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Physiological Characteristics of *Dalbergia melanoxylon* and *Dalbergia sissoo* seeds

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Abstract—The objectives of this work were to study physiological characteristics of *Dalbergia melanoxylon* (Abanous, Babanous) and *Dalbergia sissoo* seeds, and to explore a seed breaking dormancy methods. The study also aimed to compare between the indigenous species *D.melanoxylon* and exotic one *D.sissoo* in seed morphology. Seed physical and physiological characteristics were explored through the determination of seed soundness, damaged and abortive seeds, purity percentage, number of seeds per kilogram, seed moisture content percentage, viability percentage, germination rate, percentage and the phenomenon of seed dormancy. The study revealed that more than 70% of *D.melanoxylon* seeds were abortive or damaged by insects when collected. Seed characteristics of the both *D.melanoxylon* and *D.sissoo* were found to be more or less similar. Extraction of seeds from the pods, sulphuric acid for one minute and dewinging were found to be the best treatments for dormancy breaking. Seeds were found to have a combined external dormancy of chemical and physical type. The study revealed that seeds kept in the normal store and the cool store for 18 months had more or less the same germination behavior. Seed viability was kept high in both stores throughout storage period.

Keywords **- Dalbergia seeds, Dormancy breaking, Seed characteristics, Seed storage.**

I. INTRODUCTION

Trees usually come from seeds, and successful tree planting depends on healthy seeds of good genetic quality, often referred to as (genetic resources) [1]. A true seed is a fertilized mature ovule with an embryonic plant. It has protective coat and contains stored food [2]. In the seed handling the term 'seed' usually refers to the unit extracted from fruit and handled as a unit during storage, pretreatment and sowing [3]. Of all the quality measurement of seed lots, none is more important than the potential germination of the seeds [4].The main aim of a laboratory germination test is to estimate the maximum number of seeds which can germinate in optimum conditions [5]. Therefore a germination or viability test should indicate the potential germinability which with proper handling should reflect expected germination in the nursery [3]. In addition, other seed tests like moisture content, purity and number of seed per weight unit …etc have importance in seed quality evaluation. Seed dormancy refers to a state in which viable seeds fail to germinate when provided with condition normally favourable to germination [3]. Dormancy may be considered as a problem of more of afforestation programmes.

Any part of the fruit or seed, both live and dead tissue, may be involved in seed dormancy. The location and type of dormancy may be revealed experimentally by removing or treating various parts of the fruit or seed separately [6]. External seed dormancy can be divided into mechanical, chemical and physical dormancy [7]. A number of wet treatments involving; soaking seeds in water or sulphuric acid or other liquids, may combine the effects of softening hard seeds coats and leaching inhibitors [5]. Several dormancy types can be associated with the seed-coat, e.g. mechanical resistance, physical barrier to moisture absorption or gaseous exchange, temperature or chemical inhibition, and light sensitivity [8, 9, and 10] and [3].

Storage of tree seeds is becoming an important issue in world of forestry today. Most species with orthodox seed are able to be stored for long periods at room temperature with minimal losses in seed viability [11]. The principal objectives in storage are to prevent excessive loss of water from seeds and excessive respiration or other undesirable biochemical activity. In some instances, storage conditions also should be designed to break dormancy of the embryos. In general, storage involves control of moisture and temperature [1].

The objective of this research work was to study seeds characteristics of two important species, viz. *Dalbergia melanoxylon* an indigenous tree species with high economic value, but is becoming endangered. It is now diminishing out of its native habitat, even in areas, it was noticed that it has limited natural regeneration. The problem may be in the seeds physiological characteristics (viability, dormancy, insect's damage. etc) which are a necessity for the species conservation. And *Dalbergia sissoo* multipurpose an exotic tree species of high economic value with fast growing.

II. MATERIALS AND METHODS

Seed Materials

Seeds of two *Dalbergia melanoxylon* provenances were collected from El Azzaza forest 10 km Eastern El Damazin Blue Nile State, longitude 34º40 E, latitude 11º50 N, rainfall 750mm, soil type clay and El Faid Um Abdalla forest Western Sudan, South Kordofan State, longitude 30º55 E, latitude 11º45 N, rainfall 700mm, soil type clay. *Dalbergia sissoo* seeds were collected only from El Faid Um Abdalla.

Method of Seeds Collection

Pods were collected by hand from the crown of trees or hitting them by a long stick in case of the high trees. A big plastic sheet was spread on the ground under the tree. Collected seed were kept in labeled cotton sacks. Seeds from the different provenances were carried to the National Seed Center laboratory at Soba, seed processing was done. Seeds of each species and provenance were kept separate and four kilograms of each seed lot were used as study sample, using the seed halving method by hand [12].

Seed Physical and Physiological Characters

Seed Status When Collected

During seed collection from tree crown in the forest, it was noticed that the seeds of *Dalbergia melanoxlon* from the two provenances were severely attacked and damaged by insect borers (Bruchids). In addition, a big fraction of them was found abortive. But in *Dalbergia sissoo* there is very few or no damage by insects noticed, but abortive seeds were observed. To know the percentage of each component of seeds (mature seeds, abortive or empty seeds and damage seeds) the cutting test was applied. Four hundred seeds were drawn randomly by dividing the seed sample into four replicates. Pods were cut transversely into two halves and with the naked eye or by hand pressure the percentage of sound, damaged, abortive or empty seeds were count for each seed sample.

Purity Test

Two samples each of them weighting 35 g were taken separately at random from *Dalbergia melanoxylon* seed lots from the two provenances, and *Dalbergia sissoo* from South Kordofan. The weighted samples were then separated into pure seeds and inert matter. Separated fractions were weighted and the percentage of each was found. And then the purity percentage was calculated as bellow [13]:

$$P\% = pw_2 / pw_1 \times 100$$

Where: - P% is the purity percentage

pw_1 is the weight of seed sample before cleaning

pw_2 is the weight of pure seed

The Number of Seeds per Kilogram

Eight replicates of hundred seeds each, of the two *Dalbergia melanoxylon* provenances and *Dalbergia sissoo* from South Kordofan were weighted separately and the average weight for each was found. The ranges of weights were evaluated between the maximum and minimum values which were found less than 10%. Moreover, the number of seeds per kilogram was calculated as fallow [13]:-

Wt of 1000 seeds = Wt of 100 seeds x 10

If say, 1000 seeds weigh 80.5 gm

X seeds weigh 1000 gm (kg)

:.The Number of seed per kg = $\frac{1000 \times 1000}{80.5}$

Moisture Content Test

Two samples of about 10 gm each of *Dalbergia melanoxylon* seeds from two provenances and *Dalbergia sissoo* seeds from South Kordofan were taken for moisture content determination, weighed on a sensitive balance and put in an already weighed aluminum dishes, samples were put in an oven at 105 ºc for 17 hours. Samples were then taken from the oven and weighed again and the difference between the two weights was taken and the moisture content percentage at wet bases was calculated as below [13]:

$$MC\% = \frac{sw_1 - sw_2}{sw_1}$$

Where :

MC% = moisture content percentage

sw_1 = the weight of fresh sample

sw_2 = the weight of dry sample

The Viability Test

Tetrazolium test: the topographical tetrazolium test (TTZ test) was used. A colourless solution 1%, 2, 3, 5. triphenyl tetrazolium chloride was used as an indicator to reveal the reduction process which takes place within living cells by reacting with dehydrogenases taking the hydrogen and ultimately hydrogenated to a red stable and non-diffusable substance, triphenyl formazon in the living cells making it possible to distinguish red living cells and colourless dead ones. Two hundred seeds were divided into 4 replicates, and samples of 50 seeds were used for test. The seeds were treated with electric burner to punch the seed/fruit coat to allow for quick water imbibitions. Seeds were soaked in water for 6 hours and then soaked in the test solution

in glass beakers kept in the dark for 72 hours. Decision of viable or non-viable seeds was made on the bases of colour intensities and tissue soundness. At the end of the test the percent viable and non-viable seeds were recorded[13].

Germination Test

One hundred seeds of each species and provenances were drawn and divided into four reps each of 25 seeds for the determination of the germination percentage. Seeds were sawn in trays filled with moist sand in germination room temperature; 28-32ºc, 12 hours light from florescent lamps at National Tree Seed Center Laboratory, Soba. The seeds in the germination room were moistening every day. The number of germinated seeds was count weekly for germination rate calculation until the fifth week. And then the total numbers of germinated seeds were calculation, and the percentage of germination was calculated by the method below [13]:

$$G\% = \frac{\text{total number of germinated seeds}}{\text{total number of sowing seeds}} \times 100$$

Seed Dormancy Treatment

Soaking In Water

One hundred seeds of each of the three seed lots were drawn and each was divided to 4 reps of 25 seeds. Seeds were soaked in changeable plenty of water for 24, 48 and 72 hours. Seeds were taken from water and sawn in trays filled with moist sand in the germination room. Germinated seeds were recorded every week until the fifth week. The total germination percentage was measured by using the previous described method [14].

Soaking In Hot Water

One hundred seeds of each of the three seed lots were drawn and each was divided to 4 reps of 25 seeds each. Seeds were soaked in hot water (100°c) and left to cool for 1, 2, 3 hours. Seeds were taken from the water, planted in trays filled with moist sand in the germination room [14].

Sulphuric Acid (H_2So_4)

One hundred seeds of each of the three seed lots were drawn and each was divided to 4 reps of 25 seeds , and put in a conical flask, sulphuric acid was poured on the seed and left for 1, 2, and 3 minutes with continuous stirring. After that the acid was drained off and the seeds were thoroughly washed several times with plenty of water and then planted in germination room [3].

Electric Burner (Needle)

One hundred seeds of each of the three seed lots were drawn and each was divided to 4 reps of 25 seeds each. The electric burner was used to make a small burn in the seeds coat by touching the seed coat for one second with the glowing tip of the needle. Treated seeds were planted in germination room as before [12].

Dewinging

One hundred pods of each of the three seed lots were drawn and each was divided to 4 reps of 25 pods each. The pod wings were removed manually taking care not to damage or break the seeds. Dewinged seeds were planted in the germination room [15].

Extraction of Seeds

One hundred pods of each of the three seed lots were drawn and each was divided to 4 reps of 25 seeds each. Seeds were extracted from fruit by using hand taking care not to damage or break the seed. The extracted seeds were planted in the germination room and the germination percentage was recorded as above [16].

Storage Experiment

The aim of this experiment was to determine the effects of two types of storage on the viability of seed. This experiment was done in the National Tree Seed Center cold and normal store. In this experiment, 4000 seeds were drawn randomly from each species. They were then stored at two type of storage, normal store, in which seeds were put in cotton sacks and stored in room temperature at 25 – 30°c, and the cold store, in which seeds were kept in 14°c in plastic airtight container to forbid the moisture and gas exchange with the surrounding environment. Seed viability and seed moisture content were measured every six months for 18 months by testing seed germination and moisture content count. Results were analyzed to compare between the two types of storage with regard to seed germination behavior and loss in viability and vigor in addition to changes in moisture content.

Data Analysis

Results were analyzed by using JMP package statistical analysis. Analysis of variance (ANOVA) was made to determine the significance of the variation between species for seeds characteristics, germination behavior during storage for 18 months. Tukey Kramer's analysis method was used to compare means of the various traits of the seed growth parameters of the species. Percentage data was transformed before analysis to arcsin values.

III. RESULTS AND DISCUSSIONS

Table 1. The Percentage of Sound, Damaged and Abortive Seeds of Dalbergia species

Species	Sound Seed	Damaged Seed	Abortive Seed	Total
D.melanoxylon D*	25	31	44	100
D.melanoxylon K*	29	37	34	100
D.sissoo *	88	0	12	100

**D.melanoxylon D* ≡ *Dalbergia melanoxylon* from *Damazin*

**D.melanoxylon* K ≡ *Dalbergia melanoxylon* from South Kordofan

**D.sissoo* ≡ *Dalbergia sissoo* from South Kordofan

The sound seeds of *Dalbergia melanoxylon* are less than 30%, if seed collection was delayed may be more damaged seeds were found, this may be due to insects damaged which increases with time since seeds are still available for insects. This may be one of reasons why *Dalbergia melanoxylon* was not having a good natural regeneration. *Dalbergia sissoo* seed was not attacked by insects, may be because it is an exotic species, and hence not palatable to local insects. The same observation was found by Mahgoub (personal contact) in seeds of exotic species that come to the Seed Centre (*Delonex regia*, *Albizia lebbek*, *Cassia fistula*). *Dalbergia sissoo* seeds have abortive seeds and that agree with [17] who said that before storage, the pods of *Dalbergia sissoo* should be broken up into sections containing one seeds each, discarding the empty section and other foreign material. The high percentage of abortive seeds in *Dalbergia melanoxylon* from the two provenances may be attributed to a problem during pollination and fertilization of the species.

Table2. The percentage of pure seeds and innert matter in seeds samples of species under study

Species	Inert Matter	Pure Seed
D.melanoxylon D	4	96
D.melanoxylon K	7	93
D.sissoo	13	87

The purity percentage of *D.melanoxylon* from South Kordofan and *D.sissoo* is less than 95%, which is the standard purity for most species collected by the Tree Seed Centre. And this may be due to broken pod parts, mainly the wings.

Table 3. Number of seeds per kilogram and initial seeds moisture content of Dalbergia species under study

Species	Number of Seeds/Kg	Initial M.C %
D.melanoxylon D	17036	5.40
D.melanoxylon K	14145	5.51
D.sissoo	19904	5.50

The number of seed per kilogram obtained was within the range recorded for *Dalbergia sp* by [18] which was reported between10000 – 30000 seeds per kilogram. *D.melanoxylon* from South Kordofan had the lowest number of seeds per kilogram which means that seeds are bigger than those from Damazin. This may indicate that a better environment is prevailing in South Kordofan than Damazin.

It was found that the initial seed moisture content percentage of *D.melanoxylon* South Kordofan is higher than *D.melanoxylon* Damazin and *D.sissoo*. But moisture content differences did not exceed about 1% this is normal due to contact with outside atmosphere for example during sample drawing. The results showed decrease in the level of moisture content percentage of *D.sissoo* seeds than what reported by [17] who said under favorable conditions *D.sissoo* seeds can be harvested already dry enough for storage with a moisture content of 7 – 9%, and that may be attributed to the hot weather of the country which decrease seeds moisture content.

Table 4. The viability of Dalbergia species at collection and after storage for 18 months in cold and normal store

Species	Store Type	Mean of Viability	S. E.	Prob>F	Significant Level
D.melanoxylon D	At collection	91	±1.491	<0.0001	a
	Cool	90			a
	Normal	75			b
D.melanoxylon K	At collection	93	±1.791	0.1958	Ns
	Cool	90			Ns
	Normal	80			Ns
D.sissoo	At collection	97	±0.707	0.0001	a
	Cool	96			a
	Normal	90			b

The species under study showed high viability percentage, but that may not mean high germination percentage, [3] reported that seeds with relatively high viability percentage it reduced viability under test condition (e.g. seeds of 80% viability) may show poor germination % and performance when grown under field condition. After 18 months storage in the two types of storage the viability was decreased in the normal store, but it was more or less consonant in the cold store. In *D.melanoxylon* from the two provenances in normal storage the best in the initial viability was the best after 18 months, and that was in agreement with what obtained by [3], who reported that seeds with high initial viability also have a higher longevity in storage than seed with low initial viability.

Table 5. Seeds germination rates (per week) and total germination percentage of species under study

Species	Week 1	Week 2	Week 3	Week 4	Week 5	Total
D.melanoxylon D	0	38	27	6	0	71
D.melanoxylon K	0	36	26	12	0	74
D.sissoo	0	43	28	0	0	71

Results showed no germination in the first and last weeks. The highest germination rates were concentrated in the second week and the third week, followed by the fourth week for *D.melanoxylon* from the two provenances. No germination in *D.sissoo* at the fourth week which indicates that the germination rate of *D.sissoo* is faster than *D.melanoxylon*, although the total germination percentage is nearly the same. Comparing germination results of Table (5) with the initial viability in Table (4) there were about 20% viable seeds that did not germinate which may be an evidence of the presence of moderate dormancy within the seeds. The delay in germination to the second week may also refer to presence of dormancy since the seed can fully imbibe water with 24 hours.

Seed Dormancy Breaking Treatments

Table 6. Mean germination of treated seeds of *Dalbergia melanoxylon* from two provenances and *Dalbergia sissoo* from South Kordofan to improve germination versus untreated seeds (control)

Treatment	Mean Germination of *D.melanoxylon* from Damazin	Mean Germination of *D.melanoxylon* from S.Kordofan	Mean Germination of *D.sissoo* from South Kordofan
Control	71 c	74 f	71 c
EB	61 f	79 c	67 c
H_2So_4/1min	76 b	78 d	81 a
H_2So_4/2min	62 e	88 a	76 b
H_2So_4/3min	64 d	77 e	46 f
Cold Water/24h	53 h	65 g	51 e
Cold Water/48h	50 i	62 h	60 d
Cold Water/72h	41 j	59 i	42 g
Dewinging	59 g	84 b	82 a
Extracted	91 a	84 b	85 a
Hot Water/1h	3 m	65 g	45 g
Hot Water/2 h	9 l	38 j	44 g
Hot Water/3h	14 k	17 k	27 h
Standard Error	± 3.05	± 2.16	± 1.083
Probability	≤ 0.0001	≤ 0.0001	≤ 0.0001

*Different letters show pairs of means that are significantly different.

*EB ≡ Electric Burner.

In *D.melanoxylon* Damazin the germination results showed high significant differences between treatments, the best one is extraction of seed and that may indicate the pod coat of some fruits contains growth inhibitors for example the presence of external chemical dormancy. It was noticed that extraction of seed from fruit allows for the 20% of viable seeds which did not germinate in the control (untreated – whole fruits) to germination by removal of the fruit coat. Similar result were obtained by [16] who reported that in *Prunus africana* very low germination was achieved when whole fruits were sown, while 75 – 90% of the seed germinated after extraction. And the second best treatment was H_2So_4 for one minute which may have an effect by lighting the pod and seed coat by scarification. Furthermore soaking in water had the least germination results which refers also to leaching out of growth inhibiter in the soaking solution activates its function on seed. But other treatments showed less germination percentage than control and that mean it was harmful.

In *D.melanoxylon* South Kordofan results showed high significant differences in germination percentage between treatments. The most effective treatment was H_2So_4 for two minutes and that may show that the seed coat of *D.melanoxylon* South Kordofan is thicker or harder than the seed of Damazin provenance. The second best treatments were extraction, and dewinging that mean the pod coat may contain growth inhibitors in it is wings, which obstacle germination processing. It was followed by the electric burner treatment (EB) and that may mean the hole which made in the pod and seed coat increased the rate of water imbibition and also embryo emergence. This result confirms that the seed coat of *D.melanoxylon* South Kordofan is thicker or harder than that from Damazin. Acid treatment for one minute and three minute is more or less like control but since acid treatment for two minute is the best treatment, this means that acid treatment for one minute is not enough and for three minute is harmful to seed which mean soaking for three minute is more than optimum which indicated that the acid had reached the embryo. Other treatments showed lower germination percentage than control and that mean it is not effective.

Results showed high significant difference in germination percentage between treatments in *D.sissoo* seeds. The best treatments were extraction, dewinging, and H_2So_4 for one minute followed by H_2So_4 for two minutes. Other treatments were not effective since they had significantly lower germination percentage than the control. [17] reported that hot water is harmful for *D.sissoo* seeds as *D.sissoo* seeds have a thin outer layer (seed coat) and hot water can kill the seed embryo.

Seed Storage

Table 7. Initial and during storage moisture content of seeds of Dalbergia species

Species	Initial M.C%	Normal storage			Cold storage		
		6 months	12 months	18 months	6 months	12 months	18 months
D.melanoxylon D	5.40	5.02	5.3	4.4	5.12	5.5	4.5
D.melanoxylon K	5.51	5.15	5.4	4.8	5.23	5.39	4.4
D.sissoo	5.50	5.36	5.8	5.0	5.51	6.6	4.3

It was found that after six and 18 months storage, seed moisture content decreased from the initial moisture content, seed can lose moisture with time. But after 12 months the moisture content was increased and that may be due to surrounding atmosphere when the test was done (soon to the rainy season). [3] said that during seed storage, seed moisture comes into equilibrium with the humidity of the surrounding air. But moisture content differences did not exceed about 1% this is normal due to contact with outside atmosphere for example during sample drawing.

Table 8. Germination percentage of Dalbergia species during storage in two types of storage conditions

Species	Type of Storage	Months	Mean of Germination	S. E.	Prob> F	Signi. Level
D.melanoxylon D	Normal	Initial	71			a
		After 6	34	±1.258	<0.0001	c
		After12	65			b
		After 18	22			d
D.melanoxylon D	Cold	Initial	71			a
		After 6	64	±0.791	≤0.0001	b
		After12	69			a
		After 18	39			c
D.melanoxylon K	Normal	Initial	74			a
		After 6	57	±0.692	<.0001	b
		After12	72			a
		After 18	36			c
D.melanoxylon K	Cold	Initial	74			b
		After 6	34	±1.048	<.0001	d
		After12	80			a
		After 18	41			c
D.sissoo	Normal	Initial	71			a
		After 6	55	±1.099	<.0001	b
		After12	58			b
		After 18	28			c
D.sissoo	Cold	Initial	71			a
		After 6	46	±0.913	<.0001	c
		After12	67			b
		After 18	39			d

Results of table (9) showed that after six months in the normal store the germination decreased, when these results were compared with the results in Table (5) in which the viability was kept high during the storage period (18 months) this may mean that the seeds developed an induced periodical dormancy. In cold storage *D.melanoxylon* Damazin germination had increased, which may mean that the dormancy is broken, but *D.melanoxylon* South Kordofan and *D.sissoo* showed a deeper dormancy than in normal storage. It is normal to find differences between species; reference [3] reported that within any one species, dormancy may vary between seed lots and within individual seed in the same lot. After 12 months the germination increased in the two types of storage, but the cold store is better than normal store although the differences are not high. *D.melanoxylon* South Kordofan showed high germination followed by *D.melanoxylon* Damazin while *D.sissoo* is the least. After 18 months the germination decreased in the two types of storage, but in normal storage the germination was less than the cold storage. The decrease of germination may mean that the seeds have an induced periodical dormancy or relevant to the lose of viability, reference [19] reported that during storage seeds usually loose considerable viability.

Table 9. Comparison of germination in the two types of storage during 18 months

Species	Store	Mean of germination	S. E.	Significance*
D.melanoxylon D	Normal	40.333	±11.185	Ns
	Cool	57.333		Ns
D.melanoxylon K	Normal	55.000	±12.526	Ns
	Cool	51.667		Ns
D.sissoo	Normal	47.000	±8.9938	Ns
	Cool	50.667		Ns

* Ns ≡ Not Significant

IV. CONCLUSION

The study showed that more than 70% of *D.melanoxylon* seeds were abortive or damaged by insects at collection and *D.sissoo* seeds have a lower percentage of abortive seeds and no insect damage.

The seeds of the two species and provenances are more or less the same in viability, initial moisture content, and germination percentage.

Seeds of *D.*species found to have a combined coat dormancy of physical and chemical. The best treatments to break seed dormancy were extraction of seeds from pods, soaking in H_2 So_4 for one or two minutes and dewinging. Other treatments were found harmful to seeds.

With respect to seed storage moisture content, results confirmed that seed behaviour in the two types of storage was more or less the same.

Generally initial germination percentage and during the storage period, *D.melanoxylon* is better than *D.sissoo*. And *D.melanoxylon* from South Kordofan is better than *D.melanoxylon* from Damazin

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