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Seed germination of Namibian woodland tree species

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Abstract

Assisted regeneration allows for selecting desired tree qualities, such as drought resistance and good timber or fruit quality. It is also a valuable forest management tool for species with slow growth - such as most canopy tree species of northern Namibia - and limited natural regeneration, such as Pterocarpus angolensis *and* Guibourtia coleosperma*. Few nursery experiments with dry woodland tree species from northern Namibia have been published. This study aimed to test seed treatments of six indigenous tree species to improve germination rates. The seeds were incubated in germination chambers at 30°C and 26°C to establish the effects of different temperatures on germination. In general,* Dialium engleranum *and* G. coleosperma *were found to germinate well, while* Erythrophleum africanum *and* P. angolensis *germinated moderately and* Schinziophyton rautanenii *poorly. Nicking of* D. engleranum*,* E. africanum *and* P. angolensis *was found to significantly improve these species' germination rates. Soaking was noted as an inappropriate pre-treatment for both* E. africanum *and* P. angolensis*. Surface sterilisation and other pre-treatments such as nicking and soaking decreased mean germination time of* G. coleosperma *seeds. Seeds of all species, except* G. coleosperma*, need to exceed two weeks under germination conditions.* E. africanum *and* S. rautanenii *were found to have very long lasting storage durability with germination of twelve year old seeds. These results may inform seed handling practises in nurseries and the field to advance successful assistance of regeneration.*

Keywords: germination, pre-treatments, Miombo woodland, Northern Kalahari woodland, *Dialium engleranum, Erythrophleum africanum, Guibourtia coleosperma, Pterocarpus angolensis, Schinziophyton rautanenii*

Introduction

Assisted regeneration of tree species is a valuable forest management tool for species with slow growth and reduced natural regeneration. It also allows selecting desired tree qualities, such as drought resistance and good timber or fruit quality. Limited natural regeneration has been reported for several dry woodland tree species from southern Africa, especially *Pterocarpus angolensis* (Figure 1) but also *Guibourtia coleosperma* (Caro *et al.* 2005; Phiri *et al.* 2012; van Daalen 1991). Forest inventories in the Namibian Northern Kalahari dry forests and woodlands show that regeneration of these species is under-represented compared to mature tree composition (Kamwi 2003; Kanime 2003; Kanime & Laamanen 2002). More studies are needed to clarify to what extent this endangers the woodlands' future, and to what extent this is caused by fires and changing global climate (De Cauwer 2012; Geldenhuys 1977; Pröpper *et al.* 2015). Effects of reduced natural regeneration will

take a long time to become obvious as most of the canopy trees in Namibia's woodlands grow very slowly (Fichtler *et al.* 2004; Van Holsbeeck 2015). For example it takes *P. angolensis* on average about 93 years to reach the minimum diameter harvest size of 45 cm (Pröpper *et al.* 2015).

Figure 1. *Pterocarpus angolensis* trees near Nkurenkuru, Kavango West (©V. De Cauwer).

Unfortunately, nursery experiments in Namibia with indigenous woodland trees have been limited or were never published (P. Graz pers. com.). A few studies were done that yielded variable results and are only the first steps towards establishing germination protocols (Moses 2012; Van der Heyden 2014). Work outside Namibia is limited and concentrates mainly on *P. angolensis* and *Schinziophyton rautanenii* (Chisha-Kasumu *et al.* 2006; Chisha-Kasumu *et al.* 2007; Jøker *et al.* 2000; Keegan *et al.* 1989; Ronne & Jøker 2006).

The aim of this study was to assess the suitability of pre-germination treatments and to establish simple germination protocol for some indigenous Namibian trees. Treatments of seeds or pods are required to break dormancy and initiate germination and are required by many savanna tree species. Common treatments include soaking of seeds; burning of pods; exposure to smoke; mechanical or acid scarification; treatments with plant chemicals and combinations of these treatments. Six tree species were tested during this study; namely *Dialium engleranum, Erythrophleum africanum, Guibourtia coleosperma, Pterocarpus angolensis* and *Schinziophyton rautanenii*. The selected pre-germination treatment protocols are intended to suit conditions outside laboratories, to allow replication by unskilled persons and do not require special equipment or chemicals. Our main purpose is to provide well documented information that can be applied in future germination experiments in Namibia.

Materials and Methods

Most seeds were collected in northern Namibia (Hamoye, Kaisosi and Masivi) by the Namibian Directorate of Forestry (DoF) between 2003 and 2015. Seed lots originated from a single year and place of collection and were stored below 18°C. For *P. angolensis* and *S. rautanenii,* seeds were also collected by the authors in 2013 near Cuangar, in southern Angola, and south of Mashare in Kavango East, Namibia and stored below 25°C. Seeds of *Terminalia sericea* collected in 2013 were also provided by DoF, but all appeared to be non-

viable and were discarded from the experiment. Seed storage was under similar conditions to earlier Namibian studies (Moses 2012; Van der Heyden 2014).

All work was done at the National Botanic Research Institute (NBRI) in Windhoek. Pre-germination treatments included: soaking in cold, warm and hot water for 24 hours; nicking; combinations of the two; no surface sterilisation and long-term soaking for one week (Table 1). Each treatment was replicated thee times with ten seeds per replicate, and five times with six seeds for large seeded *S. rautanenii*. For *P. angolensis* each treatment was only replicated twice because the seed lot was limited (Figure 2). Nicking referred to a small incision made with a scalpel through the seed coat, away from the embryo point. For *S. rautanenii* the incision had to be made with a saw. Cold soaking was done between 23°C and 28°C. Warm and hot soaking was tested as a pre-treatment for *D. engleranum, E. africanum* and *G. coleosperma* at 50°C and with boiling water respectively.

Table 1. Overview of all pre-germination treatments and replicates per species.

At 30°C							
Treatment	*D. engleranum*	*E. africanum*	*G. coleosperum*	*P. angolensis*		*S. rautanenii*	
				2013	2014	2003	2013
Nick	3x10	3x10	3x10	2x10	2x10	5x6	
Control	3x10	3x10	3x10	2x10	2x10	5x6	
Soak cold	3x10	3x10	3x10	2x10	2x10	5x6	
Nick and soak cold	3x10	3x10	3x10	2x10	2x10	5x6	
Nick and soak warm	3x10	3x10	3x10				
Nick and soak hot	3x10	3x10	3x10				
Soak warm	3x10	3x10	3x10				
Soak hot	3x10	3x10	3x10				
No sterilisation	3x10	3x10	3x10				
Soak long						5x6	
Nick and soak long						5x6	
At 26°C							
Nick	3x10	3x10	3x10	2x10	2x10	5x6	2x5
Control	3x10	3x10	3x10	2x10	2x10	5x6	2x5
Soak cold	3x10	3x10	3x10	2x10	2x10	5x6	2x3
Nick and soak cold	3x10	3x10	3x10	2x10	2x10	5x6	2x3
Nick and soak warm	3x10	3x10	3x10				
Nick and soak hot	3x10	3x10	3x10				
Soak warm	3x10	3x10	3x10				
Soak hot	3x10	3x10	3x10				
No sterilisation	3x10	3x10	3x10				
Soak long						5x6	

Figure 2. Pods and seeds of *Pterocarpus angolensis* (©R. Younan).

Prior to incubation, all seeds, apart from the no sterilisation set, were surface sterilised in a solution of one part 15% Sodium Hypochlorite and four parts distilled water (volume). They were submerged in the solution for 5 minutes then rinsed with distilled water five times. Seeds were placed on sterilised 9cm diameter Petri dishes with two MN 618 filter papers at the bottom. Petri dishes were watered with 5ml of distilled water and closed with Parafilm "M". Replicates were placed in a germination chamber at 30°C (+/-2°C) with a 12 hour light and dark regime. In the second part of the experiment the same procedure was repeated at 26°C. The Petri dishes were checked for germination progress daily and watered when dry. Seeds with clearly emerged radicals were determined as germinated. Germination was monitored over a minimum of 14 days for each trial. Between the two temperature regimes the handling of mouldy seeds was altered. During the 30°C experiment it was realised that seeds germinate despite developing mould on the surface. Discarding seeds due to mould was detrimental to maximum germination. Therefore, in the 26°C experiment only severely mouldy seeds were discarded.

A sample of seeds remaining ungerminated after 14 days at 26°C were subjected to a Tetrazolium test for viability, using a 0.5% solution, produced from 1g of Tetrazolium salt and 200ml of distilled water. Seeds were soaked at room temperature in distilled water for 24h, split and incubated in Tetrazolium solution for 24h at room temperature in the dark, after which they were rinsed and assessed.

Germination capacity was calculated for each species as the cumulative percentage of daily germination after two weeks (Dayamba *et al.* 2014). The mean germination time was the mean number of days since sowing till germination after two to three weeks. Species were tested for significant differences in germination capacity and mean germination time in function of temperature, pre-treatments and, for *P. angolensis* and *S. rautanenii*, origin. First, the data was tested for homoscedasticity and normality with the Fligner-Killeen and Shapiro-

Wilk tests respectively. Species with normal distributed data and homogeneity of variance were subjected to a two-way analysis of variance (ANOVA). When no significant interaction was observed between factors, the ANOVA was followed by a Tukey's post-hoc analysis ($p < 0.05$) (Dayamba *et al.* 2014). For data that did not show homoscedasticity or normality, even after transformation, a non-parametric Kruskal-Wallis test was performed to verify significance of treatment, temperature and origin effects. Post-hoc test of Kruskal-Wallis consisted of pairwise comparisons with the Wilcoxon rank sum test using a Bonferroni adjustment ($p < 0.05$). Analysis was performed in R.

Results and Discussion

An overview of the effect of temperature on the germination capacity is presented in Table 2. Differences in germination success between the two temperature regimes must be considered primarily in relation to allowing mouldy seeds to germinate at 26°C while they were discarded in the 30°C experiment. As a result, over-all germination capacity was higher at 26°C than at 30°C, although this difference was only significant for two species. Across all treatments, *G. coleosperma* had the highest germination capacity, in accordance with findings by Moses (2012) (Figure 3). The percentage of *P. angolensis* seeds that germinated was far lower than the 47% obtained by Van der Heyden's (2014). This can be attributed to the fact that the latter study followed germination of the seeds for a much longer time (almost one year). Another factor that could have contributed is the use of younger pods (1 to 2 years younger than this study).

Table 2. Germination capacity and mean germination time across treatments for all species studied at both temperature regimes. Significant differences between temperature regimes are indicated for $p < 0.5$ (*) and $p < 0.01$ (**).

	Germination capacity (%)				*Mean germination time (days)*			
	Mean	*30°C*	*26°C*	*Significance*	*Mean*	*30°C*	*26°C*	*Significance*
Dialium englerianum	41	35	47	**	7.4	6.4	8.2	
Erythrophleum africanum	20	21	19		8.0	7.0	9.0	*
Guibourtia coleosperma	91	87	96		7.3	7.3	7.3	
Pterocarpus angolensis	23	14	31	*	6.6	6.2	7.0	*
Schinziophyton rautanenii	7	7	6		10.8	12.4	9.7	

Figure 3. Germinated seed of *Schinziophyton rautanenii* (front) and *Guibourtia coleosperma* (background) (©R. Younan).

Mean germination time is not affected by the different handling of mould and was in general higher at 26°C than at 30°C (Table 2). *S. rautanenii* germinated almost two days faster at 26°C than at 30°C degrees, but the difference was not significant and more tests are needed to find the optimal temperature. The shortest mean germination time was found for *P. angolensis* and *D. engleranum* at 30°C. *S. rautanenii* had the lowest germination capacity and longest germination time in both temperature regimes. This is in agreement with literature that indicates that larger seeds germinate slower than small ones and that *S. rautanenii* germinates with difficulty (Moses 2012; Murali 1997).

The species that showed significant effects for the effect of treatments on germination capacity were *D. engleranum, E. africanum* and *P. angolensis.* All treatments that included nicking of *D. engleranum* resulted in significantly higher germination rates (Figure 4). MacDonald & Omoruyi (2003) found that only hot water soaking could improve germination rates for another *Dialium* species. In this study, none of the soaking treatments showed significant improvements. However, as soaking in hot water may cause damage to the seed; it may be advisable to soak *D. engleranum* at lower temperatures in accordance to advice from the DoF. Nicking of *E. africanum* seeds resulted in significantly higher germination

(38%) compared to the treatments with the lowest germination rates: the non-surface sterilised seeds (8%) and seeds that had been nicked and soaked with cold water (12%). The combinations of nicking and soaking in water often resulted in bloated seeds with a soft core. This was not conducive for germination, as especially *E. africanum* would quickly develop mould from within. For *P. angolensis*, nicking of seeds gave significantly better results than nicking and soaking or soaking only (Figure 5).

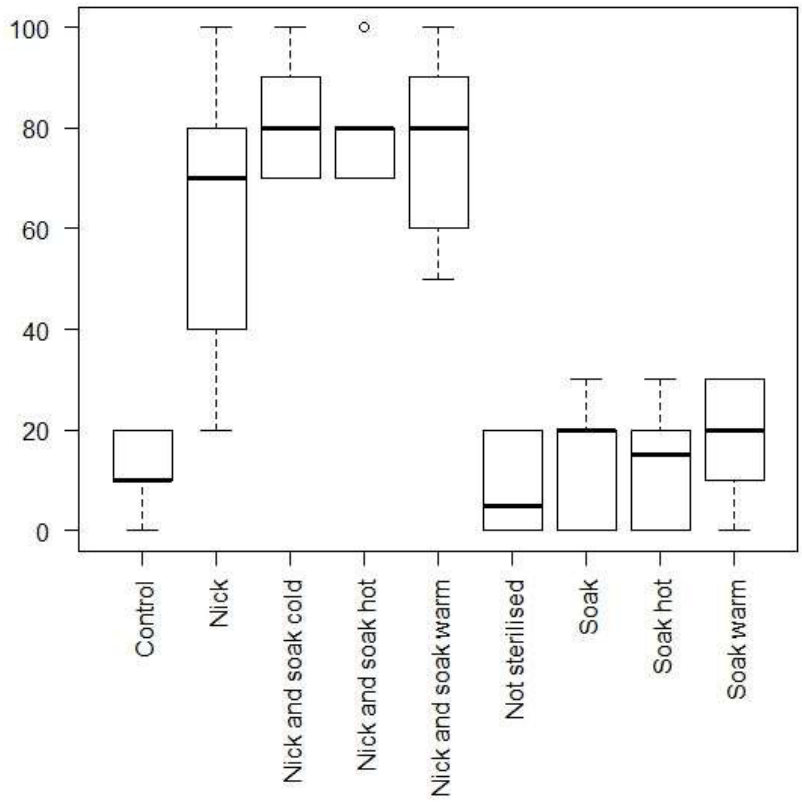

Figure 4. Germination capacity (%) of *Dialium engleranum* for the different treatments.

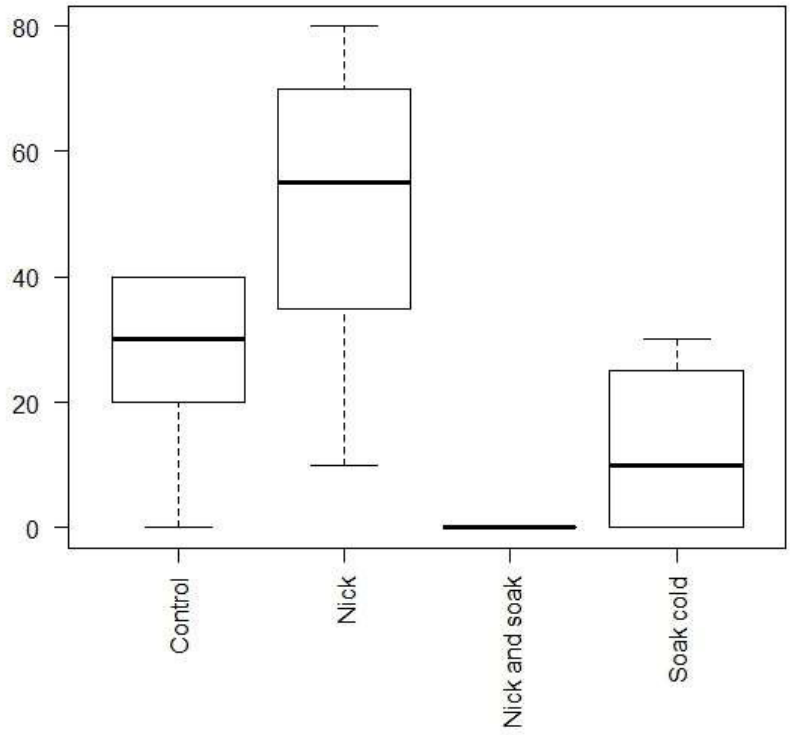

Figure 5. Germination capacity (%) of *Pterocarpus angolensis* for the different treatments.

None of the treatments used on *S. rautanenii* seeds appeared to be effective. Contrary to Ronne & Jøker (2006), soaking and nicking *S. rautanenii* seeds did not improve their germination in comparison to nicking only. According to Keegan *et al.* (1989), only ethylene can break *S. rautanenii*'s dormancy.

Origin of the *P. angolensis* and *S. rautanenii* seeds, and consequently different storage temperatures, did not have a significant effect on germination capacity nor mean germination time. Treatments did have a significant effect on mean germination time for two species. Germination of *G.coleosperma* took 11 days for the control and non-sterilised seeds compared to an average of 6 days for all other treatments. For *P. angolensis*, germination took on average 10 days for the cold soaked seeds compared to 5 or 6 days for nicking or nicking and soaking.

The Tetrazolium test revealed most seeds that hadn't germinated were viable. As the amount of non-germinated seeds was still high except for *G. coleosperma*, it can be stated that for the other species either the two week period was too short or the pre-treatments did not sufficiently break dormancy.

Seed viability of most seeds was remarkable. *E. africanum*, collected in 2003, still reached germination rates of 20%. Some seeds of *S. rautanenii* also sporadically germinated after twelve years in storage. This species can thus remain viable in storage for much longer than previously assumed (Peters 1987; Ronne & Jøker 2006). The storage durability of *P. angolensis* of at least three years as reported by Jøker *et al.* (2000) was confirmed. No literature on the effects of seed storage for several years for the other species could be found.

According to the findings, the following procedures are recommended to improve germination success:

- nicking of *D. engleranum* seeds, and optionally soaking for 24h in either cold or warm water;
- nicking of *E. africanum* seeds;
- nicking of *P. angolensis* seeds;
- optional surface sterilisation and other treatments of *G. coleosperma* seeds, especially to decrease germination time;
- nicking and a combination of nicking and soaking may promote germination of *S. rautanenii* to a certain extent, but further improvement of pre-treatments will be necessary;
- for all species studied, except *G. coleosperma*, seeds should be kept in an environment conducive to germination for longer than two weeks to achieve the maximum germination potential.

Future studies may want to investigate the germination response on more temperature regimes, as well as different day lengths and do so for a period of at least one to two months. Such an approach would allow taking the natural conditions into account better. The relationship of viability and seed floating should be further studied, especially as it is a more feasible alternative to the very expensive Tetrazolium testing. Van der Heyden (2014) found seeds of *P. angolensis* that sank to germinate better.

Other studies are now starting to build on the results of this study to improve germination and seedling development protocols for indigenous tree species and to find the most suited methods to assist regeneration in the trees' natural habitat. Much more work is needed to investigate their potential for enrichment planting in the forest, for agroforestry and inter-cropping and hereby meeting socio-economic needs.

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