corresponding to 16 and 20 weeks after maturity were not significant for the percentage seed germination (Table 5). The lowest seed germination was 13.74 % for seeds in collection date T0 corresponding to the first collection after pod maturity. Significant reduction in seed germination was observed after T2 in decreasing order with advancement of time for seed collection dates (Table 5).

Correlation analysis for seed parameters and seed morphological traits presented in correlation matrix (Table 6) revealed that highly significant positive correlation exist between pod width with seed viability; seed weight with seed germination; pod width with seed weight and number of seeds/pod with seed weight. Other observed positive correlation were between pod width with number of seeds/pod and pod length with seed weight (Table 6). Highly significant but negative correlation was observed between seed infested with seed viability. Also significant negative correlation was observed between seed infested with seed number/pod. Other correlations observed were either negative or positive but insignificant (Table 6).

Discussion

The effect of provenance

The study revealed variation in seed maturity dates among *D. melanoxylon* provenances as a result of variation in flowering time among the provenances. Flowering phenology responds to various environmental stimuli such as solar irradiance and temperature [18]. Variation for onset of flowering time and seed maturity has been demonstrated within natural populations of several species [19,20]. Pre-dispersal seed predation by insect in *D. melanoxylon* varied markedly across the provenances. The revealed significant variation on seed predation could be attributed by many factors

Table 3: Analysis of variance for the effect of provenance on seed morphological traits of *D. melanoxylon*.

Source of variation	df	Mean squares			
		Pod length (cm)	Pod width (cm)	Number of Seeds/pod	Seed weight (g/100 seeds)
Rep	4	0.15^{ns}	0.02^{ns}	0.02^{ns}	11.66^{ns}
Provenance	2	6.77*	0.27*	0.26*	472.24*
Residual	30	0.72^{ns}	0.02^{ns}	0.03^{ns}	35.69^{ns}

ns = not significant; * significant at p< 0.001; df = degree of freedom; Rep = replicate blocks.

Table 4: Analysis by DMRT for the effect of provenance on seeds parameters and seed morphological traits of *D. melanoxylon*.

	Seed parameters and seed morphological traits						
Provenance	Seeds infested (%)	Seed viability (%)	Seed germination (%)	Pod length (cm)	Pod width (cm)	No. of Seeds/ pod	Seed weight (g)
Madale	42.52^{b}	53.86^{a}	22.29^{a}	3.08^{a}	1.16^{a}	1.13^{a}	51.74^{a}
Ubena	36.25^{a}	63.62^{ab}	28.36^{a}	3.35^{a}	1.3^{b}	1.20^{a}	57.15^{ab}
Mkundi	31.54^{a}	71.92^{b}	36.62^{b}	4.53^{b}	1.47^{c}	1.41^{b}	64.25^{b}

Means indicated by the same letter on the column are not statistically different at p<0.05

Table 5: Analysis by DMRT for the effect of collection date on seeds parameters of *D. melanoxylon*.

	Seed parameters		
Collection date	Seeds infested (%)	Seed viability (%)	Seed germination (%)
T0	28.16^{a}	79.36^{c}	13.74^{a}
T1	30.5^{a}	71.52^{bc}	27.26^{b}
T2	34.91^{ab}	65.05^{b}	43.14^{c}
T3	37.93^{b}	63.053^{b}	41.97^{c}
T4	42.16^{b}	56.74^{ab}	28.18^{b}
T5	47.13^{c}	43.08^{a}	21.27^{b}

Means indicated by the same letter on the column are not statistically different at p<0.05.

Table 6: Correlation coefficients of seed parameters and seed morphological traits for *B. madagascariensis* stem Cuttings.

	Seed infested (%)	Seed viability (%)	Seed germination (%)	Pod length (cm)	Pod width (cm)	No. of Seeds/ pod	Seedweight (g)
Seed infested (%)	-						
Seed viability (%)	-0.461**	-					
Seed germination (%)	-0.181^{ns}	0.083^{ns}	-				
Pod length (cm)	-0.026 ^{ns}	0.246 ^{ns}	0.016 ^{ns}	-			
pod width (cm)	-0.105 ^{ns}	0.449**	0.237 ^{ns}	0.278 ^{ns}	-		
No. of Seeds/pod	-0.389*	0.306 ^{ns}	-0.010 ^{ns}	-0.215 ^{ns}	0.386*	-	
Seed weight (g)	-0.112^{ns}	0.099 ^{ns}	0.442**	0.347*	0.539**	0.560**	-

** Indicate significant at p< 0.01.
* Indicate significant at p< 0.05.
ns = not significant

ranging from elevation, temperature variation and anthropogenic activities. The Madale provenance which had significant high seed predation is located in lower elevation at 104 m.a.s.l, in the coastal area notably with high temperature. The provenance also being near to the city of Dar es Salaam is prone to anthropogenic activity. Other provenances are allocated a bit higher elevated area and less prone to anthropogenic activity for example Mkundi in Morogoro region at 475 m.a.s.l, thus had significant low seed predation.

Environmental conditions and habitat types are likely to affect interactions between insect seed predators and plants which often influence pre-dispersal predation [21]. It has previously been reported that increased temperature is most significant environmental factor influencing biotic interactions such as insect behavior, distribution, development, survival, and reproduction [22]. Research has shown that some host plants become more susceptible to fungi and rust diseases and insect predation with increased temperature [23]. Furthermore anthropogenic activities have also been reported to influence seed predator abundances thus causing extensive consequences for wider ecological combinations [24,25]. Pre-dispersal seed predation affects plant populations through changes in population dynamics, community structure and species diversity [26-28].

Provenance variation in seed traits revealed in *D. melanoxylon* may also be attributed either to genetic characters of source populations which has previously been reported [4] whereby Mkundi provenance which has revealed significant high seed traits had high genetic diversity compared to other populations. Provenance variation in seed traits, viability and germination could also be attributed to impact of mother plant to environmental factors such as soil, temperature, light quality, water availability and altitude as it has been reported elsewhere [29,30]. Variation could be also controlled by a given microclimate in a given geographical region or environmental factors interactions with genetic and physiological factors which play important role in determination of provenance variation in seed quality [31,32]. Similar variation in seed traits among seed population with respect to seed length, width and weight have previously been reported in other species such as Canarium schweinfurthii, Cordia australis and Pinus roxbrughii [11,33,34]. Fruit and seed dimensions can be considered as important traits for early selection of seed sources. Seed sources with higher average seed traits are likely to give higher germination and field emergence than that of lower seed traits as it has previously been reported in Pongamia pinnat and Albizzia lebbek [35,36].

Effect of collection date

Findings from this study for seed predation in *D. melanoxylon* revealed that timely seed collection after maturity is very important to obtain quality seeds. Although seed predation may occur by some insects attacking flower buds or flowers, other predation may occur during the later phase of seed development, but before the seeds mature. Still more seed predation may occur after maturity which may significantly affect pre-dispersal seed quality and certainly make seed collection wasteful and uneconomical. Pre-dispersal seed predation may cause damage to the endosperm or cotyledons which may rapidly cause the death of the seed due to shortage of food reserve [37].

High seed predation revealed in late collection dates from 16th to 20th week after fruits maturity could be due to prolonged exposure of seeds to environmental conditions which may favor fungal infections and insect predation to seeds. Fungal infection was also observed to seeds of some trees marked for this study during late seed collection dates. Findings of this study are in agreement with findings previously reported that fungal infection was not only the cause of sporadic germination in *D. melanoxylon* which infest the fruits before maturity but also affect the seeds after maturity [3]. Thus delayed seed harvesting could lead poor quality harvested seeds. The maximum seed germination revealed in this study of 43.14% for seeds collected 8 weeks after maturity is relatively higher than the trial previous reported [16]. Thus more delay in harvesting of seeds after the point of physiological maturity can be detrimental to seed quality as it has previously been reported in many trees species that can result in reduced viability and germination due to exposure to factors such as insect-pest predation and pathogen attach [15,37]. Delay seed collection may also lead to accelerated seed deterioration due to unfavorable environmental conditions or fruit may become dormant [29,38]. Several workers have also reported the importance of collection dates on seed germination markedly in different species such as Acacia catechu, Fraxinus micrantha and in Terminalia sericea [38-40].

Seed viability test with 1% tetrazolium chloride revealed high percentage of viable seeds in early seed collection dates. 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 [41,42]. 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 probably due to seed predation by insects 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 [43]. Loss of viability of seeds depends upon the time-span usually

commences at physiological maturity, pre-dispersal and continues in post dispersal, processing and storage [44]. The tetrazolium staining test (TZ) is an established method of assessing seed viability that is widely used for official and nonofficial applications [45,46].

Correlations between seed parameters are considered to be good predictor of species early selection of seed sources. It has previously been reported that seed sources with higher average seed width and seed thickness have fitness measures required and are expected to perform well for seed germination and field establishment in nursery during transplanting [47,48]. In this study positive correlation between *D. melanoxylon* seed weight and seed germination was revealed. Seed weight is considered to be an important attribute for the establishment success of plant species being linked with their seed production, establishment, and survival [49].

Conclusion

In concluding, the study has revealed variation in seed predation among *D. melanoxylon* provenances and the best timing for seed collection dates. Mkundi provenance which had the lowest seed predation, highest seed viability and seed germination has revealed to be a valuable *D. melanoxylon* seed source which should be used for seed collection. Seed collection dates between 8 weeks and 12 weeks after maturity which had a moderate seed predation of 34.91% and 37.93% with high seed germination of 43.14% and 41.97% is a recommendable time which seeds should be collected. Furthermore the study recommend a broad investigation of wider effects of seed predators in *D. melanoxylon* which may start from onset of flowering to seed maturity time consequently quantifying the effect of predation before seed maturity stage. Obtaining quality seeds of *D. melanoxylon* will be one means for successful propagation of this economically important species.

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