

## Breaking seed coat dormancy of *Acacia nilotica* seeds under simulated natural habitat conditions in Sudan

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**Abstract:** This study attempted to break the seed coat dormancy of *Acacia nilotica*, a riverine tree species, by simulating natural conditions. The effects of extended water soaking, soil burial and shade placement on seeds and pods were studied. Pods, clean-seeds and unclean-seeds were soaked in water for periods up to 24 weeks in two sites with or without forest soil. The seeds were sampled for germination every 3 weeks. The results showed that factors similar to that of the natural habitat had significant effect in breaking the seed dormancy. The length of soaking period of seeds in water was the most critical factor. Germination increased with the length of soaking time up to 18 weeks and then decreased. The 18 weeks is close to the average length of natural flooding period. Water treatment of seeds was suggested as alternative for the sulfuric acid treatment, which is expensive and hazardous.

**Resumen:** En este estudio se intentó romper la latencia de la testa de las semillas de *Acacia nilotica*, una especie arbórea ribereña, bajo condiciones naturales simuladas. Se estudiaron los efectos del remojo prolongado, del enterramiento y de la colocación en la sombra de las semillas y las vainas. Se pusieron a remojar vainas, semillas limpias y semillas no limpias en agua durante periodos de hasta 24 semanas en dos sitios con o sin suelo de bosque. La germinación de las semillas fue registrada cada tres semanas. Los resultados mostraron que factores similares a los de su hábitat natural tienen un efecto significativo en el rompimiento de la latencia de la semilla. La longitud del periodo de remojo de las semillas fue el factor más crítico. La germinación se incrementó con la longitud del tiempo de remojo hasta alcanzar un máximo a las 18 semanas y luego decreció. El periodo de 18 semanas es semejante a la longitud promedio del periodo natural de inundación. Se sugiere que los tratamientos con agua de las semillas pueden ser una alternativa al tratamiento de ácido sulfúrico, que es caro y riesgoso.

**Resumo:** Este estudo pretendeu quebrar a dormência do revestimento da *Acacia nilotica*, uma espécie arbórea ribeirinha, simulando as condições naturais. Os efeitos do embebibimento prolongado das sementes e vagens em água, e a sua colocação posterior na terra e sombra foram estudados em paralelo com o estudo da germinação das sementes no seu habitat natural. As vagens, sementes limpas e sementes sujas foram embebidas em água por períodos até 24 semanas em duas estações, com ou sem solo florestal. As sementes foram amostradas para germinação em cada 3 semanas. Os resultados mostraram que factores similares aos do habitat natural têm um efeito significativo na quebra da dormência das sementes. A duração do tempo de embebibimento das sementes em água era o factor mais crítico. A germinação aumentou com a duração do tempo de embebibimento até 18 semanas e depois disso decresceu. O tempo de 18 semanas está próximo da duração média do período de inundação natural. O tratamento das sementes com água foi sugerido como alternativa para o tratamento com ácido sulfúrico, o qual é caro e perigoso.

**Key words:** *Acacia nilotica*, riverine forest, seed coat dormancy, sulfuric acid treatment, water treatment.

## Introduction

*Acacia nilotica* (L) Willd, ex Del., commonly known in Sudan as Sunt, is an important multipurpose leguminous tree species. It is widely distributed in subtropical and tropical Africa. It has nine subspecies with distinctive geographical ranges with seven subspecies occurring in Africa and four in Sudan (El Amin 1990; Vogt 1995). The subspecies *tomentosa* is found in Sudan dominating the lower basins, commonly known as “mayaas”, along the Banks of the Blue Nile and its tributaries. The mayaas are inundated with water during the flooding season and the water can stay up to six months. *A. nilotica* is considered as a priority species in Sudan for its products and environmental role along the River Nile banks and its tributaries (Warrag *et al.* 2002). It produces fuelwood, furniture, building material for boats and railway sleepers and tannins (Badi *et al.* 1989). Also, it is known for production of ink and various local medicinal products (Vogt 1995).

*A. nilotica* and *Acacia sieberiana* have the thickest seed coat among the acacias of the Sudan imposing seed coat dormancy (Abdel Dafai 1977). Artificial germination cannot occur without treating, and loosening of the seed coat (Demel 1996; FAO 1985; Mohamed 1981). The commonly used method is by treating seeds with concentrated sulfuric acid. Other treatments at experimental levels are: use of electrical needles, rubbing and nicking with sharp tools. Soaking in water for up to 192 hrs was not successful in breaking the dormancy (Bebawi & Mohamed 1985; Mohamed 1981). Seed pretreatment with 5% carboxy methyl cellulose (CMC) medium enhanced germination and increased root and shoot growth of *A. nilotica*, *Acacia catechu*, *Albizia procera*, *Dalbergia sissoo* and *Moringa pterygosperma* (Harsh & Ojha 2000). The seed coat dormancy of *Acacia negrii* can be broken by mechanical scarification, acid treatment, boiling water and dry heat treatment (Demel 1997). It has been shown that mechanical scarification, sulfuric acid treatment, boiling or hot water and dry heat had improved germination of several *Acacia* species (Bebawi & Mohamed 1985; Danthu *et al.* 1992; Demel 1996, 1997, 1998; Masamaba 1994).

Artificially induced germination of *A. nilotica* by concentrated sulfuric acid (95%) treatment is the commonly adopted operational method in

Sudan as other treatments are suitable only for small quantities. Annually, about 15 to 20 tons of seeds are treated requiring 1.5 to 2 tons of sulfuric acid (10 kg of seeds per liter of acid (Mohamed & Abdel Majed 1996)). The acid is expensive and hazardous for people and the surrounding environment. Also, getting rid of the acid after its use adds to the environmental problems. Accordingly, there is a need for safe treatments to avoid the high cost and the problems associated with the acid use.

The seeds of *A. nilotica* germinate naturally profusely and vigorously as the floodwater subsides (Badi *et al.* 1989), indicating natural breaking of the seed coat dormancy. Mimicking the natural environment could lead to the identification of the critical factors involved in the breaking of the seed coat dormancy. Increased germination percentage of *Acacia origena* and *A. pilispinah* seeds with hot water treatment (Demel 1998) was linked to possible adaptation to frequent fires in their natural habitat (Sabiiti & Wein 1987). Also, the seasonal wet and dry tropics with frequent fires during the dry season have shown fire as a powerful natural factor in breaking the seed coat dormancy of *Tectona grandis* (Laurie 1974) and *Acacia mangium* (Bowen & Eusibio 1981).

The length of soaking of pods and seeds in the mayaas' soil with tree-shade may be the cause for breaking the seed coat dormancy. Also, it was speculated that germination occurs on seeds with damaged seed coats due to infection or other physical factors (Lamprey *et al.* 1974). The main objective of this study was to identify the factors that break the seed coat dormancy in the riverine natural habitats of *A. nilotica*, with the aim of developing suitable germination method. Specifically the effects of length of soaking seeds and pods, shade and soil were investigated.

## Materials and methods

### *Pod collection and seed handling*

Pods of *A. nilotica*, subsp. *tomentosa* were collected in March 1997 from Elgazair Reserved Forest on the Western bank of the Blue Nile, a typical riverine forest dominated by *A. nilotica*. The pods were bulked together (weight = 60 kg) and divided into three lots. One lot was directly used as pods and referred to as pod category. Seeds

were extracted from the pods of the other two lots. Seeds of the second lot were used without cleaning or removing the damaged seeds and referred to as unclean-seed-category. The third lot was cleaned from infected and damaged seeds by two sequential steps and referred to as clean-seed-category. First, the seeds were soaked in water for 24 hours, and the floating as well as swollen seeds were discarded. Secondly, the remaining seeds were dried followed by the removal of damaged and insect-infested seeds. The percentage of discarded seeds was 37%.

#### *Pod and seed treatment*

The treatments consisted of soaking in water for the three categories in containers with or without soil under two levels of shade in a El Mugran forest nursery in Khartoum State. The containers used were 40 cm x 30 cm x 30 cm in size. They were placed under low and high shade, receiving approximately 70% and 30% of the full sunlight, respectively. In each level of shade, half of the containers were filled up to 10 cm with soil from El Mugran *A. nilotica* Forest, Khartoum State while the other half were left without soil. Each lot of the three categories was divided into 12 parts and placed randomly in the containers. The total number of containers was 48 containers with 3 replicates of the 3 categories by two shade levels by two soil levels. All containers were then filled with water initially and water was added regularly to keep the containers full for 24 weeks.

#### *Germination tests*

Two samples of 100 seeds each were randomly taken from the seeds and pods of each container at day 1 and then after 3, 6, 9, 12, 15, 18, 21 and 24 weeks from soaking, representing 9 soaking periods. The seeds were directly placed in plastic dishes (25 seeds per dish) filled with sand soil. The plastic dishes were then placed in a germination incubator with average temperature of 25 °C and 12 hours photoperiod from fluorescent tubes. The dishes were kept moist by wetting them every two days. The number of germinating seeds per dish was recorded after two weeks from sowing. Parallel germination check tests were carried for untreated seeds from the three categories.

Analysis of variance procedures were carried on arcsine transformed germination percentages to

determine the effects of the tested factors (length of soaking, seed category, shade and soil). Duncan's multiple range test was used to compare differences among treatment means.

## Results and discussion

The results confirmed that prolonged immersion in water is directly related to breaking of seed dormancy of *A. nilotica*. Also, the significant effects of the length of soaking, seeds category and site on breaking of seed dormancy were identified. The parallel tests showed that the untreated seeds and pods were unable to germinate, confirming the seed coat dormancy of *A. nilotica*.

#### *Effect of length of soaking*

The average effect of length of soaking time on seed germination was highly significant ( $P = 0.0001$ ). Germination percentages increased with time and then decreased (Table 1). Seeds soaked for 1 day were unable to germinate for all the treatments. Germination increased with time and reached its maximum after 18 weeks and then decreased after 24 weeks. This trend was observed with the seed categories, soil and shade treatments (Tables 2, 3 & 4).

**Table 1.** Average effect of soaking length in water on germination of *A. nilotica* seeds.

Length of soaking week <sup>-1</sup>	Mean of germination (%)
Day 1	0.0
3 weeks	3.944 E
6 weeks	11.333 D
9 weeks	20.389 C
12 weeks	27.333 B
15 weeks	28.444 B
18 weeks	33.222 A
21 weeks	20.667 C
24 weeks	3.278 E

Means with the same letters are not significantly different ( $P < 0.05$ ).

These findings indicate that extended soaking in water is an important factor in breaking the seed coat dormancy of *A. nilotica*. In some replications 80% and 100% germination were

obtained with clean seeds under high shade after 18 weeks of soaking. This is comparable to the use of concentrated sulfuric acid for breaking *A. nilotica* seed coat dormancy with germination percentage of up to 85% (Mohamed & Abdel Majed 1996). Summing the germination percentages of the clean seeds over the soaking periods gave more than 200% germination. This shows that all viable seeds could germinate at the appropriate length of soaking with overlapping of some seeds. Also, it indicates that the variation in the response of seeds to the length of soaking.

**Table 2.** Effect of seed category and soaking length on germination of *A. nilotica* seeds.

Length of soaking	Clean seed (X)	Unclean seed (S)	Pod (F)
1 Day	0.0	0.0	0.0
3 weeks	4.500 E (a)	4.083 E (a)	3.250 D (a)
6 weeks	19.500 D (a)	8.833 D (b)	5.667 D (b)
9 weeks	33.667 BC (a)	17.833 C (b)	9.667 C (c)
12 weeks	39.500 B (a)	22.000 CB (b)	20.500 A (b)
15 weeks	41.333 B (a)	24.000 AB (b)	20.000 A (b)
18 weeks	50.833 A (a)	28.333 A (b)	20.500 A (c)
21 weeks	29.667 C (a)	18.000 C (b)	14.333 B (b)
24 weeks	2.833 E (a)	3.667 E (a)	3.333 D (a)

Means with the same upper case letters on the same column and with same lower case letters on the same row are not significantly different ( $P < 0.05$ ).

**Table 3.** Effect of site and soaking length on germination of *A. nilotica* seeds.

Length of soaking	High shade site	Low shade site
Day 1	0.0	0.0
3 weeks	4.278 D (a)	3.611 F (a)
6 weeks	15.222 C (a)	7.444 E (b)
9 weeks	25.222 B (a)	15.556 D (b)
12 weeks	33.222 A (a)	21.444 B (b)
15 weeks	34.333 A (a)	22.556 B (b)
18 weeks	36.889 A (a)	29.556 A (a)
21 weeks	23.333 A (a)	18.000 CD (b)
24 weeks	4.444 D (a)	2.111 F (b)

Means with the same upper case letters on the same column and with same lower case letters on the same row are not significantly different ( $P > 0.05$ ).

**Table 4.** Effect of soil and soaking length on germination of *A. nilotica* seeds.

Length of soaking	No Soil	Forest Soil
1 Day	0.0	0.0
3 weeks	3.611 F (a)	4.278 ED (a)
6 weeks	7.444 E (a)	9.833 D (a)
9 weeks	15.555 D (a)	19.667 C (a)
12 weeks	21.444 BC (a)	27.111 B (a)
15 weeks	22.556 B (a)	27.889 B (a)
18 weeks	29.556 A (b)	37.778 A (a)
21 weeks	18.000 CD (b)	24.111 BC(a)
24 weeks	2.111 F (a)	3.444 E (a)

Means with the same upper case letters on the same column and with same lower case letters on the same row are not significantly different ( $P < 0.05$ ).

#### *Effect of seed category*

The effect of the seed categories was significant within the periods between 6 and 21 weeks ( $P < 0.05$ ). At each soaking period, the clean seeds had higher germination percentages followed by the unclean seeds and pods (Table 2). The maximum germination percentage occurred after 18 weeks. The pods and unclean seed categories gave closer percentages. The highest germination percentages of the clean seeds reject the idea that the natural germination is due to damaged and infected seeds. The similarity between the unclean seeds and the pods further showed that the presence of pod tissue doesn't affect the germination. The difference between the clean seed category and the other two categories (unclean seeds and pods) was due to the damaged and insect- infected seeds.

#### *Effect of shade*

Generally, the high shade site had higher germination percentages compared to low shade site with significant differences at the soaking periods after 6, 9, 12, 15, 21 and 24 weeks (Table 3). The higher germination percent of the high shade site reflected similarity to the natural environment with the presence of shading from big trees and low evaporation.

### *Effect of soil*

Similar to the seed categories and sites, the presence or absence of soil followed the general trend of germination with time. The maximum germination percentage occurred after 18 weeks of soaking. The effect of the presence of soil at each soaking period was not significant (Table 4).

The trend of the germination with the length of soaking shows the relation between the species and its natural riverine habitat. Naturally, seeds could be immersed in water for varying length of time according to the seasonal flooding. Abdel Dafai (1977) observed that seed coat becomes thicker as the species inhabits wetter habitat. It appears that natural germination occurs due to soaking of pods and seeds of *A. nilotica* during the flood season. The seeds would gradually be affected by water until the seed coat becomes permeable to water with large variation between seeds. As a result, germination occurs as the floodwater subsides with high percentage after the length of the normal flooding season, which normally ranges between 4 to 6 months (Badi *et al.* 1989). The observed decrease in natural regeneration after flooding for more than 6 months and the decrease in germination percent after 18 weeks obtained in this study may be due to the effect of water on the embryonic tissues.

The findings of this study indicated the ecological relationships between the species and the ecosystem. Extended soaking of seed in water has gradual softening effect on the seed coat until it becomes permeable to water. Accordingly, germination percentage increased gradually with time until week 18 and then decreased gradually till it reached its minimum level after 24 weeks of soaking. The variation in germination reflects variation in seed coat. For operational planting program, it is recommended to soak clean seeds in water for about 18 weeks under shade and sow them immediately in the intended forest site. Use of cheap containers at the forest site for water treatment can be adopted.

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