SEED STORAGE BEHAVIOUR AND PRETREATMENT METHODS FOR *ACOKANTHERA SCHIMPERI* (A. DC.) SCHWEINF. USING A COMBINATION OF EXPERIMENTAL APPROACH

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ABSTRACT: *Acokanthera schimperi* (A. DC.) Schweinf is one of the known medicinal plants in the rural community of Ethiopia. However, there is no information on seed storage behaviour and the appropriate conservation method. The aim of the present study is to determine the appropriate seed pretreatments and to investigate the impact of moisture content, storage temperature, and storage duration on seed germination and storage behaviour of *A. schimperi*. To provide information on the seed storage behaviour of the species, we combined an experimental technique based on critical moisture content, storage temperature, and duration analysis. The initial moisture content of the seeds was determined and the effects of dehydration and cold storage on seed viability, germination, and subsequent seedling vigour were examined. The best pretreatment method and seeding medium for the species were also determined. The results showed that the initial moisture content of *A. schimperi* seeds was 55.8%, with almost 100% viability. Seed germination ranged from 75% (mechanical scarification) to 95% (cold water treatment). The *A. schimperi* seed exhibits non-physiological dormancy, allowing for simple germination in a controlled environment using filter paper or sand media. Viability of *A. schimperi* seeds was significantly affected by seed moisture content ($p < 0.01$). However, the interaction effect of storage temperature and duration on seed viability was not significant (P=0.06). These results provide the first concrete evidence of *A. schimperi*'s recalcitrance and practical guidance for handling the seeds in a practical way and developing sustainable conservation strategies for the species.

Key words: *Acokanthera schimperi*, Moisture content, Recalcitrance, Seed viability, Storage behaviour

INTRODUCTION

Seed storage refers to keeping seeds alive for a long time by preserving them in a controlled environment. Before selecting the best storage options, it is necessary to assess the seed storage behaviour between species which allows to distinguish among the orthodox, intermediate, and recalcitrant nature of the seeds (Hong et al., 1996). Storage behavior of seeds ascertains whether the species can be successfully maintained over the

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long term, the medium term, or only the short term for practical seed storage purposes, particularly genetic resource conservation (Hong and Ellis, 1996). Seeds can be classified according to seed storage behavour as Orthodox (seeds that do not lose viability when stored at low temperatures and with low seed moisture content), Recalcitrant (seeds that lose viability at low moisture content level), and Intermediate (seeds that exhibit storage characteristics between orthodox and recalcitrant seeds) (Hong and Ellis, 1996). Most recalcitrant seeds, unlike orthodox seeds, are unable to withstand dehydration or cooling (less than 10 °C) and could not be kept for a long period of time (Shih et al., 2008).

Acokanthera schimperi (A. DC.) Schweinf belongs to the Apocynaceae family, which is a shrub or tree with a height of 1 to 9 m. It is found in dry evergreen ecosystem and is a well-known species in East Africa distributed in Ethiopia, Eritrea, Tanzania, Uganda, Rwanda, DR Congo and Yemen (Hedberg, 1996). *Acokanthera schimperi*, also known as "kararu" locally, is a small, densely rounded tree with glabrous branches, cuspidate leaves, soft, brown bark, and fragrant flowers (Bussmann et al., 2021). The species' leaf extract is widely used in the conventional treatment of malaria, wounds and a number of illnesses, including common cold tonsillitis, bacterial nail infections, leprosy, tinea capitis, dermatitis, and sexually transmitted diseases (Mohammed et al., 2014). Additionally, root fusion is used for the treatment of swelling, elephantiasis, and headache. The stem and bark extracts are used as anti-venom for snake bites and rheumatoid pain, headaches, scabies, and warts (Kassa et al., 2020). Therefore, it is important to preserve the genetic resource of this multipurpose plant species. Seed storage is one of the conservation methods of genetic resources.

Due to a number of challenges, such as deforestation, habitat degradation, climate change, and the introduction of invasive species, many tree species in the tropics, including Ethiopia, are in danger of going extinct. Thus, knowledge of the seed storage behaviour of a target species is required in order to determine whether or not seed storage is suitable as a method of genetic conservation, and to handle seeds appropriately during collection and germplasm exchange (Hong and Ellis, 1996). However, there is a dearth of

information available on the seed storage behaviour for the majority of the numerous forest types that exist in Ethiopia (Teketay, 2005). Similarly, no information is available on seed storage behaviour and appropriate conservation method pertinent to *A. schimperi*. Therefore, the objective of the present study is to fill this gap by examining pretreatment conditions for germination and the seed storage behaviour of this medicinal and economically valuable tree species.

MATERIALS AND METHODS

Site description

The *A. schimperi* seed was collected from the Bishan Gari dry Afromontane natural forest located in the Heban Arsi district of the West Arsi Zone of the Oromia National Regional State (Figure 1). The area is 200 km away from Addis Ababa, Ethiopia's capital, in southeast direction. It lies between the coordinated 07°32'30.16"–07°35'34.52"N and 38°47'43.93"–38°49'46.51"E. The elevation of the seed collection site ranged from 1591 to 1626 m a.s.l. The soil has a sandy texture and has a deep brown colour. It has an annual temperature of 10 to 25 °C and annual precipitation of 500 to 1000 mm. The vegetation type of the collecting site is found in dry Afromontane forest (Mekonnen et al., 2018). *Acacia* species, *Mimusops kummel, Ficus* species, *Celtis africana, Olea species, Podocarpus falcatus*, *Cordia Africana*, *Calpurnia aurea, Euclea racemosa* and *Carissa spinarum* are common and associated tree and shrub species in this vegetation (Mewded et al., 2022).

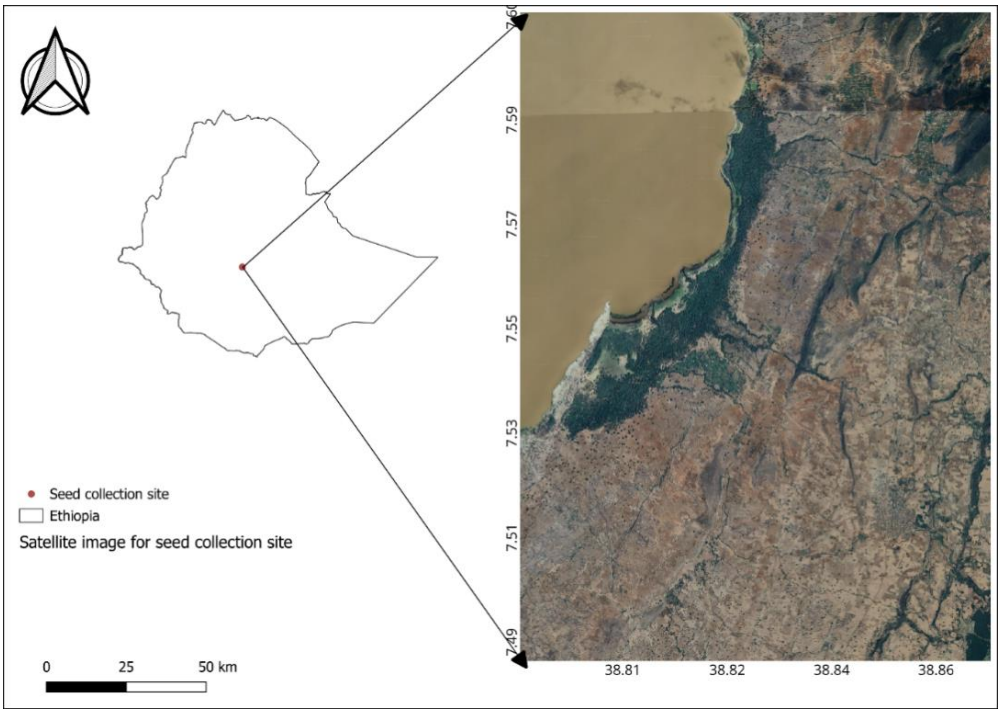

Figure 1. Map showing *A. schimperi* seed collection site.

Seed harvesting and cleaning

The potential geographic location and phenology of the species (flowering, fruiting and seed-bearing times) was identified before seed collection by reconnaissance survey. Individual mother trees were randomly and evenly selected from the same population of *A. schimperi* to collect seeds at the same site. After selecting and marking the mother trees, tarpaulin under the plant was used to catch fallen seeds and fruit when the branches are shaken. Seeds at the stage of dispersal were collected by hand and pruner, and secateur was used to cut fruit on the branch. Unripen and infested seeds were removed from the composite sample. A composite sample was obtained by mixing the primary sample, which was taken randomly from each individual tree (IBPGR, 1982). The seeds were transported to the Ethiopian Biodiversity Institute's seed laboratory in an aerated plastic bag. The seeds were separated from the fruit, rinsed and cleaned under pressure with running tap water to remove any gelatinous coatings and vegetative parts (Figure 2). The seeds were spread thinly on the plastic sheet and allowed to air dry.

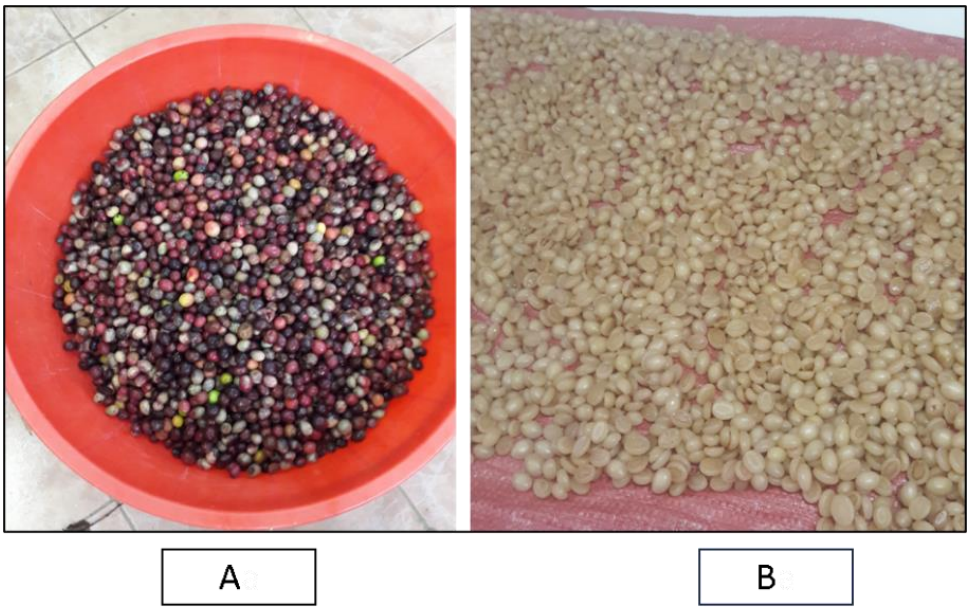

Figure 2. Seeds of A. schimperi with fleshy fruit (A) and seeds after cleaning (B).

Thousand-seed weight (TSG) determination

Cleaned 1000 *A. schimperi* seeds were counted using seed counter and the weight of the 1000 seeds was recorded. The procedure was repeated for the second trial and the mean of the two trials was recorded. The whole working sample was put through the seed counting machine and the number of seeds in the sample was noted. The total weight of the seed sample was weighted to the same number of decimal places as in the purity analysis. The thousand seed weight was determined using the following calculation:

$$TWS = W/S * 1000$$

Where TWS- is the Thousand Seed Weight (grams per 1000 seeds)

W- the total weight of seeds (gram)

S- the total number of seeds

Seed moisture content determination

After seed cleaning and thousand seed weight determination, the initial moisture content of the seed was determined using destructive (Oven drying) method as described in ISTA (2005). Low constant temperature method of oven drying method was adopted to determine the initial moisture content of the seed. To do this, sample from the cleaned seed was taken and ground using seed grinder to promote uniform and complete

drying. Small stainless-steel containers, including the lids, were labelled and weighed using a sensitive balance. Half (0.5) gram of sub-sample was taken from the ground sample and placed in two separate containers which will serve as two replicates. The drying oven was adjusted to 103 ° C following the low constant temperature method of ISTA (2005). The sample-containing containers were placed in a drying oven and the lids were removed to subject the sample to temperature. The samples were dried for 17 hours at 103 °C. The lids were replaced on each container after the end of drying. The containers were moved to the desiccator and allowed to cool for 45 minutes. The weights of the containers, including the samples, were recorded, and the initial moisture content of the sample was calculated using the following formula (Rao et al., 2006).

$$Moisture\ content\ (MC)(\%) = \frac{\mathrm{W2} - \mathrm{W3}}{W2 - W1} * 100$$

Where, W1 = weight of container with lid; W2 = weight of container with lid and sample before drying; and W3 = weight of container with lid and sample after drying.

Seed drying

After determining the initial moisture content of the seed, seeds were placed in drying room (chamber) with the temperature of 15 ^{o}C and 15% humidity. The moisture determination procedure was repeated weekly until the seeds reach the final moisture content level. Seeds with initial MC were taken and placed in three different environments (room temperature (20 ^{o}C), +4 ^{o}C and -10 ^{o}C) (IBPGR, 1982).

Pretreatment and growth media

Mechanical scarification, cold water soaking, and hot water soaking were the three pretreatment techniques used to determine the best way for seed germination. Forty seeds were used for each type of pretreatment. For mechanical scarification, the seed coats were mechanically opened with sterile pliers to allow water to enter and promote germination. For the cold-water treatment, seeds were immersed for 24 hours in a beaker filled with cold water at room temperature. Following the removal of the water, the seeds were sown on the top filter paper using a petri dish. For the hot water treatment, seeds were placed in beakers with water that

had been heated to about 100 ° C. The beakers were then allowed to stand for five minutes before the seeds were sown.

Germination and viability test

The top of paper method was used to test the viability of the seeds as the germination test is the most accurate and reliable method in the viability test. Petri dishes, forceps, and other materials were sterilized by wiping with 80 % alcohol to limit fungal contamination (Sutherland et al., 2002). Filter paper was placed on labelled petri dish. The seeds were spread uniformly on the surface of the filter paper using forceps, ensuring that none of the seeds touch each other and the appropriate amount of distilled water was added. The petri dish was covered to ensure that there is no air lock resulting from excess moisture on the covers and placed in incubator maintained at 30^{o}C. For the germination test using sand, a plastic tray was filled with enough amount of sterilized sand and seeds were placed in holes and covered with sand (Figure 3).

The test was run for a month and germinated seeds were recorded in every five days. At the end of the experiment, germinated seeds, viable but not germinated seeds and seeds that failed to germinate, and dead seeds were counted. The percentage of germination and viability were calculated using the following formula (FAO and IPGRI, 1994).

$$\%\ of\ germination = \frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds\ sown} * 100$$

$$\%\ of\ viability = \frac{number\ of\ germinated\ seeds + number\ of\ viable\ seeds}{total\ number\ of\ seeds\ sown} * 100$$

Figure 3. Germination test for the selection of best germination media and pretreatment. Germination test using top of paper method (A) and seed sowing using sterilized sand (B).

Data analysis

The R statistical software version 4.2.2 was used for data analysis. Analysis of variance (ANOVA) was used to evaluate the percentages of seed germination and effects of pretreatment, moisture content, storage temperature, and storage period. One-way ANOVA was used to determine the interaction effect of two and more factors on the germination of seeds.

RESULTS

Thousand seed weight determination

The mean thousand seed weight (TSG) of *A. schimperi* was 657.5 grams. The total number of *A. schimperi* seeds was 4550 (Table 1). About 2991 grams of *A. schimperi* seed was required for base and active collection.

Table 1. Thousand seed weight determination and amount of seeds required for storage of *A. schimperi*.

TSG Trial 1	TSG Trial 2	Mean TSG	Total number of seeds	Total amount of seed required for storage
651.64	663.36	657.5	4550	2991

TSG – thousand seed weight (in grams)

Seed moisture content

The initial moisture content (MC) of *A. schimperi* was 55.8 %. This MC declined significantly to 13.5%, 8.9 %, 8.4 % and 8.1 % in the first, second, third and fourth drying week, respectively (Figure 1). In this experiment, the final MC of *A. schimperi* seed was 7.4% at constant temperature and humidity. The decrease in moisture content of the seeds showed an inverted J-shaped pattern from initial to final MC. Four MC levels were selected for storage behaviour investigation; initial (55.8 %), intermediate (13.5%), intermediate (8.4%) and final (7.4%) MC.

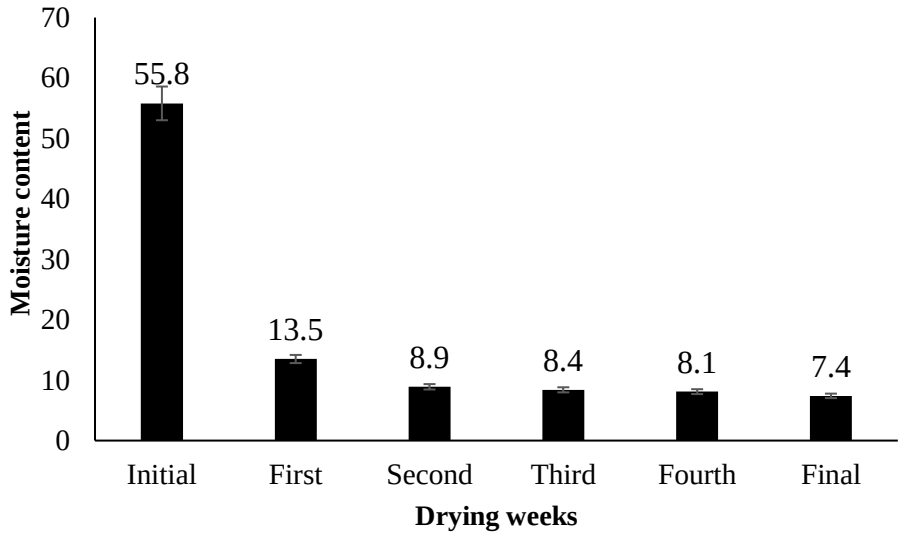

Figure 4. Moisture content of *A. schimperi* seeds in different drying weeks including initial and final MC.

Germination pretreatment and media

The initial germination test indicated variation in germination and viability among different pretreatment methods ($p < 0.01$). The highest seed germination and viability was recorded in cold water treatment with 95% of germination followed by the control (without any treatment) with 90% of germination (Table 2). The lowest seed germination was recorded in scarification method with 75% of germination. Seed germination in sand media was 85%. Since seed germination using on top of paper method and cold-water treatment gave better results, these were selected for the rest of the investigation.

Table 2. Different pretreatments used and percent of germination.

Treatments	Total seeds sown	Germinated seeds	Fresh seeds	Dead seeds	Germinated seeds (%)	Viable seeds (%)
Scarification	40	30	0	10	75	75
Coldwater	40	38	0	2	95	95
Hot water	40	34	0	6	85	85
Control	40	36	0	4	90	90
Sand	40	34	2	4	85	90
Total	200	172	2	26	86	87

Effect of moisture content on seed viability

The percentage of viability of *A. schimperi* seed was significantly affected by moisture content level (p = 0.01). The highest percentage of viability was recorded in MC level of 55.8 (90%) followed by MC 13.5 and 8.4 MC with 2.5% of viability (Figure 5). At final MC level of 7.4%, the percentage of viability becomes zero. That means the seed has totally died and indicated that the species was sensitive to change in MC level.

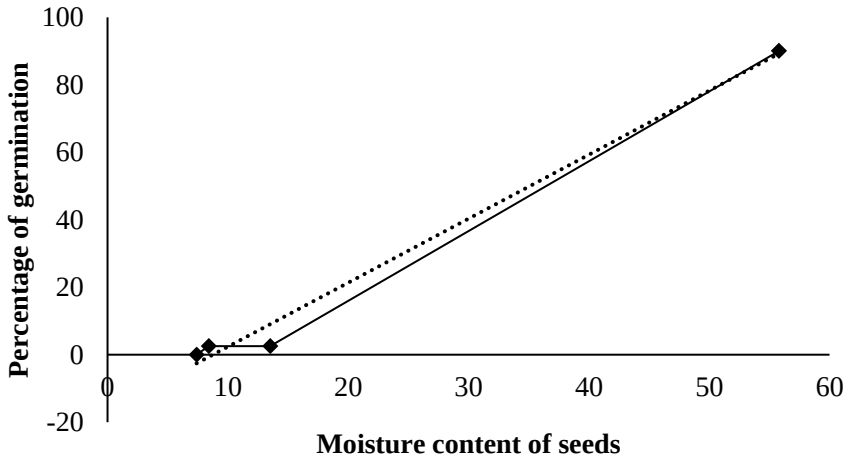

Figure 5. Percentage of germination in different moisture content level (55.8%, 13.5%, 8.4% and 7.4%) of *A. schimperi* seeds.

Effect of storage temperature and duration on seed viability

The interaction effect of storage temperature and duration on seed viability was not significant (P=0.06). At the third month of storage, 2.5% of seed germination was recorded in all storage temperatures (-10 ^{0}C, + 4

^{0}C and +20 ^{0}C) tested. In the rest of storage durations, total loss of seed viability was observed except in the sixth month at room temperature (+20 ^{0}C) (Table 3). This indicated that the seeds have lost their viability with decrease in temperature and increase in storage duration.

Table 3. Effects of storage temperature (-10^{0}C, +4^{0}C and 20^{0}C) and duration (3, 6, 9, 12 months) on seed germination and viability.

Storage environment	Storage months	Percentage of germination
- 10^{0}C	3	2.5
	6	0
	9	0
	12	0
+ 4^{0}C	3	2.5
	6	0
	9	0
	12	0
+20^{0}C (Room temperature)	3	2.5
	6	2.5
	9	0
	12	0

Effect of seed storage duration on seed viability

Seed storage duration was the other determinant factors that affected seed germination and viability of *A. schimperi* seeds. However, the effect is not significant ($p = 0.44$) (Figure 6). The percentage of seed viability in *A. schimperi* seed at initial moisture content (55.8) was 90%. The percentage of viability of the seed decreased significantly at the third month of storage to 7.5% of germination. At the sixth, ninth and twelfth month of storage, the percentage of seed germination and viability become nearly zero.

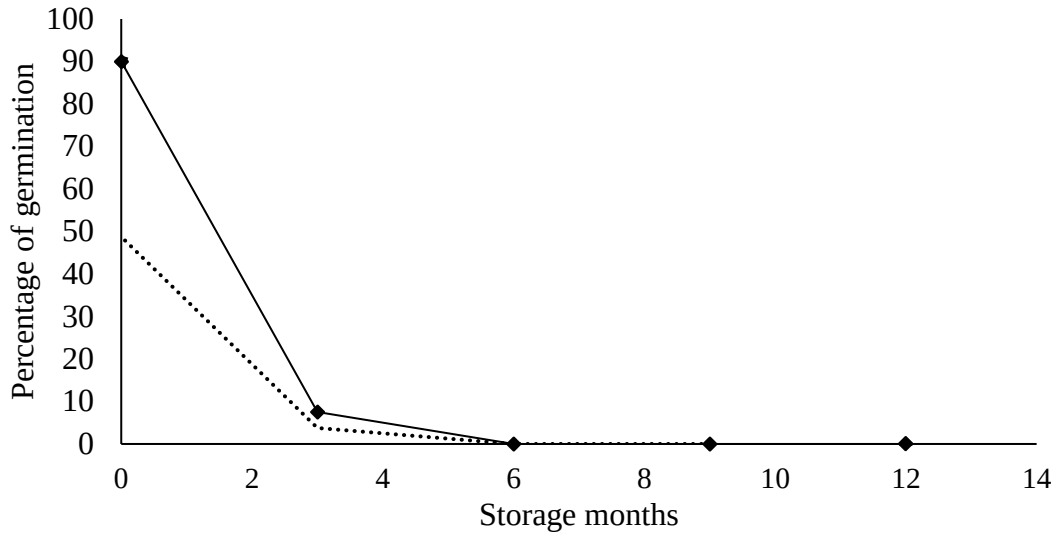

Figure 6. Percentage of germination in different storage duration (months).

DISCUSSION

The findings in this research showed that the seed of *A. schimperi* are most probably recalcitrant seed type in terms of storage behaviour. As a result, long term storage of *A. schimperi* seed in cold storage is not feasible. Species with recalcitrant seeds were often harvested at or above 30% moisture content, and they rarely sustained desiccation below 12–10% moisture level (Hong and Ellis, 1996). Once dried to a moisture content below a reasonably high critical value, recalcitrant seeds no longer have a chance of surviving (Chin et al., 1989). In this study, the initial moisture content of *A. schimperi* (55.8) reflected behaviour of recalcitrance and it has lost its viability almost totally within a week of dehydration. Seed death may result from either the moisture content dropping below a particular critical level or just a normal physiological decline over time (Chin et al., 1989). Recalcitrant seeds depend on plasmodesmata for intercellular transport because of their large seed size, which can be interrupted by drying and result in a loss of viability subsequently (Livingston, 1964). Numerous elements, such as plant species, environmental conditions during seed development and maturation, physiological state of the seed at maturity, and seed storage techniques, have an impact on seed viability (Hong et al., 1996; Pritchard and Dickie, 2003; Probert et al.,

2009). However, seed moisture content, temperature, and oxygen concentration in the storage environment have the greatest impact on how long seeds last (Ibrahim and Roberts, 1983; Roberts, 1972; Roos, 1980).

The viability of *A. schimperi* seeds was highly affected by a decrease in seed moisture level. Seed moisture content is highly influenced by change in temperature and relative humidity (Kauth and Biber, 2015). As seed moisture content decreased, longevity and viability increased with exception of highly desiccation sensitive species (Finch-Savage, 2003). Decrease in moisture content leads to oxidation of lipids and protein resulting in seed deterioration (Ranganathan and Groot, 2023). Additionally, dehydration alters the structure of enzymes, damages cell membranes, and releases phenolic chemicals that decrease enzyme activity (Loomis and Battaile, 1966).

The seed germination of *A. schimperi* was affected by the time of storage and the storage temperature (P=0.06). Recalcitrant tropical species seeds typically lose viability from a few weeks to several months (Berjak and Pammenter, 2008). Though seed viability potential of many species is increased by decrease in temperatures, it has been observed that low temperatures to have negative effects on the viability of severs recalcitrant species (Ranganathan et al., 2015). The occurrence of a temperature-dependent, rate-limiting process, the lack of which results in lethal metabolic disruption; the lack of a protective component in seeds resistant to chilling; and the release of a toxic substance as a result of alterations in membrane permeability brought on by freezing are suggested to be the possible reasons for the rapid decline in viability of recalcitrant seeds due to decrease in temperatures (Chin et al., 1989).

CONCLUSION

The present study examines the seed germination pretreatment and storage behaviour of *A. schimperi* tree species. To our knowledge, recalcitrance has never been previously documented in the *A. schimperi* tree species. Hence this study will contribute to selecting appropriate methods and strategies for conservation of the species. The seed of *A. schimperi* exhibits non-physiological dormancy, which makes it simple to germinate in a controlled setting with media such as sand or filter paper. The results also showed that the

seed germination potential of *A. schimperi* is significantly influenced by the seed moisture content, storage temperature, and their interactions. Low storage temperature and dehydration reduced the species' seed vigor. Before being dehydrated and stored at low temperatures, *A. schimperi* seeds with a moisture content of 55.8% can germinate to a significant degree. We recommend the option of using cryopreservation for the long-term storage of *A. schimperi* seeds.

ACKNOWLEDGMENT

We would like to express our gratitude to the local community of Bishangari communities of Heban Arsi district for their assistance and permission of the seed collection. The laboratory technician for the forest seed gene bank, Helen Worku, deserve special thanks for her unwavering assistance with seed preparation and germination. We also thank the Ethiopian Biodiversity Institute for the financial support.

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