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Effect of different mechanical seed scarification methods on germination and emergence dynamics of baobab (*Adansonia digitata* L.)

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Abstract

The African baobab (*Adansonia digitata* L.) is a multipurpose fruit-producing tree indigenous to African savannahs. Commercial interest in the species has grown in recent years. The major obstacle of seed-based propagation of baobab is its inherent seed dormancy. Therefore, this study tested the effects of different mechanical seed scarification methods on seed germination parameters and seedling development of *A. digitata*. The results indicate that mechanical scarification had a significant effect on germination and emergence dynamics of *A. digitata*. The highest total emergence percentage with 61.7 % was achieved by scarifying the seeds with a saw on the hilum side. Cotyledon damage due to mechanical scarification occurred in all treatments. Proportions of damage categories depended significantly on treatment. The largest proportion of undamaged cotyledons was with 63.6 % achieved by scarifying the seeds with a saw on the hilum side. This precise technique may be suitable for propagation of baobab in a rural setting, however, the effects of scarification methods on seedling emergence needs to be further investigated.

Keywords: baobab, cotyledon damage, propagation, seed dormancy, seedling development

1 Introduction

The African baobab (*Adansonia digitata* L., Malvaceae) is a multipurpose fruit-producing tree indigenous to African savannahs and well adapted to dry environments (Wickens & Lowe 2009; De Smedt *et al*., 2012). Commercial interest in this taxon has grown in recent years (Buchmann *et al*., 2010; Gebauer *et al*., 2014; Jäckering *et al*., 2019; Darr *et al*., 2020) but exploitation of the species' resources still depends mainly on wild stands (Gebauer *et al*., 2016) which are increasingly threatened (Sanchez *et al*., 2011; Gebauer & Luedeling, 2013; Venter & Witkowski, 2013). While adult baobabs possess great longevity and belong to the oldest deciduous trees on earth with ages of up to 2,000 years (Patrut *et al*., 2018), recruitment events are episodic and seem to be limited to exceptional rainy seasons, making juvenile trees a rarity (Venter & Witkowski, 2013). It is therefore imperative to abet recruitment of the tree by actively propagating baobab from seeds e.g. in tree nurseries, home gardens or agroforestry systems.

The major obstacle of sexual or seed-based anthropogenic propagation of baobab is its inherent seed dormancy (Esenowo, 1991). Germination and subsequently emergence and recruitment in this taxon is inhibited by the seed coat's impermeability to water and oxygen, a typical mechanism of primary seed dormancy found in many woody species of arid savannah zones (Danthu *et al*., 1995; Schmidt, 2000; Falemara *et al*., 2014; Yousif *et al*., 2019). *A. digitata* produces orthodox seeds with strong physical dormancy for zoochoric dispersal (Razanameharizaka *et al*., 2006; Gebauer *et al*., 2014; Gebauer *et al*., 2016) or hydrochoric dispersal (Tsy *et al*., 2009; Kempe *et al*., 2018).

Choosing the correct seed pre-treatment method for field cultivation of *A. digitata* has to find a balance between applicability and efficacy and may depend on local germination and morphometric characteristics of available seed stock and environmental conditions. While acid seed scarification often has produced the best results it is not recommend for use in the field or outside laboratory settings due to sulphuric acid's harmful effects on both humans and the environment coupled with low availability to farmers (Danthu 1995; Assogbadjo *et al*., 2011; Falemara *et al*., 2014). Mechani-

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Dedicated to the memory of Prof. Dr. Chimuleke Munthali, enthusiastic baobab researcher.

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cal seed scarification has long been established as a method leading to significant positive germination rates for species with hard seed coats including baobab (e.g. 75 % in Esenowo, 1991; 68 % in Danthu, 1995; data are means) and has been used as a positive control in more recent studies as well (Razanameharizaka *et al*., 2006; Lautenschläger *et al*., 2020). Furthermore, mechanical scarification is recommended as the method of choice in baobab propagation tutorial videos aimed at laypersons (Pander, 2016; Ratana, 2018; Matthew, 2020). However, methods are either not described in detail or differ from source to source. Detailed studies on the effect of different mechanical scarification methods on germination parameters and seedling development of *A. digitata* are missing.

Mechanical scarification directly disrupts the water-impermeable seed coats, representing the biological effect of mastication, the initial stage in endozoochory (Razanamandranto *et al*., 2004). In mechanical or manual scarification a small piece of the seed coat is removed ('nicking' or 'filing') or the seed coat as a whole is cracked ('cracking') using tools such as secateurs, files, sand paper or a hammer (Danthu, 1995; Razanameharizaka *et al*., 2006; Saied *et al*., 2008). Hansohm *et al*., (2020) recommend mechanical scarification in a technical baobab cultivation manual aimed at supporting rural farmers, as any abrasive surface can be used to scarify the seed in absence of suitable tools. However, as germination speed or emergence times are of major relevance in agronomic settings as well, germination rates do not suffice as the only germination characteristic relevant in decision-making. Therefore, our study was designed to test the effect of mechanical scarification methods on different germination parameters and seedling development characteristics of *A. digitata*.

2 Materials and methods

2.1 Plant material

Baobab seeds were collected from wild stands in Kilifi (latitude: 3° 30' S, longitude: 39° 54' E, 5 m a.s.l.), Kenya in December 2019, extracted from fruits and stored at room temperature. Prior to the start of the experiments seeds were manually depulped. Nonviable seeds were removed by applying the flotation method (Sacande *et al*., 2006; Hansohm *et al*., 2020).

2.2 Experimental design and seed pre-treatments

The experiments were conducted in a growth chamber of the Tropical Greenhouse at the Faculty of Life Sciences, Rhine-Waal University of Applied Sciences, Germany. Day temperature of the chamber was set to 35 °C and night temperature to 25 °C. Due to varying outside weather conditions daily mean temperatures in the two experiments varied between 27.9 °C and 30.3 °C in experiment 1 and 2, respectively.

The experimental setups were two randomized complete block designs with three blocks and 60 seeds per treatment. Seeds were sown at a depth of 4 cm in quartz sand in June/July 2020 in randomized 10×10 grids in cell trays with dimensions of $4 \times 4 \times 8$ cm. Daily irrigation averaged 5 ml tap water per cell and day.

Experiment 1

In total 300 seeds were randomly assigned to the following treatments: Control = no scarification, T01 = mechanical scarification via hand saw, T02 = T01 + soaking in ambient water for 48 h, T03 = T02 + complete removal of seed coat and T04 = T02 + complete removal of seed coat with subsequent storage on wet tissue paper in a sealed plastic box for 24 h.

Mechanical scarification was carried out using a PUK hand saw (Josef Haunstetter Sägenfabrik, Germany) on the rounded side opposite the hilum region of the seeds, which were fixed in a vice using cardboard to facilitate secure fixation of the seeds without damaging them. Scarification was done until the white seed embryo started to become visible. In the respective treatments (T02-T04), seeds were soaked in tap water, which was changed once after 24 hours. Seed coats of soaked seeds were removed per hand immediately after taking them out of the water by splitting the softened seed coats along the sawed fissure.

Experiment 2

300 seeds were randomly assigned to the following treatments: T05 = mechanical scarification via hand file on opposite side of hilum, T06 = mechanical scarification via hand file on side of hilum, T07 = mechanical scarification via hand saw on side of hilum. As in experiment 1 the seeds were fixed in a vice for scarification. Scarification treatments on the hilum side was done on both outer ends of the seed.

2.3 Data collection

Data collection was identical for both experiments. Seed emergence and seedling death were recorded daily as days after sowing (DAS). Seedling emergence was measured as emergence initiation (E_{ini}) when the hypocotyl hook first protruded from the soil and as emergence completion (E_{comp}) when the seedling had either emerged fully and unfolded its cotyledons or the seedling had emerged fully and started developing its first true leaves, in case cotyledons did not unfold either due to being obstructed by the unshed seed coat

or stuck together due to damage or malformation. Cotyledon damage was recorded at the end of each experiment as an ordinal variable ranging from damage category 0 = no damage on cotyledons, damage category 1 = slight damage on cotyledons (cuts, margins torn, but cotyledon largely unaffected; Fig. 4d, e, h), to damage category 2 = heavy damage on cotyledons (deeply torn margins, necrotic cotyledons, fused together precluding unfolding; Fig. 4b, c, g).

The following variables were calculated based on recorded E_{ini} and E_{comp} after 30 DAS for the first experiment and after 26 DAS for the second experiment for each treatment × block combination separately: Total emergence percentage (*TEP*), days to first emergence (E_{1st}), days to 50 % emergence (E_{50}), emergence spread (E_s), emergence length (E_l), defined as the number of days between E_{ini} and E_{comp} for each seedling and mean emergence time (*MET*), defined according to Watkins & Cantliffe (1982) as

$$\frac{\sum(D_i \times N_i)}{S}$$

where D_i = DAS, N_i = number of seeds that initiated germination on the i^{th} day and S = total number of seeds in treatment.

2.4 Statistical analyses

Data were analysed in R studio using R 4.0.3 (R Core Team, 2020). For descriptive statistics and data manipulation the *tidyverse* package was employed (Wickham *et al.*, 2019). Data were tested for normality using QQ-plots and histograms of model residuals. Homogeneity of variances was tested graphically and using Bartlett's test. Percentages were arc-sine transformed before analysis. Parametric data were analysed using a Type I ANOVA. Separation of means at $p < 0.05$ was achieved using the Tukey Honest Significant Difference Test from the multcompView package (Graves *et al.*, 2019). Non-parametric data were analysed using the Kruskal-Wallis rank-sum test and separation of means was achieved with Dunn's Test of Multiple Comparisons from the FSA package (Ogle *et al.*, 2020). Damage categories were analysed for independence using the Asymptotic General Independence Test or Ordinal Chi-Square Test from the coin package (Hothorn *et al.*, 2006) using pooled data from both experiments. Separation of dependent means at $p < 0.05$ was performed using the Pairwise Permutation Test using the rcompanion package (Mangiafico, 2020).

3 Results

3.1 Pre-treatments' applicability

Mechanical scarifications were successfully and homogeneously applied using the clamped vice-method and yielded 60 scarified seeds per hour. Seeds were more difficult to scarify with a file than with a saw. Scarified and subsequently soaked seeds swelled to twice their original size over the course of two days. Decoating of seeds took more time per seed compared to sawing or filing them.

3.2 Germination and emergence dynamics – Experiment 1

T01 yielded with 53 % the highest *TEP* of all scarification treatments (Table 1). Significant differences were observed for *TEP*, with T01 yielding a significantly higher *TEP* then control, T02 and T04 ($p < 0.01$, Table 1). The control treatment only produced one germination in one block. E_{ini} was significantly influenced by the different treatments as well with T02, T03 and T04 emerging significantly faster than the control ($p < 0.001$, Fig. 1). Excluding the control due to lack of emerged seedlings still left T01 to be significantly different from T02, T03 and T04 ($p < 0.001$, data not shown).

Table 1: *Means ± standard deviations of calculated germination parameters of* A. digitata *seeds: total emergence percentage (TEP), mean emergence time (MET), days to first emergence (E_{1st}), days to 50 % emergence (E_{50}), emergence spread (E_s) and emergence length (E_l) of treatments.*

Treatment	*TEP (%)*	*MET (days)*	E_{1st} *(days)*	E_{50} *(days)*	E_s *(days)*	E_l *(days)*
Control	1.7 ± 2.4^{b}	-	-	-	-	1.0 ± 0.0^{a}
T01	53.3 ± 21.1^{a}	13.7 ± 2.3	9.3 ± 0.5	13.2 ± 2.3	10.3 ± 3.4	3.4 ± 2.1^{a}
T02	18.3 ± 6.3^{b}	9.6 ± 2.2	5.3 ± 0.9	9.0 ± 1.9	9.7 ± 5.6	5.0 ± 1.3^{a}
T03	33.3 ± 2.4^{ab}	6.3 ± 0.6	4.0 ± 0.0	5.8 ± 0.2	6.3 ± 2.1	8.4 ± 3.0^{b}
T04	8.0 ± 4.75^{b}	9.4 ± 6.1	8.0 ± 5.7	8.3 ± 5.5	4.0 ± 5.7	3.2 ± 0.5^{a}

Superscript letters within a column indicate significant differences between treatments at $p < 0.05$. Hyphens indicate non-available parameters due to insufficient (n < 2) germination. Control = no scarification, T01 = mechanical scarification via hand saw, T02 = T01 + soaking in water for 48 h, T03 = T02 + complete removal of seed coat and T04 = T02 + complete removal of seed coat with subsequent storage on wet tissue paper in a sealed plastic box for 24 h.

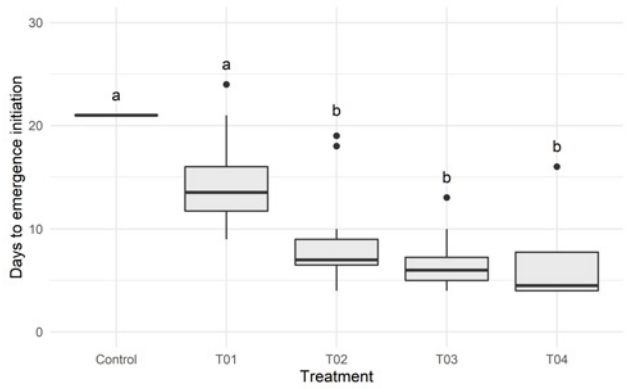

Fig. 1: *Boxplots of days to emergence initiation (E_{ini}) categorised by treatment of* A. digitata *seeds. Letters above boxplots indicate significant differences at $p < 0.05$. Control = no scarification, T01 = mechanical scarification via hand saw, T02 = T01 + soaking in ambient water for 48 h, T03 = T02 + complete removal of seed coat and T04 = T02 + complete removal of seed coat with subsequent storage on wet tissue paper in a sealed plastic box for 24 h.*

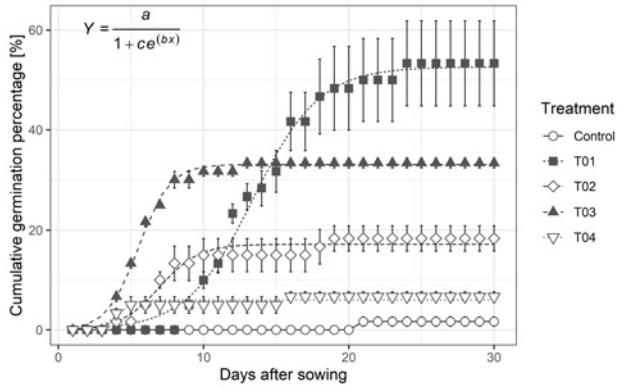

Fig. 2: *Effect of various scarification treatments on germination of* A. digitata *seeds. Data are means with standard deviation as error bars. Non-linear regressions are given as lines based on the displayed logistic growth function formula. Control = no scarification, T01 = mechanical scarification via hand saw, T02 = T01 + soaking in water for 48 h, T03 = T02 + complete removal of seed coat and T04 = T02 + complete removal of seed coat with subsequent storage on wet tissue paper in a sealed plastic box for 24 h.*

MET, E_{1st}, E_{50} and E_s were not found to be significantly different between treatments but show a trend with T01 taking the most days to reach its *TEP* and having the highest *MET* and E_{50} of all treatments at 13.7 days and 13.0 days, respectively (Table 1). Differences at E_{1st} are also reflected in the different onsets of cumulative germination curves (Fig. 2). Once E_{1st} was observed, 50 % emergence was reached quickly for all treatments with less than two days between E_{1st} and E_{50} for T02 and T03 and less than a day for T04, yet levelled off quickly as well, leading to few days of sustained emergence and low overall *TEP* except for T01 (Fig. 2). E_s was generally shorter than a week with exceptions of T01 and T02 where the latest germination occurred at 24 DAS and 18 DAS, respectively (Fig. 1). For T03, El was found to be significantly different compared to other treatments with 8.4 days ($p < 0.001$, Table 1).

All treatments had a significant effect on seed viability of ungerminated seeds ($p < 0.001$, data not shown) and ungerminated seeds of all treatments were found soft and rotten while all control seeds were found to be not rotten and hard (data not shown).

3.3 Germination and emergence dynamics – Experiment 2

T07 yielded the highest *TEP* at 61.7 % while *TEP*s of both filing treatments were significantly lower at 31.7 % for T05 and 20.0 % for T06, respectively ($p < 0.01$, Table 2). E_{ini} did not vary significantly between treatments (data not shown). *MET*, E_{1st} and E_{50} were also not found to be significantly different between treatments. *MET* was similar across treatments featuring a 1.8 days difference between the fastest *MET* of 10.7 days for T07 and the slowest *MET* of 12.5 days for T06. T06 had the highest E_{50} with 12.3 days compared to T05 and T07. E_s was significantly lower for T06 compared to T05 and T07 ($p < 0.01$, Table 2). E_l for T06 and T07 differed significantly from each other with T07 featuring the lowest value of 2.7 days ($p < 0.05$).

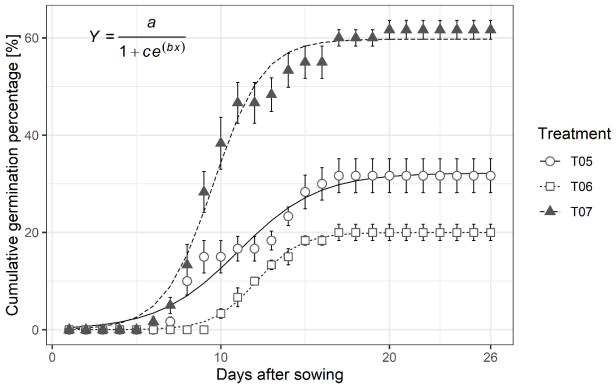

Fig. 3: *Effect of various scarification treatments on germination of* A. digitata *seeds. Data are means with standard deviation as error bars. Non-linear regressions are given as lines based on the displayed logistic growth function formula. T05 = mechanical scarification via hand file on opposite side of hilum, T06 = mechanical scarification via hand file on side of hilum, T07 = mechanical scarification via hand saw on side of hilum.*

Cumulative germination curves (Fig. 3) show *TEP* plateaus at 16 DAS for T06 and T07 and 19 DAS for T05. T05 and T07 regressions vary only in steepness while T06 shows a delayed onset of emergence which is reflected by its significantly smaller E_s. T05 data shows high variation with

Table 2: *Means ± standard deviations of calculated germination parameters of* A. digitata *seeds: total emergence percentage (TEP), mean emergence time (MET), days to first emergence (E_{1st}), days to 50 % emergence (E_{50}), emergence spread (E_s) and emergence length (E_l) of treatments.*

Treatment	TEP (%)	MET (days)	E_{1st} (days)	E_{50} (days)	E_s (days)	E_l (days)
T05	31.7 ± 8.6^a	11.3 ± 1.5	7.7 ± 0.5	10.3 ± 2.6	7.7 ± 1.3^b	4.0 ± 2.1^{ab}
T06	20.0 ± 4.1^a	12.5 ± 1.2	10.7 ± 0.9	12.3 ± 1.9	4.0 ± 0.8^a	4.8 ± 2.5^a
T07	61.7 ± 4.7^b	10.7 ± 1.4	7.3 ± 1.3	9.7 ± 0.9	9.7 ± 1.3^b	2.7 ± 1.8^b

Superscript letters within a column indicate significant differences between treatments at $p < 0.05$. T05 = mechanical scarification via hand file on opposite side of hilum, T06 = mechanical scarification via hand file on side of hilum, T07 = mechanical scarification via hand saw on side of hilum.

two distinct 'jumps' in emergence at 6 and 13 DAS, whereas T06 and T07 rise continuously once emergence starts. At the end of the experiment all ungerminated seeds were found soft and rotten.

3.4 Post-emergence behaviour and cotyledon damage

Seedlings in both experiments were often unable to discard their seed coats after emergence leading to precluded unfolding of the cotyledons (Fig. 4a). Dried up seed coats stayed attached to the cotyledons while the first true leaves developed unobstructed (Fig. 4f).

Slightly damaged cotyledons followed an 'angel cut' pattern with diametric diagonals along the midrib on the cotyledons in the first experiment (Fig. 4d, e), whereas heavy damage did not follow a specific pattern for both experiments and ranged from torn off cotyledon margins (Fig. 4c) to fully necrotic cotyledons hardened to a point inhibiting unfolding over the course of the experiment (Fig. 4b). Seedlings with slight damage in the second experiment exhibited a mirrored 'angel cut' pattern reflected along the orthogonal of the cotyledon midrib coined a 'butterfly cut' (Fig. 4h) which was associated with T06 and T07 (scarification on the hilum side of the seed).

Cotyledon damage occurred in all treatments across both experiments except for the control (Fig. 5). Proportions of damage categories depended significantly on treatment

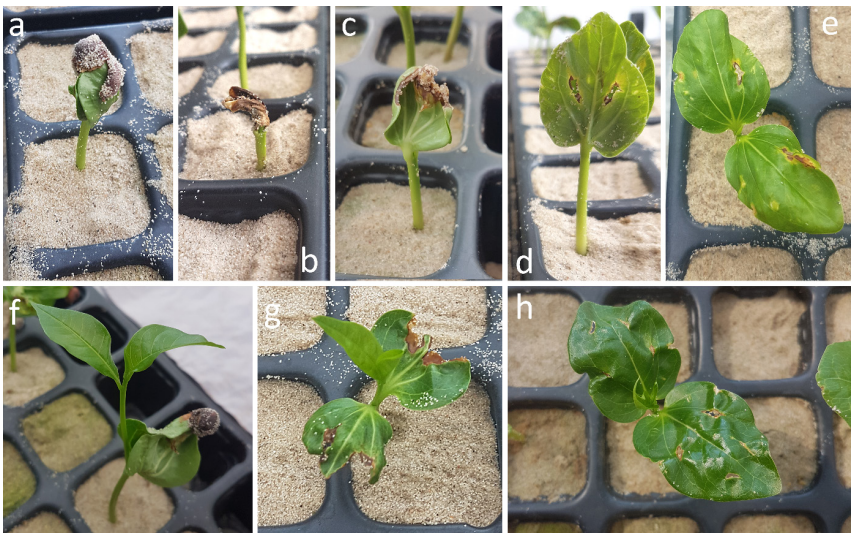

Fig. 4: A. digitata *seedlings in various stages of development with different levels of cotyledon damage. a) Seedling with unshed seed coat at 22 DAS. Seed coat split by growth of cotyledons but not shed. b) Heavily damaged (category 2) cotyledons on seedling at 15 DAS. Cotyledons fused together and fully necrotic. c) Heavily damaged (category 2) cotyledons at 26 DAS. Only parts of cotyledons are necrotic, but fused together. d) Slight damage (category 1) on cotyledons at 15 DAS. Cuts show typical pattern observed for seedlings scarified on the opposite of the hilum. e) Slightly damaged (category 1) seedling at 12 DAS with unilateral cut pattern. f) Seedling at 21 DAS with first true leaves. Cotyledons have not unfolded due to unshed seed coat. g) Heavily damaged (category 2) cotyledons on seedling at 13 DAS. Cotyledons are not fused together. Leaf tips have been torn off completely with necrotic margins. h) Slightly damaged (category 1) cotyledons at 15 DAS. Cuts show typical mirror pattern associated with scarification on the hilum side of the seed.*

($p < 0.001$, Fig. 5) as well as the side on which the scarification was applied (hilum or opposite side, ($p < 0.001$, Fig. 6a) and the tool used (file or saw, $p < 0.05$, Fig. 6b). Slight damage was present for all treatments except control and heavy damage was observed in all treatments except control and T04. Damage category 0 was not observed in T03, T04 and T05 (Fig. 5). Excluding the control, the largest proportion of undamaged cotyledons was with 63.6 % achieved in T07 with scarification by saw. However, the proportion of heavily damaged cotelydons was similar for both scarifications using either saw or file (Fig. 6b). Decoating led to a significantly higher proportion of heavily damaged cotyledons in T03 compared to T02 and the highest proportion of heavily damaged cotyledons of all treatments. Neither T03, T04 nor T05 produced seedlings with undamaged cotyledons, with T05 yielding the highest proportion (68.5 %) of seedlings with only slightly damaged cotyledons of all treatments (Fig. 5).

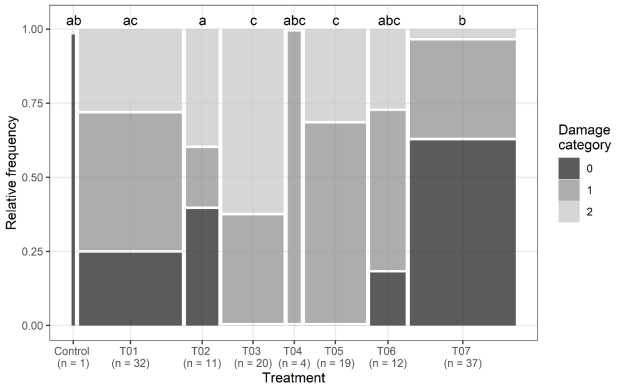

Fig. 5: *Mosaic plot of damage category proportions by treatment of* A. digitata *seedlings. Data of experiment 1 and 2 are pooled. Column width indicates relative proportion of the treatment group to all treatment groups. Absolute treatment group size is given as n = number of germinated seedlings per treatment group. Damage category 0 = no damage on cotyledons, damage category 1 = slight damage on cotyledons (cuts, margins torn, but cotyledon largely unaffected), damage category 2 = heavy damage on cotyledons (deeply torn margins, necrotic cotyledons, fused together precluding unfolding). Control = no scarification, T01 = mechanical scarification via hand saw, T02 = T01 + soaking in water for 48 h, T03 = T02 + complete removal of seed coat and T04 = T02 + complete removal of seed coat with subsequent storage on wet tissue paper in a sealed plastic box for 24 h, T05 = mechanical scarification via hand file on opposite side of hilum, T06 = mechanical scarification via hand file on side of hilum, T07 = mechanical scarification via hand saw on side of hilum. Thin bar lines in control, T03, T04 and T05 indicate zero numbers of that category in the dataset of the respective treatment. Letters above stacked bars indicate significant differences using the Pairwise Permutation test at $p < 0.001$.*
Absolute values of proportions are available in table SB1 in the supplement.

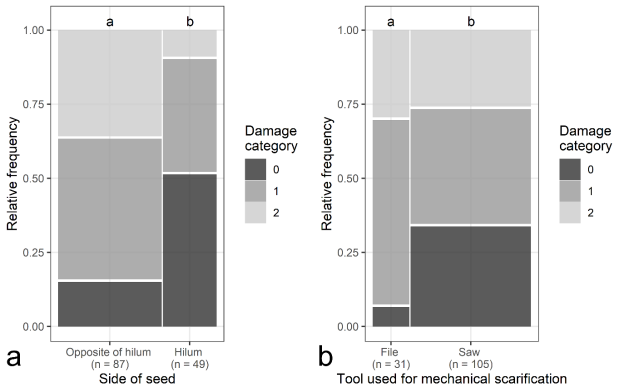

Fig. 6: *a) Mosaic plot of damage category proportions of* A. digitata *seedlings by side where scarification was applied. Data of experiment 1 and 2 are pooled. Absolute treatment group size is given as n = number of germinated seedlings per treatment group. Letters above stacked bars indicate significant differences using the Pairwise Permutation test at $p < 0.001$. b) Mosaic plot of damage category proportions of* A. digitata *seedlings by tool used for mechanical scarification. Data of experiment 1 and 2 are pooled. Letters above stacked bars indicate significant differences using the Pairwise Permutation test at $p < 0.05$. Damage category 0 = no damage on cotyledons, damage category 1 = slight damage on cotyledons (cuts, margins torn, but cotyledon largely unaffected), damage category 2 = heavy damage on cotyledons (deeply torn margins, necrotic cotyledons, fused together precluding unfolding).*
Absolute values of proportions are available in tables SB2 and SB3 in the supplement.

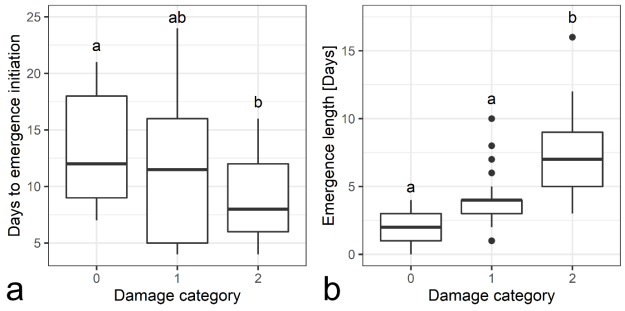

Fig. 7: *a) Boxplot of E_{ini} of the first experiment by damage category of* A. digitata *seedlings. Letters above boxplots indicate significant differences using Tukey's HSD test at $p < 0.05$. b) Boxplot of E_l of the first experiment by damage category of A. digitata seedlings. Letters above boxplots indicate significant differences using Tukey's HSD test at $p < 0.05$. Damage category 0 = no damage on cotyledons, damage category 1 = slight damage on cotyledons (cuts, margins torn, but cotyledon largely unaffected), damage category 2 = heavy damage on cotyledons (deeply torn margins, necrotic cotyledons, fused together precluding unfolding).*

Interactions between damage categories and treatments for emergence variables E_I and E_{ini} were not found to be significant in the pooled data of both experiments, but effects of

damage category on E_I and E_{ini} parameters were found to be significant for the first experiment ($p < 0.001$ for E_I and $p < 0.05$ for E_{ini}, Fig. 7a, b). E_{ini} were significantly lower for seedlings with high damage on cotyledons compared to seedlings with no cotyledon damage ($p < 0.05$), with a difference of 4.6 days (Fig. 7a). An opposite effect was observed for seedlings with heavy cotyledon damage with these featuring significantly higher E_I than seedlings with no cotyledon damage ($p < 0.001$; Fig. 7b), with a difference of 5.9 days. Effect of damage category was not present on E_I in the second experiment, but significantly influenced E_{ini} as well ($p < 0.05$; data not shown).

4 Discussion

Mechanical scarification of baobab seeds had an effect on seed germination rates. However, T07 with a *TEP* of more than 60 % was the only treatment reaching levels found in literature for mechanically scarified baobab seeds (Esenowo 1991; Danthu *et al*., 1995). Untreated control seeds yielded no germinations except of one late case at 21 DAS which is in accordance with results of Esenowo (1991), Kempe *et al*. (2018) and Lautenschläger *et al*. (2020), where untreated seeds did not germinate at all. Yet these findings contradict results from Danthu *et al*. (1995), Razanameharizaka *et al*. (2006) and Assogbadjo *et al*. (2011), where non-scarified seeds reached up to 57 % *TEP*. These discrepancies confirm high variabilities in baobab seed germination characteristics between provenances, as seed morphometric characteristics have been found to relate to germinability and to be highly heterogeneous, even within provenances or individual trees (Assogbadjo *et al*., 2011; Munthali *et al*., 2012; Niang *et al*., 2015).

Signs of initiated germination were present for T02, T03 and T04 before sowing but these did not translate into increased emergence success, as T04 produced the lowest *TEP* of all treatments in both experiments excluding control. Falemara *et al*. (2014) posited that the faster the seed coat is ruptured, the faster the rate of germination. However, it is likely that seeds decayed before they could emerge from the substrate as emergence does not indicate initiated germination, but rather the endpoint of germination (El-Siddig *et al*., 2004). Soaking scarified baobab seeds surprisingly induced detrimental effects on emergence: as seeds featuring these treatments had already soaked up water before being sown which was apparent due to their swelling, they were anticipated to germinate more quickly, since water uptake is considered the basis of inducing germination in physically dormant species (Baskin & Baskin, 2004; Yousif *et al*., 2019). However, soaked treatments yielded significantly lower *TEP*s with the exception of T03 compared to the unsoaked treatment T01 (Table 1). Soaked seeds might have been prone to rot and pathogen infection which was corroborated by exhumed seed coats being covered in fungal mycelia and seeds having decayed. Jansen *et al*. (2020) observed fungal mycelia on exhumed seed coats of field-sown *A. digitata* seeds that had germinated as well, indicating that fungal rot of the seed coat is a common phenomenon and may not imperil the seed embryo per se. Danthu *et al*. (1995) found that soaking of seeds post-scarification for more than 6 hours reduced germination rates to 2 % and ungerminated seeds decayed in their seed coats, theorising that excessive imbibition of water led to asphyxia and necrosis of seed embryo tissues. This points to asphyxia due to excessive moisture to be a more likely candidate for early seed death. Another avenue of explanation would be rancidification of seed fats and oils: *A. digitata* seeds have high mono- and polyunsaturated fatty acid content (Sacande *et al*., 2006; Nouruddeen *et al*., 2016). These fatty acids may oxidise under air exposure which could deplete seed embryo reserves. Kaboré *et al*. (2011) noted that cold-water soaking of decoated seeds decreased tannin and phytate activity, but did not report effects on germinability, as it is likely that soaking of decoated seeds leads to asphyxia. Esenowo (1991) reported that decoated seed embryos gave high germination rates of 85 %, but only germination under in vitro conditions was measured, not emergence.

Using a file to abrade the seed coat instead of just fissuring it results in larger amounts of the seed coat being removed in order to expose the seed embryo, thus baring an increased surface area of the seed embryo to ambient air before planting. Saied *et al*. (2008) found significantly longer emergence times for scarification by sand paper compared to cracking the seed coat for seeds of *Ziziphus spina-christi* L., but no significant difference in *TEP*. Wang *et al*. (2011) also found no significant differences in germination between scarification by nicking or using sand paper for multiple *Vigna* species, but observed a significantly faster germination rate for mechanical scarification compared to thermal scarification. A file may introduce an increased amount of foreign particles to the seed embryo due to the larger area of contact with the seed coat during scarification as well as facilitate lower fissility for the developing embryo compared to the more linear and narrower area of contact when applying a saw. Sand paper has the added drawback of wearing out too rapidly to be of practical use, as seeds of *A. digitata* can be considered to be very hard and, thus, abrasion-resistant. Thermal scarification has recently been demonstrated to induce germination in 78 % of tested baobab seeds by Lautenschläger *et al*. (2020) which could potentially allow effective and simul-

taneous scarification of larger seed quantities, but the tested method relied on thermo-stable and temperature-controlled ovens applying a homogenous heat regime and thus deliver repeatable and comparable results using consistent application timings. Access to these devices may well be a serious obstacle for widespread adoption of this method in rural sub-Saharan Africa, as the alternative of using wood-fire or charcoal stoves commonly owned by many households in the region poses the problem of achieving a both temporally and spatially controlled heat regime and thus risks overheating and subsequently killing the seed embryos. Kempe *et al*. (2018) achieved a germination rate of 25 % of baobab seeds embedded in baobab fruits using a charcoal grill. Yet, the use of firewood for scarification competes with the more pressing use of firewood for cooking while additionally contributing to deforestation. Hand files and vices as used in this study may still be considered specialised tools inaccessible to rural households, however, Hansohm *et al*. (2020) suggested using coarse stone surfaces for abrasion as a tool-independent and commonly accessible alternative for mechanically scarifying baobab seeds.

In our experiments cotyledon damage varied significantly across treatments while being correlated with significantly different E_{ini} and E_l independent of treatment, but not experiment. This suggests a significant interaction between cotyledon damage, environmental factors and emergence. It also has to be mentioned that the effect of damage category on E_{ini} and E_l as well as the non-significant interaction with the type of treatment is purely based on data from seedlings that managed to emerge: heavy cotyledon damage might lead to seedling death in the majority of cases and reduce emergence overall, but as non-emerged seedlings are recorded as not available (NA) in the underlying dataset of this study, they are not represented in the analysis.

Cotyledon damage as a result of scarification treatments in baobab has not been specifically reported in the literature beyond mentions of general seedling viability; however, implications of scarification on cotyledon development and emergence can be derived from seed anatomy: *A. digitata* is a species from the Malvaceae family producing foliate axile seed embryos with developed cotyledons as characterised by Martin (1946). Baobab cotyledons are extensively expanded and folded in multiple layers along the hypocotyl axis with most of the cotyledonic tissues being oriented opposite of the hilum. This makes injuries of the cotyledons very likely during mechanical scarification on the side opposite the hilum, as the cotyledons are the first embryonic tissue encountered when breaking the seed coat. Damage category proportions between scarification on the hilum side and the opposite side were significantly different, with scarification on the hilum side yielding a higher proportion of undamaged seedlings and scarification on the opposite side yielding a higher proportion of heavily damaged seedlings. This is compounded by T05, where a file was used on the opposite side of the hilum, yielding not a single seedling with no cotyledon damage.

In conclusion, it could be shown that mechanical scarification had a significant effect on germination and emergence dynamics of *A. digitata* seeds. The best germination and consequently emergence rate combined with the highest proportion of undamaged seedlings was achieved by scarifying with a saw on the hilum side of the seed (T07). This approach should be tested when propagating *A. digitata* from seeds. This precise technique may be suitable for propagation of baobab in a rural setting, however the effects of scarification methods on seedling emergence dynamics as applied by Saied *et al*. (2008) need to be further investigated as even recent studies (e.g. Niang *et al*., 2015; Kempe *et al*., 2018; Lautenschläger *et al*., 2020) still measure germination as relative percentage data but do not consider germination characteristics beyond emergence of the radicle or run-on effects of scarification method on early seedling development. Considering emergence dynamics might elucidate some of the contrasting results obtained by different studies testing the same scarification methods and help disseminate a more complete indicator set and approach for scarification method selection across species.

Supplement

The supplement related to this article is available online on the same landing page at: https://doi.org/10.17170/kobra-202107134322 .

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Assogbadjo, A. E., Glèlè Kakaï, R., Edon, S., Kyndt, T., & Sinsin, B. (2011). Natural variation in fruit characteristics, seed germination and seedling growth of *Adansonia digitata* L. in Benin. *New Forests*, 41, 113–125.

Buchmann, C., Prehsler, S., Hartl, A., & Vogl, C. R. (2010). The Importance of Baobab (*Adansonia digitata* L.) in Rural West African Subsistence – Suggestion of a Cautionary Approach to International Market Export of Baobab Fruits. *Ecology of Food and Nutrition*, 49, 145–172.

Danthu, P., Roussel, J., Gaye, A., & El Mazzoudi, E. H. (1995). Baobab (*Adansonia digitata* L.) seed pretreatments for germination improvement. *Seed Science & Technology*, 23, 469–475.

Darr, D., Chopi-Msadala, C., Namakhwa, C. D., Meinhold, K., & Munthali, C. (2020). Processed Baobab (*Adansonia digitata* L.) Food Products in Malawi: From Poor Men's to Premium-Priced Specialty Food? *Forests*, 11, 698.

De Smedt, S., Sanchez, A.C., Van Den Bilcke, N., Simbo, D., Potters, G., & Samson, R. (2012). Functional responses of baobab (*Adansonia digitata* L.) seedlings to drought conditions: Differences between western and south-eastern Africa. *Environmental and Experimental Botany*, 75, 181–187.

El-Siddig, K., Gebauer, J., Ebert, G., Ali, A. M., & Inanaga, S. (2004). Influence of Salinity on Emergence and Early Seedling Growth of *Tamarindus indica* L. *European Journal of Horticultural Science*, 69, 79–81.

Esenowo, G. J. (1991). Studies on germination of *Adansonia digitata* seeds. *Journal of Agricultural Science*, 117, 81–84.

Falemara, B. C., Chomini, M. S., Thlama, D. M., & Udenkwere, M. (2014). Pre-Germination and Dormancy Response of *Adansonia digitata* L. Seeds to pre-treatment techniques and growth media. *European Journal of Agriculture and Forestry Research*, 2, 31-41.

Gebauer, J., & Luedeling, E. (2013). A note on baobab (*Adansonia digitata* L.) in Kordofan, Sudan. *Genetic Resources and Crop Evolution*, 60, 1587–1596.

Gebauer, J., Assem, A., Busch, E., Hardtmann, S., Möckel, D., Krebs, F., Ziegler, T., Wichern, F., Wiehle, M., & Kehlenbeck, K. (2014). Der Baobab (*Adansonia digitata* L.): Wildobst aus Afrika für Deutschland und Europa?! *Erwerbs-Obstbau*, 56, 9–24.

Gebauer, J., Adam, Y., Cuni Sanchez, A., Darr, D., Eltahir, M., Fadl, K., Fernsebner, G., Frei, M., Habte, T.-Y., Hammer, K., Hunsche, M., Johnson, H., Kordofani, M., Krawinkel, M., Kugler, F., Luedeling, E., Mahmoud, T., Maina, A., Mithöfer, D., & Kehlenbeck, K. (2016). Africa's wooden elephant: the baobab tree (*Adansonia digitata* L.) in Sudan and Kenya: a review. *Genetic Resources and Crop Evolution*, 63, 377–399.

Graves, S., Piepho, H.P., Selzer, L., & Dorai-Raj, S. (2019). multcompView: Visualizations of Paired Comparisons., Part R package version 0.1-8, CRAN.

Hansohm, N., Jansen, L., Mandala, K., Kandiado, P., Meinhold, K., Darr, D., Wichern, F., & Gebauer, J. (2020). Instruction Manual for Baobab Cultivation - Based on Experiences in Mangochi, Malawi. OPUS Publication Server of the Rhine-Waal University of Applied Sciences, 1-21. URN:urn:nbn:de:hbz:1383-opus4-5859.

Hothorn, T., Hornik, K., van de Wiel, M. A., & Zeileis, A. (2006). A Lego system for conditional inference. *The American Statistician*, 60, 257–263.

Jäckering, L., Fischer, S., & Kehlenbeck, K. (2019). A value chain analysis of baobab (*Adansonia digitata* L.) products in Eastern and Coastal Kenya. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, 120, 91–104.

Kempe, A., Neinhuis, C., & Lautenschläger, T. (2018). *Adansonia digitata* and *Adansonia gregori*i fruit shells serve as a protection against high temperatures experienced during wildfires. *Botanical Studies*, 59, 7.

Lautenschläger, T., Teutloff, N., Gunther, M., & Neinhuis, C. (2020). *Adansonia digitata* germination tests. Elephants or heat: what causes scarification of seed to facilitate germination? *Botanical Studies*, 61, 19.

Mangiafico, S. (2020). rcompanion: Functions to Support Extension Education Program Evaluation.

Martin, A. C. (1946). The comparative internal morphology of seeds. *American Midland Naturalist*, 36, 513–660.

Matthew, R. (2020). Planting Baobab seeds. Available at: https://www.youtube.com/watch?v=7SoGjPZwhWc. Last accessed on 08.02.2021.

Munthali, C. R. Y., Chirwa, P. W., & Akinnifesi, F. K. (2012). Genetic variation among and within provenances of *Adansonia digitata* L. (Baobab) in seed germination and seedling growth from selected natural populations in Malawi. *Agroforestry Systems*, 86, 419–431.

Niang, M., Diouf, M., Samba, S. A. N., Ndoye, O., Cissé, N., & Van Damme, P. (2015). Difference in germination rate of Baobab (*Adansonia digitata* L.) provenances contrasting in their seed morphometrics when pretreated with concentrated sulfuric acid. *African Journal of Agricultural Research*, 10, 1412–1420.

Ogle, D. H., Wheeler, P., & Dinno, A. (2020). FSA: Fisheries Stock Analysis.

Pander, H. (2016). Baobabs Samen keimen lassen - so gehts. Available at: https://baobabstories.com/baobab-samen-keimen-lassen. Last accessed on 08.02.2021.

Patrut, A., Woodborne, S., Patrut, R. T., Rakosy, L., Lowy, D. A., Hall, G., & Von Reden, K. F. (2018). The demise of the largest and oldest African baobabs. *Nature Plants*, 4, 423–426.

Ratana, P. (2018). How to grow Baobab from seeds in 5 days. Available at: https://www.youtube.com/watch?v=PUkyq8CL2Ko. Last accessed on 08.02.2021.

Razanameharizaka, J., Grouzis, M., Ravelomanana, D., & Danthu, P. (2006). Seed storage behaviour and seed germination in African and Malagasy baobabs (*Adansonia* species). *Seed Science Research*, 16, 83–88.

R Development Core Team (2020). R: A language and environment for statistical computing., Part 4.0.2. Vienna, Austria:R Foundation for Statistical Computing.

Sacande, M., Rønne, C., Sanon, M., & Jøker, D. (2006). *Adansonia digitata* L. *Forest and Landscape Denmark, Seed Leaflet*, 109, 1–2. Available at: http://curis.ku.dk/portallife/files/20497189/adansonia_109.pdf. Last accessed on 08.02.2021.

R Studio Team (2020). RStudio: Integrated Development for R. PBC, Boston, MA:RStudio.

Saied, A. S., Gebauer, J., & Buerkert, A. (2008). Effect of different scarification methods on germination of Ziziphus spina-christi seeds. *Seed Science & Technology*, 36, 201–205.

Sanchez, A. C., Osborne, P. E., & Haq, N. (2011). Climate change and the African baobab (*Adansonia digitata* L.): the need for better conservation strategies. *African Journal of Ecology*, 49, 234–245.

Schmidt, S. (2000). Guide to handling of Tropical and Subtropical forest seed. Denmark:Danida Forest Seed Centre.

Tsy, J. M. L. P., Lumaret, R., Mayne, D., Vall, A. O. M., Abutaba, Y. I. M., Sagna, M., Raoseta, S. O. R., & Danthu, P. (2009). Chloroplast DNA phylogeography suggest a West African centre of origin for the baobab, *Adansonia digitata* L. (Bombacoideae, Malvaceae). *Molecular Ecology*, 18, 1707–1715.

Venter, S. M., & Witkowski, E. T. F. (2013). Where are the young baobabs? Factors affecting regeneration of *Adansonia digitata* L. in a communally managed region of southern Africa. *Journal of Arid Environments*, 92, 1–13.

Wang, Y. R., He, X. Q., Hanson, J., & Mariam, Y. W. (2011). Breaking hard seed dormancy in diverse accessions of five wild *Vigna* species by hot water and mechanical scarification. *Seed Science & Technology*, 39, 12–20.

Watkins, T. J., & Cantliffe, D. J. (1982). Mechanical resistance of the seed coat and endosperm during germination of *Capsicum annuum* at low temperature. *Plant Physiology*, 72, 146–150.

Wickens G. E., & Lowe P. (2008). The baobabs: pachycauls of Africa, Madagascar and Australia. Springer, Dordrecht.

Wickham, H., Averick, M., Bryan, J., Chang, W., D'Agostino, L., Francois, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Kohske, T., Vaughan, D., Wilke, C., Woo, K., & Yutani, H. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, 4, 1686.

Yousif, M. A. I., Wang, Y. R., & Hu, X. W. (2019). Seed dormancy and dormancy breaking of selected *Acacia* species from sub-Saharan Africa. *Seed Science & Technology*, 47, 131–144.