

# Effect of storage temperature and packing materials on seed germination and seed storage behavior of *Schefflera abyssinica*

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**Abstract.** Bareke T, Addi A, Roba K, Kumsa T. 2022. Effect of storage temperature and packing materials on seed germination and seed storage behavior of *Schefflera abyssinica*. *Nusantara Bioscience* 14: 141-147. Knowledge of seed storage behavior is crucial for developing appropriate ex-situ conservation strategies. The main objective of this study was to determine the seed storage behavior of *Schefflera abyssinica* (Hochst. ex A. Rich.). A factorial combination of three temperatures (-10, 0, and 22°C) levels, two types of packing containers (polythene bag and aluminum bag), and eight periods of storage (0, 30, 60, 90, 120, 150, 180 days, and for a year) level was used to determine the germination capacity and storage behavior of the seeds. Accordingly, seed storage temperatures and storage period (up to 1 year) have a significant effect on the germination ( $p < 0.01$ ) of *S. abyssinica*. The highest average germination percentage of *S. abyssinica* seeds was obtained after 2 months of storage under all conditions. The seed storage period influences the germination of *S. abyssinica* by 47.8%. The germination percentages of *S. abyssinica* seeds have shown significant differences in storage temperatures. Seeds stored at -10°C showed the highest germination percentage in all storage periods compared to the two storage temperatures. Generally, the highest germination capacity of *S. abyssinica* seeds was between 7% and 9% moisture content after 60 and 90 days of storage. Packing materials have no significant difference in the survival of seeds. No stored seeds germinated after one year of storage at room temperature, 0°C, and -10°C. Based on the definition of seed storage behavior, we conclude that seeds of *S. abyssinica* have intermediate storage.

**Keywords:** Germination, moisture content, seed, storage behavior

## INTRODUCTION

Knowledge of the seed storage behavior is a key feature for determining the success of seed storage protocols used to develop appropriate ex-situ conservation strategies (Hong and Ellis 1996; Jaganathan et al. 2019). Successful storage enables the maintenance of seed viability over time to improve the plant breeding program (Ibraheem et al. 2021). Seed storage behavior varies from one species to another and with the storage environment. Hence, proper seed storage minimizes the rate of deterioration and the loss of viability (Singh et al. 2017). Environmental storage conditions such as temperature and seed water content are the most relevant factors for conserving seed viability (Silva et al. 2019). The impact of moisture content and temperature on seed quality is, therefore, of particular significance in tropical countries where ambient conditions tend to lead to a rapid loss in seed quality (Singh et al. 2017; Ibraheem et al. 2021).

Seed storage behavior is classified as orthodox, intermediate, or recalcitrant based on the responses to moisture content and storage temperature (Hong et al. 1998; Chmielarz 2009). In addition, seed storage behavior is affected by seed origin (provenance) and seed storage containers (Bareke et al. 2018). Orthodox seeds tolerate severe desiccation from 2 to 5% without damage (Roberts 1973), while recalcitrant seeds survive high moisture content (>31%) (ISTA 1999). Recalcitrant seeds do not survive drying to low moisture content and are sensitive to

desiccation (Pammenter and Berjak 2014). Due to this, recalcitrant seeds are viable only for a very limited period and exhibit an inability for medium- and long-term storage (Hong and Ellis 1996). Intermediate seed storage behavior is found between orthodox and recalcitrant (Bareke 2018). The difference between the three types of seed storage behavior is presented based on evolution, environmental influences, and seed maturation (José 2018).

*Schefflera abyssinica* (Hochst. ex A. Rich.) is an indigenous tree belonging to Araliaceae that is branched and small/medium to 30 m tall in height. It produces creamy-yellowish or creamy-white flowers from March to April. It grows in Afromontane forests, secondary forests, and woodlands within the altitudinal range of 1,450-2,800 masl, often in association with *Hagenia abyssinica* (Bruce) J.F.Gmel. (Addi et al. 2014). It is also usually left as scattered trees when forests are cleared for farmlands.

The *S. abyssinica* is one of Ethiopia's most important honey plants (Bareke and Addi 2019). It is a high producer of nectar and significantly contributes to honey production. One hectare of *S. abyssinica* plants has the potential to produce 895.5 kg of harvestable honey (Bareke et al. 2020). Due to its high potential, monofloral honey can be produced from this species which has high demand in the market and could generate high income (Addi et al. 2014; Bareke and Addi 2018).

The *S. abyssinica* is considered an epiphyte, which grows on another tree species, finally overwhelms it, and becomes an independent tree in highland areas.

Currently, *S. abyssinica* can be propagated by seed using an aqueous smoke solution (Bareke et al. 2014), and smoke treatment improve seedlings' capacity to survive the effect of aphids. However, the appropriate seed storage temperature, storage material, storage periods, and seed storage behavior of *S. abyssinica* are not known, which is detrimental to developing conservation strategies for the species. Therefore, the study was designed to identify the appropriate storage temperature, materials, and period and determine the seed storage behavior of *S. abyssinica*.

## MATERIALS AND METHODS

### Study site

Seeds were collected from the Munessa forest in Ethiopia, which was recommended as a good provenance for the multiplication of *S. abyssinica* by seedlings (Bareke et al. 2014). The experiment was conducted at Holeta Bee Research Center, Oromia region, Ethiopia.

### Collection of fruits/seeds and processing

After 5 to 10 preferred (elite) mother trees were randomly selected, mature fruits (Figure 1) were collected from each tree's crown's top, middle and lower parts (ISTA 2007). Moreover, to ensure maximum genetic variation within the population, the selected trees were at least 100 m apart (FAO 1975). The mixture of fruits was packed in perforated sacks, transported to Holeta Bee Research Center, and placed on the laboratory bench at room temperature for about a week. Seeds from dehiscing fruits were manually extracted and allowed to dry for 1 day on the same bench.

### Seed storage behavior testing

For practical seed storage purposes, the difference among the orthodox, intermediate, and recalcitrant categories of seed storage behavior enables one to determine whether the species can be maintained successfully over the long term, the medium term, or only the short term, respectively. The two-stage procedure of Hong and Ellis (1996) was used to classify seed storage

behavior: desiccation tolerance and cold tolerance. Furthermore, seeds were dried to about 12-18% moisture content (MC) to determine desiccation tolerance using ambient relative humidity and room temperature (Hong and Ellis 1996). Tolerance of desiccation to these levels of seed MC is usually sufficient to differentiate recalcitrant seed storage behavior from orthodox and intermediate seed storage behavior (Hong and Ellis 1996).

### Determination of seed moisture content

Samples of fresh seeds were used to determine the initial MC. Then, for three replicates of 5 g of seeds each, seeds were weighed, dried in an oven for 2 hrs at 120°C, and then placed in a desiccator for cooling before weighing again. Finally, dry weight was measured, and calculation was done for MC determination based on a fresh weight basis (ISTA 2005; Schmidt 2007), as shown in the following equation.

$$MC (\%) = \left( \frac{W_2 - W_3}{W_2 - W_1} \right) \times 100$$

Where MC is moisture content,  $w_1$  is the weight of the container,  $w_2$  is the weight of the container with seed sample before oven drying, and  $w_3$  is the weight of the container with seed sample after oven drying.

### Identifying orthodox and intermediate storage behavior

Seeds were stored over a range of temperatures to determine cold tolerance, i.e., the second step distinguishes orthodox and intermediate storage behavior (Hong and Ellis 1996; ISTA 2007). A factorial combination of three temperatures (-10, 0, and 22°C) levels, two types of packing containers (Polythene bag and aluminum bag), and eight periods of storage (0, 30, 60, 90, 120, 150, 180 days, and for a year) was used to determine the germination capacity and storage behavior of the seeds. If all or most seeds die during 12 months of storage, then the seeds have intermediate storage behavior. On the other hand, if no loss in viability is evident during this period, then the species shows orthodox seed storage behavior (Hong and Ellis 1996).



Figure 1. Seeds of *Schefflera abyssinica* during collection

### Germination experiment

The germination test was conducted using Whatman filter paper in 12-cm-diameter Petri dishes. Four replicates of 25 seeds each for each storage condition were incubated in a lightroom at room temperature. Distilled water was added to the Petri dishes to moisten the filter paper. The germination percentage was computed using the following equation (ISTA 1999; Davies et al. 2015).

$$G (\%) = \frac{n}{N} \times 100$$

Where G is germination, n is the number of seeds germinated, and N is the sum of the number of germinated seeds, fresh seeds, and those destroyed by fungi.

### Data collection methods

For the entire experiment, seed germination counts were made every three days after the commencement of the seed germination. Furthermore, to facilitate future counts, germinated seeds were removed after recording. The experiment was continued until at least 90% of the replication from each treatment showed no new germination for 3 consecutive counts. A seed is considered

germinated when the radicle protrusion occurs on the Petri dish's surface (Tigabu et al. 2007; Dayamba et al. 2016).

### Data analysis

Data were analyzed using factorial analysis, Oneway ANOVA, and regression analysis to see their effect on the seeds' germination capacity. Therefore, the effect of each factor was analyzed independently using One way ANOVA.

## RESULTS AND DISCUSSION

### Effect of storage temperature, containers, and storage period on seed germination

Seed storage temperatures and storage period significantly affected the germination capacity of *S. abyssinica*, whereas storage containers and the interaction between temperature and containers did not (Table 1).

The germination percentages of seeds stored at -10°C were significantly higher than those stored at the other two temperature treatments (Table 2). These results indicated that -10°C is an appropriate temperature for storing *S. abyssinica* seeds.

**Table 1.** The combined effect of storage temperature, storage container, and time of storage on the germination capacity of *S. abyssinica* seeds

Treatment	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Storage temperature	1	849	849	9.406	0.00238 **
Storage container	1	4	4	0.041	0.83999
Storage period	6	12913	3228	35.749	< 2e-16 ***
Storage temperature* storage container	1	43	43	0.477	0.49045
Residuals	276	24924	90		

Note: Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table 2.** Effect of storage temperature on mean germination percentage of *S. abyssinica* seeds

Storage temperature	Mean germination %	Minimum germination %	Maximum germination %
-10°C	56.0 <sup>a</sup>	41.20	91.00
0°C	46 <sup>c</sup>	5.00	88.00
22°C	50 <sup>b</sup>	12.50	90.00

Note: Since all seeds were not germinated at a year of storage, the data of this result is only up to six months of storage

**Table 3.** The mean moisture content (MC) and germination capacity of *S. abyssinica* seeds after different periods of storage

Seed longevity	Mean MC	Mean germination %	Minimum germination %	Maximum germination %
Before storage	9.5 <sup>a</sup>	56 <sup>bc</sup>	45	65
First month (30 days)	8.58 <sup>ab</sup>	56 <sup>bc</sup>	45	91
Second month (60 days)	8.37 <sup>b</sup>	70 <sup>a</sup>	46	91
Fourth month (120 days)	7.65 <sup>bc</sup>	48 <sup>cd</sup>	43	73
Third month (90 days)	7.58 <sup>bc</sup>	65 <sup>ab</sup>	44	85
Fifth month (150 days)	6.75 <sup>c</sup>	40 <sup>d</sup>	20	71
Sixth month (180 days)	6.5 <sup>c</sup>	20 <sup>e</sup>	5	30
Year	6.00 <sup>d</sup>	0 <sup>f</sup>	0	0

Note: Treatments with the same letter are not significantly different along a column of mean moisture content and germination percentage.

**Seed moisture content and germination**

The highest average germination capacity of *S. abyssinica* seeds was seen after 60 days of storage. On the other hand, the lowest germination capacity of *S. abyssinica* was seen at the end of the fifth month (after 150 days) of seed storage (Table 3). After 180 days of storage, germination capacity was less than 20%. The maximum germination capacities of the seeds were seen from the first to third months of storage with 7.58 to 8.58% of average seed MC. After 1 year of storage, no seeds germinated, which indicates that the storage behavior of *S. abyssinica* seeds is intermediate since the seed MC is within the intermediate seed category.

**Effect of storage period on germination**

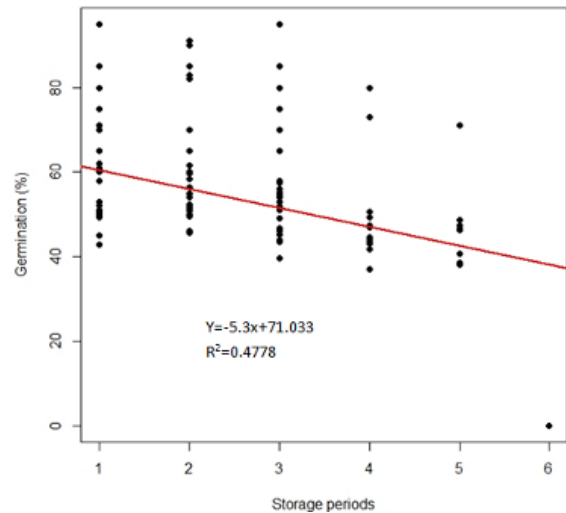
The effect of the seed storage period on the germination capacity of *S. abyssinica* was about 47.8% (Figure 2). The peak germination time was in the first and second months of the storage period. Therefore, the seed germination capacity of *S. abyssinica* has an indirect relationship with the seed storage period, and as the storage period increases, the germination capacity of the seeds decreases.

Most of the seeds (50%) stored at -10°C were germinated up to 120 days of storage (Figure 4). This result is statistically similar to that for seeds stored at room temperature. On the other hand, seeds stored at 0°C had the highest germination capacity only after the second and third months of storage. At the end of 6 months of storage, the lowest germination percentage was seen for the seeds stored at 22°C. For all months of storage, the germination capacity of seeds stored at -10°C was the best compared to the other two storage temperatures. After 1 year of storage, the seeds of *S. abyssinica* did not germinate.

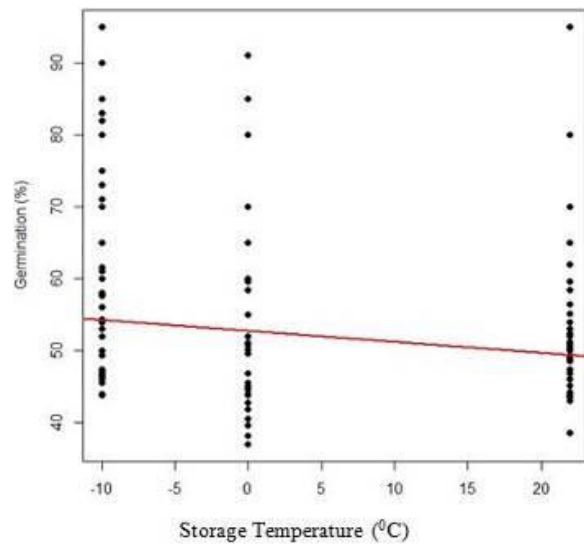
Most seeds stored at -10°C germinated 50% after 120 days of storage (Figure 3).

**Seed storage behavior**

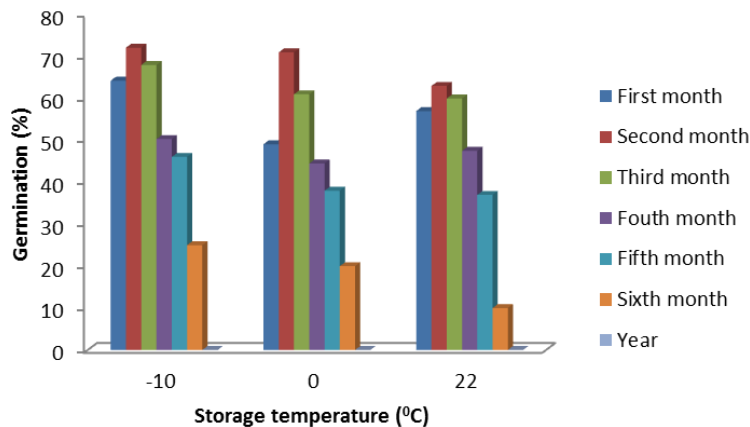
The highest germination capacity of *S. abyssinica* seeds was stored at an MC between 7% and 9% (Figure 5). Thus, most seeds tolerate desiccation to about 7-9% MC. Further, desiccation lowered the MC and reduced the germination capacity of *S. abyssinica* seeds. Therefore, this shows intermediate seed storage behavior.



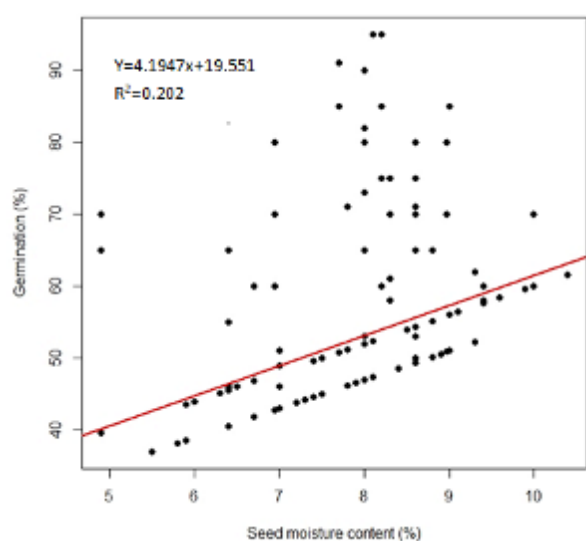
**Figure 2.** The effect of storage duration on the germination of *S. abyssinica* seeds



**Figure 3.** The overall germination percentages of *Schefflera abyssinica* seeds stored at -10°C, 0°C and 22°C for 6 months



**Figure 4.** The effect of storage temperature on germination percentages of *S. abyssinica* seeds after different periods of storage



**Figure 5.** The relationship between seed moisture content and germination capacity of *S. abyssinica*

## Discussion

### *Seed moisture content and germination capacity of S. abyssinica seeds*

The growth of a new plant from a seed is called germination, the protrusion of the radicle from its enclosing tissue (Rai and Kim, 2020). The germination process starts with imbibition (initial absorption of water to hydrate seed) and activation of metabolism (Bareke 2018). On the other hand, seed germination is a multifaceted physiological process controlled by genetic and environmental factors (Marcos 2015; Guo et al. 2020). Seed germination is affected by temperature, water potential, oxygen, light, pH, seed storage period, and storage container (Guo et al. 2020; Rai and Kim 2020).

Seed MC had no significant variation between storage containers but varied among storage periods. Parimala et al. (2013) reported that the MC of seeds is the most important factor influencing the germination capacity of seeds during storage.

### *Seed storage containers*

Polythene and aluminum bags were the two packing materials used for this study. Statistically, the two packing materials had no significant variation. Both packing materials are waterproof. For seed preservation, types of containers that can provide suitable conditions to maintain seed quality for a longer period are preferred. Packing material or container normalizes relative humidity, seed moisture content, and temperature (Akter et al. 2014). The appropriate storage container is varied from plant species to species. For example, Akter et al. (2014), tin containers for Soybean (Akter et al. 2014) and mungbean (Mohammad et al. 2017), and sealed containers for *Lens culinaris* Medik. (Kamrul et al. 2017) and polylined bags for *Trifolium alexandrinum* L. (Bahukhandi et al. 2017).

### *Seed storage behavior*

Seed storage behavior is the way to identify whether the seeds of a plant species can be maintained successfully over a long, medium, or short period (Hong and Ellis 1996). This information is essential for developing appropriate ex-situ conservation strategies (Hong et al. 1996). The MC of *S. abyssinica* seeds was an average of 9.5% after they were allowed to air dry at room temperature for 1 week. At the end of the fifth month of storage, seed MC had decreased to an average of 6.75%. A similar study conducted by Zheng et al. (2016) on Kapok (*Ceiba pentandra* (L.) Gaertn.) also indicated that the moisture content was 11.1% at the initial measurement and decreased to 4.98% after 5 days of desiccation. As the storage period increased, the MC of *S. abyssinica* seed decreased, while germination capacity increased somewhat and decreased after reaching a germination peak. After the fifth month of seed storage, the MC and germination capacity of *S. abyssinica* seeds decreased. In a study conducted by Joshi et al. (2019) on seed germination and seed storage behavior of *Pittosporum eriocarpum* Royle, the MC and germination capacity of stored seeds gradually decreased with an increase in storage period. The highest germination capacity of *S. abyssinica* seeds was 7 and 9% MC. That shows the intermediate seed storage behavior. However, the determination of desiccation tolerance does not alone enable the determination of seed storage behavior.

### *Seed storage period and temperature*

The germination capacity of *S. abyssinica* increased from the first month (30 days) of storage to the end of the third month (90 days) of storage and then decreased. After the fifth month of storage, most seeds did not germinate. Mohammad et al. (2017) and Boadu and Siaw (2019) also reported that the duration of seed storage of *Triplochiton scleroxylon* K.Schum. and mungbean, respectively, had a significant influence on their germination capacity. In addition, Olosunde et al. (2017) also mentioned that the seed storage period influences the final germination percentage and the health of the stands of the *Abelmoschus esculentus* (L.) Moench plant.

Duration of seed storage period and temperature are very important in determining seed storage behavior associated with desiccation tolerance. For instance, seeds of *Cattleya aurantiaca* (Bateman ex Lindl.) P.N.Don tolerated desiccation to 3.7 and 2.2% MC with 94% germination; however, only 10% germinated after storage for 90 days at -18°C with 3.7% MC (Pritchard and Seaton 1993).

Many authors have mentioned that seed storage longevity is affected by many factors. Some of them are seed maturation and ways of pre-harvesting handling. Also, harvesting time and weather conditions affect seed storage longevity (Hong and Ellis 1996; Hay and Probert 2011; Hay et al. 2013; Bareke 2018; Ellis et al. 2018). If matured seeds are not collected from mother trees, they have drastic consequences on the quality of seeds. As a result, seeds rapidly deteriorate when exposed to less favorable

environmental conditions (Bareke 2018). Visual identification of the physiological maturity of seeds is used to identify the maturity of seeds.

The germination capacities of *S. abyssinica* seeds have shown significant differences in storage temperatures. Accordingly, seeds stored at -10°C had the highest germination capacity in all storage months compared to the two storage temperatures. Many authors also reported that low temperature and low relative humidity are required to maintain the quality of the seeds (Schwallier et al. 2011; Silva et al. 2019). Hong and Ellis (1996) also reported that the storage environment influences the response of seed storage behavior. According to Harrington's rule, seeds with an MC of 5-14% will have a double germination potential when the MC of the seeds decreases by 1% (Parimala et al. 2013). In addition, moisture content above 14% increases the chance that seeds will be attacked by insects and mold, whereas an MC below 5% causes physiochemical changes in the seeds.

Orthodox seeds can be dried to an MC of about 5% without damage; if most or all seeds tolerate desiccation to about 10-12.5% MC, they are said to have intermediate seed storage behavior. On the other hand, if most or all seeds are killed by desiccation to 15-20% moisture content, they have recalcitrant seed storage behavior (Hong and Ellis 1996).

In conclusion, on average, the MC of *S. abyssinica* seeds was 9.5% after they were dried at room temperature for 1 week. At the end of the sixth month of storage, seed MC decreased to an average of 6.75%. The germination capacity of *S. abyssinica* seeds indirectly correlates with the seed storage period and MC. The seed storage period influences the germination capacity of *S. abyssinica* by 47.8%. The germination capacities of *S. abyssinica* seeds have shown significant differences in the storage temperature. Seeds stored at -10°C had the highest germination capacity in all storage months compared to the other storage temperatures. Generally, the highest germination capacity of *S. abyssinica* seeds was found to be between 7 and 9% moisture content at the end of the second and third months of storage. All stored seeds were not germinated after 1 year of storage at room temperature, 0°C, and -10°C. Therefore, based on the definition of seed storage behavior given by Hong and Ellis (1996), we conclude that seeds of *S. abyssinica* have intermediate storage behavior.

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