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# Germination of West African Ebony (*Diospyros mespiliformis* Hochst) Seeds: Effects of Dehydration and Different Pre-sowing Treatments

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## Abstract

*Diospyros mespiliformis* is a highly valued and threatened tree species within the Sahelo-Sudanian zone of Africa, but its seed germination requirements under cultivation are not well researched. In a first experiment which aimed at determining germination response of seeds to dehydration, fresh seeds were dried at room temperature for 26 days during which their moisture content, their germinability, and their viability were monitored at two-day intervals. In the second experiment, 14 pre-germination treatments were tested for their effect on the germination of dried seeds. Results showed that fresh seeds had 52.7% moisture and achieved 97.7% germination. As seeds were dried, percentage germination gradually decreased with decreasing moisture content and reached 0% when moisture content had dropped to 18%. Meanwhile, seed viability remained at 100% over drying duration. Seeds that were not germinated after air dry also recorded 100% viability. The most effective treatment for inducing germination of dried seeds was scarification using 98% sulfuric acid for 30 min which resulted in 96.6% germination. This study reports for the first time in *D. mespiliformis* seeds a desiccation-induced dormancy which can be efficiently alleviated by acid scarification. This study provides useful information that will contribute to efficient management of *D. mespiliformis* seed resources for propagation.

**Key Words:** desiccation-induced dormancy, domestication, propagation, seed germination, West African Ebony

## Introduction

In the Sahelo-Sudanian zone of Africa, rural and urban populations remain dependent on goods and services supplied by forest trees and multiple use woody plants. These trees of the dry-land forests provide timber, fuel-wood and a wide range of non-wood products; including human foods, forage for domestic animals, medicinal products and raw material for craftsmen (FAO 1983). The Sahelo-Sudanian tree species also have added values of conservation for scenic purposes, stabilization of climate, maintenance of water supply and preservation from erosion (Goudie 2006). In this ecosystem, most of the forest natural resources with direct relevance to the well being of the people are subject to strong pressures both of climatic and man-made origins. While climatic pressure is related to the successive droughts that the zone has experienced since 1965 (Gonzalez 2001), the anthropic pressure is exerted through overexploitation coupled with the lack of appropriate management strategy (FAO 2001). These pressures have led to the progressive decline of many high valued tree species indigenous to the area (Lapido et al. 1994). In order to prevent the extinction and derive maximum benefits from these indigenous trees, it is necessary to promote their

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conservation in the environment. One important approach for the conservation of plant species is cultivation (Sanchez et al. 2010). However, most of these forest tree species remain essentially wild and information on propagation techniques is scantily available. Therefore, there is a huge need for developing methods for growing and propagating such indigenous species. Indeed, the domestication of indigenous trees producing high-valued products is a strategic component for the intensification and diversification of smallholder farming systems in the tropic and subtropics (Leakey and Tchoundjeu 2001).

*Diospyros mespiliformis* Hochst, a member of the Ebenaceae family (The Angiosperm Phylogeny Group 2009) is one of the many tree species of the Sahelo-Sudanian zone of Africa that are of socio-economic importance. Its common names include: West African Ebony, jackalberry and Monkey guava. It is a tall, upright tree that can reach a height of 25 m, with a trunk circumference of more than 5 m (Mbuya et al. 1994). It grows mostly in savanna and woodland, often on termite hills. The tree is widely distributed throughout the eastern, the western, and the southern parts of the African continent where it is high-valued for its good quality wood and for its different plant parts which are used extensively as food, traditional medicine and fodder (Burkill 1994). The tree produces black heartwood called "ebony" which is heavy, hard, strong, fine-grained and is fungus and termite resistant. This wood is used in making tool handles, gunstocks, furniture and carving. The tree is one of the most valuable luxury timber species and thus frequently exploited (von Maydell 1986). *D. mespiliformis* also makes good fuelwood and charcoal (FAO 1983; Mbuya et al. 1994). As food, the fruit is edible. It can be either eaten fresh or dried and stored for later consumption in times of food shortage. The fruit is also used in making brandy (FAO 1983). As a traditional remedy, the leaves are used to treat fever, as wound dressing, and as a poison antidote. The bark and roots are used for diseases such as malaria, syphilis and leprosies. Different parts of the tree are also used to treat headaches, toothaches and other body pains. Indeed, extracts of various parts of the plant are believed to have antibiotic properties (von Maydell 1986). As fodder, leaves and fruits of *D. mespiliformis* are eaten by a wide range of domestic and non domestic animals.

Despite its socio-economic importance, *D. mespiliformis* is still exploited in the wild because the species has not been domesticated yet, and very little scientific knowledge is available on its propagation needs. The literature gives contradictory information concerning the germination of *D. mespiliformis* seeds. Authors describe *D. mespiliformis* seed as easy to germinate (Prins and Maghembe 1994), while others report that seed dormancy caused by an impermeable seed coat is the main problem to overcome if propagating this species is to be successful (von Maydell 1986). Although the requirements for seed germination is not clearly established, most of the research works related to this plant aimed at improving plant growth rather than seed germination (Jegede et al. 2015). Therefore, as a contribution to the domestication of the species, research is required to determine the germination's requirements of its seeds.

The present study, which aimed at identifying and recommending the appropriate protocol for the germination of African ebony seeds in nursery condition, investigated the effects of an intrinsic factor (i.e. initial seed moisture content) and an extrinsic factor (i.e. pre-germination treatment) on the germination of *D. mespiliformis* seeds.

## Materials and Methods

### *Seed material*

Mature and disease-free fruits of *D. mespiliformis* were harvested from 12 randomly selected trees in the administrative Division of Mayo Louti (8°-10° latitude North and 12°-15° longitude East), in the Far-North Region of Cameroon and immediately brought to the laboratory of Applied Botany of the University of Dschang, Cameroon. In the laboratory, mature seeds were extracted from fresh fruits, mixed in a single batch and used for the determination of initial characteristics (viability percentage and moisture content) and for further experiments.

### *Viability test*

The viability of seed was determined using floatation method. This consisted in soaking seed in water for a few minutes. Those that sank were collected as viable while those that floated were considered unviable and discarded (Ambebe and Achankeng 2019). Six replications each of 25

seeds were used for viability test.

### *Seeds' moisture determination*

Seeds' moisture determination was done by the oven dry method which consisted in weighing seed samples before and after drying them in the oven at 103°C for 24 h. Each sample contained 20 seeds. Moisture content, which was expressed as a percentage of fresh weight, was calculated using the formula: $MC=[(FW-DW)\div FW]\times 100$ where $FW$ (fresh weigh) is the weight of sample before drying and $DW$ (dry weigh) is the weight of sample after drying (ISTA 2004). The value of the moisture content was the mean of six measurements (six replications of 20 seeds).

### *Experiment 1: Determining the effect of seed dehydration on the germination*

To determine whether dehydration of seeds affects germination, fresh seeds were extracted from fruits and spread in a single layer on the laboratory bench top and left to dry under shade at laboratory temperature (25±2°C). At 2-days intervals, seed samples were withdrawn for moisture content measurement, viability test and germination test.

Moisture content measurement and viability test were done as described above. For the germination test, six replications each of twenty-five viable seeds were also withdrawn at 2-day intervals and allowed to germinate in the nursery at 25±4°C, in polythene bags filled with river sand as substrate. The seeded polythene bags were watered daily so that the medium (substrate) was kept moist without getting waterlogged. Seeds which emerged above the surface of the substrate as a consequence of hypocotyls elongation were recorded as having germinated. The record of germination was done daily and the experiment was finished when no further germination was recorded over a period of four consecutive weeks. The percentage germination was calculated as follows: $\%G=(nsg\div nss)\times 100$ Where $\%G$ is the percentage of germination $nsg$ is the number of seeds that germinated and nss is the number of seeds sown. At the end of the germination test, seeds that were not germinated after air dry were once more checked for viability using floatation method as described above.

### *Experiment 2: Testing the effect of pre-sowing treatments on dried seeds' germination*

#### Seed material and treatments

To investigate on the effects of pre-sowing treatments on the germination of dried seeds, seeds which were dried under shade in laboratory for 26 days [period after which the seed's moisture was lowest (18%) and remained constant over time] were used. 14 pre-germination treatments were tested for their effects on germination. The 14 pre-germination treatments included a control (seeds sown without any treatment); a mechanical scarification (MS) which was done by peeling off partially the seed coat using a sharp blade; a cold water treatment which was done by soaking seeds in clean tap water at ambient temperature (25°C) for 24 h; a hot water treatments which was done by soaking seeds in hot water (100°C) in which they remained till the temperature dropped to room temperature; five hydrochloric acid treatments which were done by soaking seeds in 98% HCl for 5, 10, 15, 30 and 60 min; and five sulfuric acid treatments which were done by soaking seeds in 98% $H_2SO_4$ for 5, 10, 15, 30 and 60 min. Immediately after acid treatments, seeds were washed thoroughly with tape water before sowing. Seeds were sown 2 cm depth in perforated polythene bags (20 cm high and 15 cm diameter) filled with fine river sand as substrate.

#### Experimental design and conditions

For each treatment, twelve polythene bags each of 25 seeds were used (twelve replications of 25 seeds), making a total of 300 seeds per treatment. The treatments were identified with permanent ink on the polythene bag and randomly distributed in the nursery, at 25±4°C and 12 h/day photoperiod. Using a sprayer, water was applied daily to the seeded polythene bags so that the medium (substrate) was kept moist without getting waterlogged as indicated above. The number of germinating seeds was recorded every day. For each treatment the percentage germination was calculated as indicated above, and the mean germination time (MGT) was calculated following the formula by Bewley and Black (1994), as follows: $MGT=\Sigma(j\times nj)/\Sigma nj$ Where $MGT$ is the mean germination time (days); $j$ is the number of days starting from the date of sowing; $nj$ is the number of seeds that germinated at the $j^{th}$ day from sowing.

### Data analysis

Data analyses were performed using SPSS 21.0 software package. The dependent variables were mean germination percentage and mean germination time. Prior to analysis, percentage data were subject to arcsine transformation as follows: $y = arcsin\sqrt{p/100}$ where $y$ is the transformed data and $p$ is percentage (Snedecor and Cochran, 1980). Statistical significance was determined by analysis of variance (ANOVA). The mean values were separated using Duncan Multiple Range tests ($p \leq 0.05$).

## Results

### *Germination response to seeds dehydration*

The determination of initial characteristics of fresh seeds revealed moisture content of 52.7±3.1% fresh weight, a viability rate of 100% and a germination rate of 97.7±2.1%. The germination was spread out over the period from day-49 to day-98 after sowing. From the 98$^{th}$ day after sowing, there was no further germination over a period of four consecutive weeks. As seeds were dried, their moisture content gradually decreased and reached the value of 18±2.0% fresh weight after 26 days of drying. At the same time, germination percentage gradually decreased from 97.7±2.1% recorded with fresh seeds to 0% recorded with seeds which were dried for 26 days (Fig. 1). Meanwhile, the viability rate remained constant at 100% over drying duration. At the end of the germination test, seeds that were not germinated after air dry also recorded 100% viability, indicating that the decrease in germination percentage was not associated with a loss of viability.

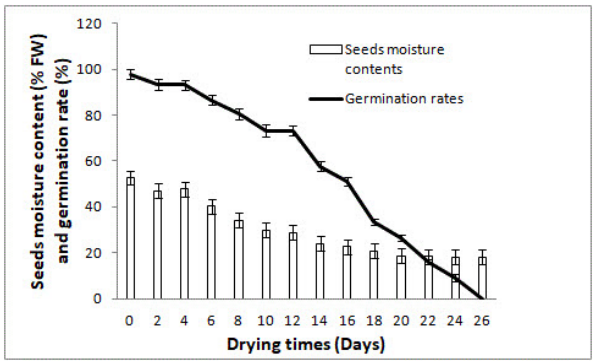


**Fig. 1.** Moisture content and germination percentage of *Diospyros mespiliformis* seeds over drying time.

### *Effects of pre-sowing treatments on the germination of dried seeds*

Pre-sowing treatments significantly ($p<0.001$) affected both germination percentage and mean germination time (Table 1). Control seeds and seeds soaked in hot water failed to germinate. Manually scarified seeds and seeds soaked in cold water for 24 hours germinated at 8±1.4% and 4±1.4% respectively, which were not significantly different from the 0% recorded with untreated seeds. Germination percentages higher than that recorded with untreated control seeds were obtained only with seeds soaked in either concentrated hydrochloric acid or concentrated sulfuric acid. The highest germination percentage (96.6±1.5%) was recorded with seeds treated with 98% $H_2SO_4$ for 30 min. Above the soaking duration of 30 min, the percentage of germination decreased with increasing duration of treatment (Fig. 2). Scarification with sulfuric acid for 30 min also resulted in the lowest mean germination time (58.2±3.7 d) while the highest mean germination time (98±0 d) was recorded with cold water treatment (Fig. 3).

## Discussion

Fresh seeds with high initial moisture content (52.7%) performed the highest germination percentage (97.7%).

**Table 1.** Results of the analysis of variance showing the degree of freedom (*df*) and thelevel of significance (*F* and p-values) at whichtreatments affected germination percentage and mean germination time in *D. mespiliformis* seeds

| Source of variation | Germination percentage | | | Mean germination time | | |
|---|---|---|---|---|---|---|
| | *df* | *F* value | p-value | *df* | *F* value | p-value |
| Treatments | 13 | 47.19 | <0.001 | 11 | 14.34 | <0.001 |

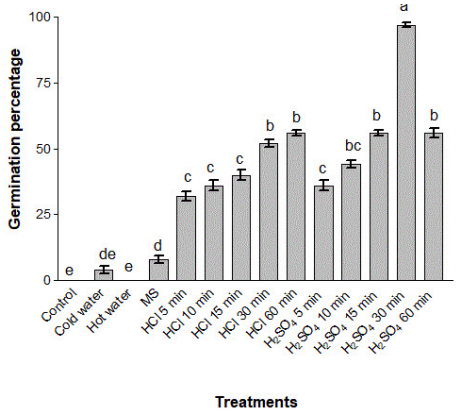


**Fig. 2.** Effects of different pre-sowing treatments on the germination percentage of *D. mespiliformis* seeds (bars with same letters are not significantly different at $p \leq 0.05$; MS, mechanical scarification).

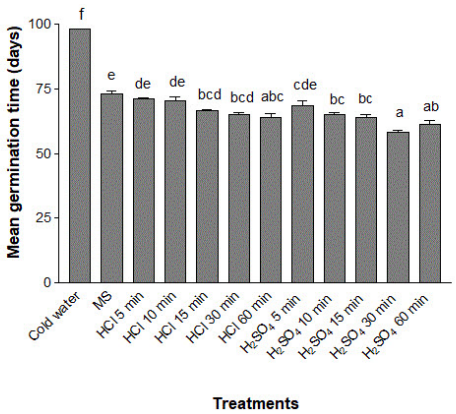


**Fig. 3.** Effects of different pre-sowing treatments on the mean germination time of *D. mespiliformis* seeds (bars with same letters are not significantly different at $p \leq 0.05$; MS, mechanical scarification).

The high germination percentage recorded with fresh seeds in the present study is consistent with previous work by Prins and Maghembe (1994) who reported that more than 80% germination could be achieved with untreated *D. mespiliformis* seeds. The mean germination percentage declined as the moisture content decreased, and below 18% moisture no seed germination was recorded. In desiccation-sensitive (recalcitrant) seeds, the loss in germination capability as a result of seed dehydration is a common phenomenon which is attributable to a loss of viability. In the present study nevertheless, there was no loss in viability as seed were dried, indicating that the desiccation of seeds had induced seed dormancy rather than viability loss. Desiccation-induced dormancy has also been reported in seeds of many other species including Sorghum (Nutile and Woodstock 1967), Sitka spruce (Jones et al. 1998) and papaya (Wood et al. 2000), and could be alleviated only after appropriate treatments.

Although they were viable at 100%, untreated dried seeds failed to germinate. This is in accordance with von Maydell (1986) who reported that seed dormancy is the main problem to overcome if propagating this species is to be successful. Pre-sowing treatments significantly affected mean germination percentage, whose highest value (96.6%) was recorded with seeds soaked in H2SO4 for 30 min. The fact that this chemical scarification induced germination without any further additional treatment shows that the seed coat is the sole barrier to germination in *D. mespiliformis* dried seeds. The dehydration of *D. mespiliformis* seed may have rendered its coat impervious to water and gazes thereby limiting imbibitions and germination. The successful alleviation of coat-imposed seed dormancy by mechanical or acid scarification treatments in the present study have also been reported for a wide range of plant species including *Abelmoschus esculentus* (Musara et al. 2015), *Xylopia aethiopica* (Kanmegne et al. 2017), *Xylopia parviflora* (Kanmegne et al. 2018), *Castanopsis indica* (Hasnat et al. 2019) and *Quercus gomeziana* (Nandi et al. 2020). With *Diospyros ebenum* that is related species to *Diospyros mespiliformis*, although treatment of dormant seeds with $H_2SO_4$ had no effect on final percentage germination, it resulted in one hand in significant increase of germination rate index, germination value, germination energy, coefficient of velocity of germination and, in another hand, in significant shortening of germination mean time, imbibitions period and time spread germination (Jeyavanan et al. 2016). This effect of $H_2SO_4$ could be attributed to successful removal of several lignified layer in the testae, which are packed tightly together and content water repelling compounds (Baskin 2003). These layers act as physical barrier to water absorption and gaseous exchange (Colling 2009). Soaking seeds in concentrated sulfuric acid for duration longer than the optimal treatment duration resulted in lower germination percentage as compared to that exposed for the optimal duration. This is consistent with previous findings of Gbadamosi and Oni (2004) on *Enantia*

*chloantha*, Kanmegne et al. (2017) on *Xylopia aethiopica* and Kanmegne et al. (2018) on *Xylopia parviflora*, and may be due to the corrosive nature of the acid which undoubtedly damaged the embryo of the seeds when they were left therein for long period of time.

The present study showed that hot water treatment of dormant *D. mespiliformis* seeds resulted in 0% germination. Similar results have been reported with other species including *Canarium resiniferum* (Hasnat et al. 2017) and *Castanopsis indica* (Hasnat et al. 2019), indicating that in these species, seeds with heat-sensitive embryos may be killed by boiling treatment.

## Conclusion

The results from the present study revealed for the first time the existence of a desiccation-induced dormancy in *D. mespiliformis* dried seeds. While fresh seeds germinate at high percentage with no need of pre-sowing treatment, dried seeds suffer from a coat-imposed dormancy which can be successfully broken by soaking seeds for 30 min in 98% sulfuric acid. For an efficient germination of *D. mespiliformis* seeds, it is recommended that fresh seeds should be sown in sand substrate without any pre-sowing treatment whereas dried seeds should be soaked in 98% sulfuric acid for 30 min and thoroughly rinsed with water prior to sowing.

This study has shed light on the controversy of the literature about germination requirements of *D. mespiliformis* seeds, and provides useful information that will be used for efficient management of *D. mespiliformis* seed resources for propagation and in the environmental conservation effort.

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