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Viability of *Moringa oleifera* seeds stored at different temperatures and recent status of *Moringa oleifera* collections in seed banks worldwide

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Summary

Rapid decline in seed viability of *Moringa oleifera* Lam. due to storage severely reduces its capability to germinate. This study aimed to evaluate the viability of moringa seeds stored at various temperatures through seed germination on a selected medium and provide insights into its conservation in seed banks. The research consisted of two experiments. (1) Optimization of moringa seed germination using different media: A) sand 100%; B) mixed sand, soil, manure, rice husk charcoal (1:1:1:1); C) mixed sand, soil, and manure (1:1:1); D) mixed soil, manure, rice husk charcoal (2:1:1); and E) soil 100%. (2) Determination of moringa seed viability stored at various temperatures (-35, 5, 20, and 25 °C) every three months for 24 months. The information on the moringa seed banks was compiled via a literature review. Germination variables (percentage, rate, time, and index) were observed and recorded. The best germination medium for moringa seeds was medium B. Storage temperature at 5 °C was the most suitable temperature to maintain viability, indicated by the highest germination percentage, rate, and index, and the shortest germination time of only 6 days. There are moringa seed collections of 11 seed banks published online.

Keywords: Drumstick tree; germination media; seed banks; storage temperature

Introduction

Moringa or the drumstick tree (*Moringa oleifera* Lam., family Moringaceae) is widely known to have high nutritional content and to be rich in bioactive compounds (Lin et al., 2018). Thus, it has been widely used as a functional food ingredient and medicine. It occurs across many soil types and climatic conditions (Devkota and Bhusal, 2020). However, *M. oleifera* cultivation activities are currently minimal, even though the demand for this plant (especially the leaves) has recently increased and is projected to continue to increase in the years to come (MRF, 2019). To meet the high demand, more widespread and efficient *M. oleifera* cultivation is needed. One factor that determines the success of plant cultivation is the quality of the seeds.

Seeds that exhibit orthodox storage behavior, meaning they are desiccation tolerant, have the ability to be stored for extended periods of time. Orthodox seeds can survive a dormant period caused by drying and cooling, where their metabolism slows down considerably (Colville and Pritchard, 2019). Therefore, seeds are one of the most valuable and widespread approaches to ex-situ plant conservation (Liu et al., 2018). However, *M. oleifera* seedlings grown either from vegetative organs like stem cuttings or generative from seeds have both advantages and disadvantages. Seedlings from cuttings grow faster than those grown from seed and have the same characteristics as the parent. However supplying stems of a certain size as the cutting material for large planting areas can be a problem. Planting material using seeds could be disadvantageous if higher genetic diversity leads to a lack of uniformity (Sahwalita and Muslimin, 2015), yet they are more effective and applicable than seedlings from cuttings for massive cultivation activities. Mature plants of *M. oleifera* regenerated from seeds are sturdier and less prone to collapse because they develop deep taproots, making them suitable for conservation purposes. However, growing *M. oleifera* from seeds is often constrained by low viability especially post-storage (Sauveur and Broin, 2010).

Seed viability of *M. oleifera* decreases drastically after 6-12 months of storage depending on storage conditions (Sauveur and Broi, 2010; Fotouo-M. et al., 2015). Bezerra et al. (2004) reported that storing the seeds at 10 °C maintained the viability of *M. oleifera* seeds better than storing at ambient temperature for up to 24 months. Mubvuma et al. (2013) used more variations of storage temperatures in their experiment, but the investigated storage period was only up to 4 months. The results obtained were also different with the highest viability of *M. oleifera* seeds being obtained at 25 °C for two months of storage. Fotouo-M. et al. (2015) used a longer storage period (36 months) at room temperature (25-30 °C), resulting in a significant decrease in seed viability after being stored for twelve months. These studies did not consider the growing media for germination testing even though it is known to significantly affect seed germination (Mariappan et al., 2014; Handayani and Yuzammi, 2019). On the other hand, the temperature range of 20-30 °C was discovered to be the most advantageous for both the sprouting of seeds and the growth of seedlings of *M. oleifera* (Muhl et al., 2011). Ahmed et al. (2014) recommended providing about 50% shade during nursery conditions for optimal germination and seedling growth of *M. oleifera*.

Our study aimed to test the viability of *M. oleifera* seeds after being stored at multiple temperatures for varied periods of time using different growth media. In addition, we summarize the recent status of *M. oleifera* in seed banks because, so far, this species was considered less important for conservation, whereas *M. oleifera* cultivation activities are currently minimal (MRF, 2019). The study of seed viability after being stored at multiple temperatures can support *M. oleifera* conservation in seed banks due to one such challenge of seed banks is seed longevity (Dickie, 2021). Adequate seed storage after collecting is a critical key to maintaining seed viability (Vitis et al., 2020). The information on the viability of the seeds offered by seed banks is usually unavailable. For this reason, research on seed viability after being stored for long period needs to be carried out regularly.

Materials and methods

The materials used in this study were *M. oleifera* seeds obtained from the Bina Bhakti Farmers Group through a CV. Pusaka Madura, Sumenep Madura, East Java, Indonesia. This study consisted of two experiments. First, we tested the effects of different growth media

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on *M. oleifera* germination. Second, we tested the viability of seeds stored at various temperatures. The first experiment aimed to identify a growing medium to support the germination of *M. oleifera* seeds. The second experiment used growing media from the first experiment to find a suitable storage temperature for seeds and extend their shelf life with high viability. All experiments were conducted at the Research Center for Genetic Engineering, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN).

Seeds conditions prior to storage were observed including the moisture content, the weight of 1000 seeds, and the number of seeds in 1 kg. The seed moisture content was expressed in percent by the weight delta of fresh and dry weight from 20 seeds and compared with their fresh weight. The weight of 1000 seeds was calculated by counting the weight of 100 seeds and repeated eight times. The value was then converted to the weight of 1000 seeds. The weight of 1000 seeds was determined as the number of seeds per kilogram and was calculated according to the formula SUDRAJAT et al. (2015) as equation 1:

$$\text{Number of seeds/kg} = \frac{1000}{\text{Weight of 1000 seeds}} \times 1000 \quad (1)$$

Experiment I. Effect of the combination of growing media on *Moringa oleifera* seed germination

This experiment was designed as completely randomized design. The growing media contains organic and inorganic matter, viz. sand, soil, manure, and roasted rice (*Oryza sativa*) husks. The soil used in this study was loamy soil and taken from the topsoil (20 cm). Growing media treatments were applied using five different weight ratios: medium A (sand 100%), medium B (sand: soil: manure: husk, 1:1:1:1) (v/v/v/v); medium C (sand: soil: manure, 1:1:1) (v/v/v); medium D (soil: manure: husk, 2:1:1) (v/v/v); and medium E (soil 100%). The seeds were embedded in the growing media using seedling tray and protected from direct light exposure within a greenhouse.

The wings of *M. oleifera* seeds were removed manually. After washing with running water, the seeds then were soaked for 1 hour to allow imbibition. Before sowing, the seeds were soaked in a fungicide and bactericide solution (2 g/L each) for 30 minutes (RIDWAN et al., 2021). The number of seeds used in each treatment was 50 with three replicates, thus the total number of *M. oleifera* seeds used was 750. Watering was carried out every day from the beginning to the end of the observation period to maintain the humidity of the growing media. Observations of the number of germinating seeds were carried out every day for 14 days which were then used to calculate the germination percentage (GP), germination rate (GR), germination time (GT), and germination index (GI). GP was calculated for 14 days using the formula of SAJIMIN et al. (2017), meanwhile, GI, GT, and GR were obtained by counting the number of germinated seeds and calculated using the formula of RANAL and SANTANA (2006) as equation 2, 3, 4, and 5. Germination of seeds was defined as radicle emergence.

$$GP\,(\%) = \frac{G_T}{G_0} \times 100 \quad (2)$$

$$GI = \frac{G_1}{T_1} + \frac{G_2}{T_2} + \ldots . + \frac{G_n}{T_n} \quad (3)$$

$$GT\,(\text{day}) = \frac{(G_1T_1 + G_2T_2 + \ldots + G_nT_n)}{(G_1 + G_2 + \ldots + G^n)} \quad (4)$$

$$GR\,(\%/\text{day}) = \frac{(G_1 + G_2 + \ldots + G_n)}{(G_1T_1 + G_2T_2 + \ldots + G_nT_n)} \times 100 \quad (5)$$

Where *GP* is germination percentage (%); G_T is number of normal germinated seeds in the time *T* (not the accumulated number, but the number correspondent to the T^{th} observation); G_0 is total number of seeds planted; *GI* is germination index; *GT* is germination time (day); *GR* is germination rate (%/day); *G* is number of germinated seeds in the time *T*; *T* is time time of observation; and *n* is the last day of observation.

Experiment II. Effect of storage temperatures on the viability of *Moringa oleifera* seeds

This experiment was designed as completely randomized design with one factor. The varying factor was the storage temperature (-35, 5, 20, and 25 °C), which was chosen in order to obtain a wide enough temperature range. Air-tight containers were used for storing seeds in a dark place, and the moisture content of the seeds was measured before storage. Each treatment used 50 seeds with three replicates. A total of 150 seeds in each treatment were taken and tested for viability every three months for 24 months by germinating them on a growing media consisting of a mixture of sand, soil, manure, and roasted husks in a ratio of 1:1:1:1 (v/v/v/v). Seed preparation before sowing and germination observations were carried out in the same way as Experiment I.

Data analysis

We identified differences among treatments with ANOVA and post-hoc tests (Duncan Multiple Ranges Test or DMRT). Statistical analysis was performed using R software 4.0.3 (TRF, 2022). The information on the recent status of the seed banks of *M. oleifera* was compiled via a literature review.

Results and discussion

Seeds condition prior to storage

We found that the moisture content of *M. oleifera* seeds before storage measured 7.67%, which was reported as ideal for storing orthodox seeds. TATIPATA (2009) reported that soybean seeds stored with 8% moisture content resulted in a higher germination percentage compared to those stored in 10% and 12% moisture conditions. In *M. oleifera*, seeds stored in 4 to 8% moisture conditions showed a higher germination percentage and germination index compared to those stored in more than 10% moisture conditions (AFZAL et al., 2020). Low moisture content in storing orthodox seeds maintains seed viability as it inhibits respiration which can damage the seeds and reduce food reserves (AFZAL et al., 2020).

Genetic and environmental factors influence the weight and size of seeds (WIDIASTUTI and LATIFAH, 2016). In this study, the weight of 1000 *M. oleifera* seeds was 0.204 kg and the number of seeds in 1 kg was 4,889 seeds, indicating good quality similar to previous studies by LÓPEZ et al. (2020). Heavier seeds generally have better germination rates due to their higher nutrient content and energy levels (SCHMIDT, 2002; RAHMAWATI and SAENONG, 2010). VALDES-RODRÍGUEZ et al. (2018) also found that heavier and larger *M. oleifera* seeds produce taller seedlings with more roots. Similarly, GOMAA and PICÓ (2011) observed that larger seeds in another *Moringa* species, *M. peregrina* (Forssk.) Fiori, had faster germination and better seedling growth rates. The weight and size of the seed also affects the total number of seeds per kg, with heavier and larger seeds resulting in fewer seeds per kg. This information is important for estimating the amount of seeds required for planting in a specific area (SAHWALITA and MUSLIMIN, 2015).

Effect of growing media composition on *Moringa oleifera* seeds germination

In this study, several organic and inorganic materials, such as sand, soil, manure, and rice husk charcoal were used to determine the best

media for germinating *M. oleifera* seeds (experiment I). The selected medium was then used as a basal medium to test the viability of *M. oleifera* seeds from different temperature treatments (experiment II). The parameters observed in these experiments were germination percentage, germination rate, germination time, and germination index.

The germination percentage of medium B and D was above 60% and significantly different from medium A and E, which enabled about 20% germination (Fig. 1a). Medium B and D also resulted in a higher germination rate (14.46 and 14.26%/day, respectively), as well as germination index (4.81 and 4.60, respectively) (Fig. 1b, 1d). Germination time represents the time required for seeds to germinate indicated by plumule emergence. In this experiment, *M. oleifera* seeds sown in all media treatments began to germinate on the 7th and 8th days after sowing (Fig. 1c). Seeds sown in medium B and D germinated faster compared to medium A, C, and E, however, the results were not significantly different.

Medium A (sand only) and E (soil only) resulted in a low percentage of *M. oleifera* seed germination. This indicated that the medium containing only one component could not support germination well. This result was similar to a previous study, where media consisting of 100% river sand and 100% topsoil showed a low percentage of plumule emergence of 53 and 40%, respectively (Saani et al., 2020). Dharmveer et al. (2016) also reported that seeds of medicinal plant *Angelica glauca* Edgew. (Apiaceae) sown in media containing 100% topsoil yielded low germination (51%). Seeds require sufficient water content for water imbibition in the early stage of germination, however sand is highly porous which results in low water and nutrient holding capacity (Isa et al., 2021). On the other hand, topsoil containing high soil moisture content causes the soil to become too compact and humid when it is not mixed with porous materials causing germination inhibition.

Contrary to this study, Gairola et al. (2011) reported that *Jatropha curcas* L. (Euphorbiaceae) seed sown on vermiculite and sand showed high germination percentage (85 and 82%, respectively) compared to media containing cocopeat, perlite, normal soil, or filter paper. Mariappan et al. (2014) reported that the seeds of *J. curcas* and *Pongamia pinnata* (L.) Pierre (Fabaceae) sown at 2 cm depth in river sand media resulted in the highest germination percentage (82 and 88%, respectively) compared to other treatments (rolled paper towel, top of the sand, detoxified white quartz sand, and vermiculite). In this study, the most suitable medium for the germination of *M. oleifera* seeds contained a mixture of organic and inorganic matter, medium B, C, and D. Medium B and D showed the highest percentage of germination (65 and 64%, respectively), followed by medium C (46%). Medium B and D were superior to germinate *M. oleifera* seeds as indicated by the higher value of germination percentage, rate, and index with the fastest germination time compared to the three other media (Fig. 1). These media contained soil, manure, and rice husk charcoal indicating that *M. oleifera* seeds required sufficient organic matter to initiate germination. Soil with a crumb structure is good for supporting plant growth and development because it contains organic matter as a source of nutrients for plants. Charcoal husk has light and coarse characteristics causing high water holding capacity in the media. Its black color can absorb thermal energy effectively, which may accelerate germination (Damayanti et al., 2019).

Seed germination in medium B containing sand was slightly higher than in medium D which did not contain sand. Sand can be used as a planting medium because it does not contain toxic materials, has a pH of 6.0-7.5 and a size of 0.05-0.8 mm, thus can create good porous and aerated conditions. However, sand has a low moisture capacity and nutrient content. On the other hand, soil and manure supplemented in the media played an important role as macro and micronutrient sources as well as moisture holders. The best-growing media should provide a balance between aeration and moisture (Handayani and Yuzammi, 2019) to promote water imbibition and subsequently accelerate the germination rate.

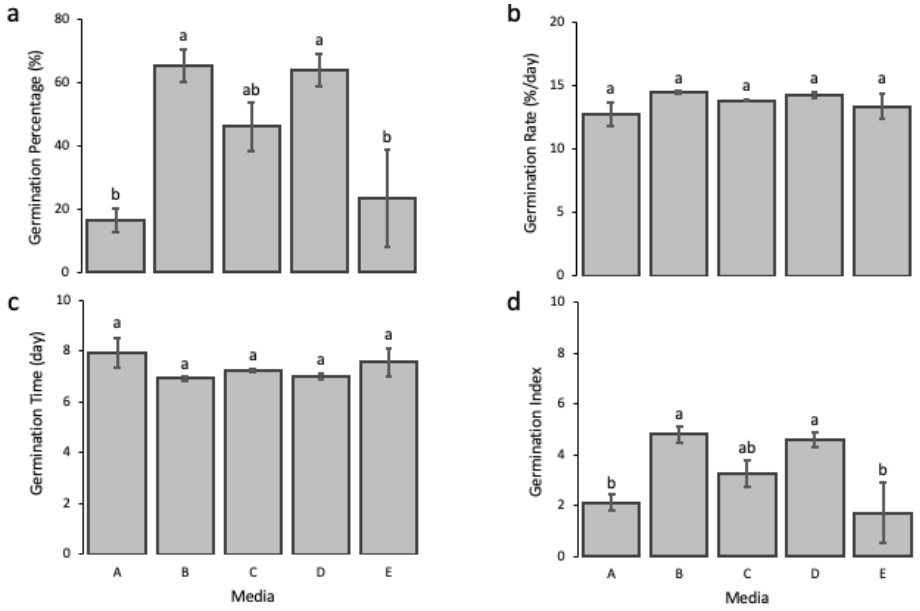

Fig. 1: Seed germination parameters of *Moringa oleifera* including germination percentage (a), germination rate (b), germination time (c), and germination index (d) observed on day 14 after sowing in some growing media. Value (means ± standard error) marked with the same letter are not significantly different at $p \leq 0.05$ by Duncan's test.

Effect of storage temperatures on the viability of *Moringa oleifera* seeds

Seed viability is defined as the ability of the embryo to germinate. It is affected by several factors including temperature, light, oxygen and water and is also species dependent (GEBEYEHU, 2020). Several tests for seed viability have been developed through seed germination (WAWRZYNIAK et al., 2020), X-rays (AL-TURKI and BASKIN, 2017), and tetrazolium staining test (PRADHAN et al., 2022). The most reliable way to evaluate viability is to germinate seeds under ideal conditions, although this may require several days or weeks to be done. This study determined *M. oleifera* seeds viability through seeds germination using medium B which consisted of sand, soil, manure, and rice husk charcoal (1:1:1:1, v/v/v/v) from the previous experiment.

Based on the percentage of germination for 24 months, the viability of *M. oleifera* seeds stored at 25 °C began to decrease after being stored for 12 months and dropped drastically after the 15 months until the percentage of germination reached 0% at 24 months of storage. Seeds stored at 20 °C showed a relatively constant percentage of germination but decreased sharply at the 24th month of storage. Meanwhile, seeds stored at -35 °C showed relatively constant viability for 24 months of storage. The storage temperature of 5 °C was able to maintain the viability of *M. oleifera* seeds as indicated by the percentage of germination at the beginning of storage was more than 80% and at the end of observation (24th month) was still 92% (Tab. 1; Fig. 2a).

Seed germination rate and time showed constant results for 24 months, except for the treatment at 25 °C (Tab. 1; Fig. 2b-c). The highest germination rate (14.47 %/day) and the fastest germination time (6.91 days to emerge) were obtained from 5 °C treatment in all periods of storage. The germination index of seeds stored at 25 °C began to decrease at the 18th month of storage, while 5 °C treatment still yielded the highest germination index (6.83) (Tab. 1; Fig. 2d). These results indicate that *M. oleifera* seeds were best stored at 5 °C as they successfully maintained seed viability and obtained the highest value of germination percentage, rate, and index and the shortest germination time (Fig. 3).

Studies of short-term seed shelf-life (<18 months) show that temperatures between 0 and 5 °C are sufficient to maintain dry seed viability (HONG et al., 1996). BEZERRA et al. (2004) stated that the viability of *M. oleifera* seeds stored at room temperature decreased by 65% after 12 months of storage and completely lost viability after 24 months of storage. As the respiration rate continues to increase due to enzyme activation, the oxygen consequently runs out and carbon dioxide accumulates, thus resulting in high relative humidity. Furthermore, high temperatures caused faster seed deterioration and subsequently affected germination and seedling vigor (GEBEYEHU, 2020). To summarize, a temperature of 5 °C is recommended for storing *M. oleifera* seeds for more than 2 years as it successfully maintained seed viability.

Recent status of the seed banks of *Moringa oleifera*

A seed bank is urgently needed to support plant breeding programs and conservation efforts of *M. oleifera*. A seed bank, a place where seeds are stored to preserve genetic diversity, is an ex-situ technique that implicates the preservation of germplasm away from where they naturally occur for a relatively long period using methods of drying and deep-frozen. Long-term storage of seeds and conservation in botanic gardens are the most valuable and widespread approaches to ex-situ plant conservation (LIU et al., 2018). Until recently, seed banks only collected the seeds from crop plants (LESTARI and ASIH, 2015). Since the Global Strategy for Plant Conservation was adopted in 2002, many samples of wild species seeds have been collected (HAY and PROBERT, 2013). A seed bank is most widely used for conservation due to it offers the preservation of high levels of genetic diversity at a comparatively low expense, for a relatively long term, and in minimal space (LESTARI and ASIH, 2015). Furthermore, seed banks also provide well-documented seeds from a wide variety of species, cultivars and ecotypes for future research.

There are more than 1750 seed banks in the world (HAY and PROBERT, 2013; LIU et al., 2018). However, only a few publish their *M. oleifera* seed collections online (Tab. 2). We suspect that several other seed banks also have collections of *M. oleifera* seeds. Seed

Tab. 1: Seed germination of *Moringa oleifera* after being stored at various temperatures for 18, 21, and 24 months.

Storage temperature (°C)	Germination percentage (%)		
	18 months	21 months	24 months
-35	53.33 ± 3.53 b	62.67 ± 11.10 b	88.00 ± 2.00 a
5	85.33 ± 1.76 a	86.67 ± 0.67 a	92.67 ± 2.40 a
20	78.00 ± 7.21 a	82.00 ± 1.15 a	40.00 ± 6.43 b
25	44.67 ± 7.06 b	10.00 ± 2.00 c	0.00 ± 0.00 c
		Germination rate (%/day)	
-35	10.51 ± 0.34 ab	12.41 ± 0.30 b	13.67 ± 0.30 b
5	11.49 ± 0.27 a	14.73 ± 0.44 a	14.47 ± 0.16 a
20	10.02 ± 0.13 b	12.52 ± 0.14 b	11.37 ± 0.12 c
25	10.28 ± 0.68 ab	10.26 ± 0.31 c	0.00 ± 0.00 d
		Germination time (day)	
-35	9.54 ± 0.32 ab	8.07 ± 0.20 b	7.32 ± 0.16 b
5	8.71 ± 0.20 b	6.80 ± 0.21 c	6.91 ± 0.08 c
20	9.98 ± 0.13 a	7.99 ± 0.09 b	8.80 ± 0.09 a
25	9.81 ± 0.61 ab	9.76 ± 0.30 a	0.00 ± 0.00 d
		Germination index	
-35	2.88 ± 0.11 bc	4.06 ± 0.77 b	6.16 ± 0.26 a
5	5.05 ± 0.21 a	6.48 ± 0.21 a	6.83 ± 0.13 a
20	4.00 ± 0.38 ab	5.20 ± 0.09 ab	2.32 ± 0.38 b
25	2.43 ± 0.60 c	0.51 ± 0.09 c	0.00 ± 0.00 c

Data were expressed as means ± standard error (n = 5). Means of each column for each parameter followed by the same letter/s are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

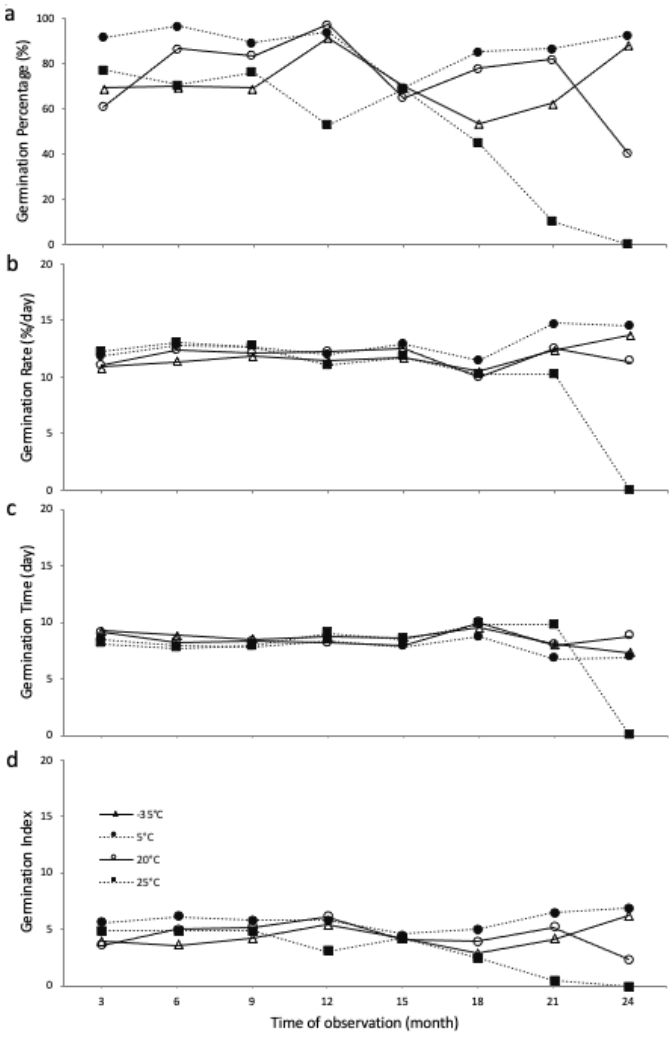

Fig. 2: Effect of storage temperature treatments on *Moringa oleifera* germination percentage, rate, time, and index over 24 months of storage.

Centre of Tamil Nadu Agricultural University, for example, reported that they have 76 kg (last update 10 October 2022) of *M. oleifera* seeds preserved (TNAU, 2022). It is not surprising, since *M. oleifera* is massively distributed throughout India (PANDEY et al., 2019), was originally cultivated by the Dravidians in South India (MAINENTI, 2018) and reportedly became a superfood (SARKAR and PANDA, 2021). The World Vegetable Center in Taiwan has conserved around 90 accessions (last update 20 November 2022) of *M. oleifera*, the majority collected from Thailand and others from Cambodia, India, Indonesia, Laos, Malaysia, Philippines, Taiwan, Tanzania, Nigeria, and USA (WVS, 2022a). The World Vegetable Center is an international nonprofit institute that is engaged in combating poverty and malnutrition through increased production and consumption of vegetables (WVS, 2022b). Other seed banks (Tab. 2) reportedly conserved seeds of around 1-33 accessions or 20-150,000 seeds of *M. oleifera*. There are only two accessions of *M. oleifera* in the Millennium Seed Bank (last update 20 November 2022) (MSB, 2022a). The Millennium Seed Bank has over 2.4 billion seeds preserved from around the world (MSB, 2022b) and is the culmination of ex-situ seed conservation that began in the 1960s at the Royal Botanic Gardens, Kew - UK (LIU et al., 2018, 2020).

According to our investigation (Tab. 2) there were no seed banks from Indonesia reported. Seeds, however, have been collected and stored in the seed banks of the Botanic Gardens of Bogor, Cibodas, Purwodadi, and "Eka Karya" Bali. LATIFAH et al. (2019) reported that there was a total of 1355 accessions of seed collections (mostly orthodox seeds) in Indonesian Botanic Garden Seed Banks. There was a total of 749 accessions in Bogor Botanic Gardens, 61 accessions in Cibodas Botanic Garden, 413 accessions in Purwodadi Botanic Garden, and 132 accessions in "Eka Karya" Bali Botanic Garden. These seed collections were stored in cold storage or a freezer (LATIFAH et al., 2019). However, there was no collection of *M. oleifera* seeds reported in Bogor Botanic Gardens (Hutabarat PWK 2022, pers. comm.) because *M. oleifera* is widely distributed in Indonesia, especially in Java and the Lesser Sunda Islands (RIASTIWI et al., 2018) and not a priority for conservation. We suspect something similar has also occurred in other botanic gardens in Indonesia. Herbarium Bogoriense (BO) in Cibinong - Indonesia has collected seeds (and fruit) as a carpological collection. The carpological col-

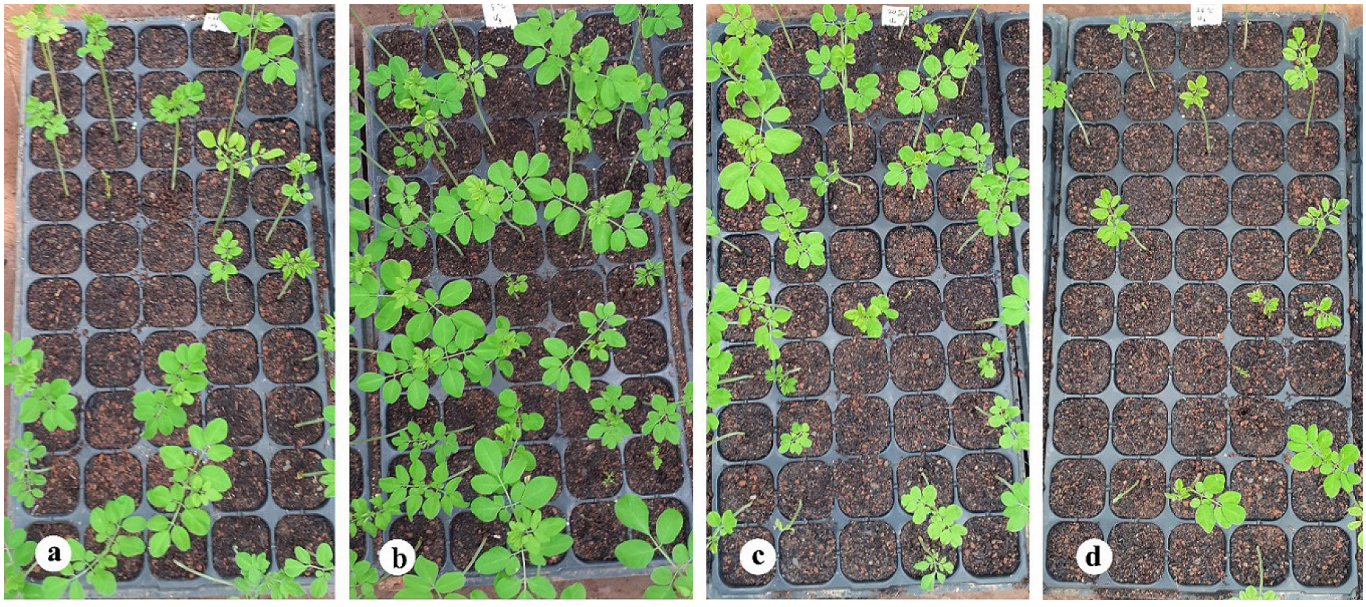

Fig. 3: Seed germination of *Moringa oleifera* in media containing sand, soil, manure, and rice husk charcoal (1:1:1:1) being after stored at several temperatures: -35 °C (a), 5 °C (b), 20 °C (c), and 25 °C (d).

Tab. 2: List of seed banks that collected *Moringa oleifera* seeds around the world.

Country	Seed banks	Institutions and address	Website	Number of collections
Brazil	Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)	Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Parque Estação Biológica - PqEB, s/nº, Brasília	https://www.genesys-pgr.org/a/v293j61qexk	33 accessions (20 November 2022)
Ethiopia	International Livestock Research Institute (ILRI)	International Livestock Research Institute – Ethiopia. Addis Ababa, Ethiopia, 5689	https://www.genesys-pgr.org/a/v2XaX22xyRG	2 accessions (20 November 2022)
India	Seed Centre of Tamil Nadu Agricultural University	Tamil Nadu Agricultural University. Lawley Road, Coimbatore, 641003	https://tnau.ac.in/seed-availability	76 kg (10 October 2022)
Kenya	Genetic Resources (GeRRI)	Kenya Agricultural and Research Institute Organization. City Square, Nairobi, 00200, Kenya, 57811	https://www.genesys-pgr.org/a/v2BMjLRkl6d Livestock Research	21 accessions (20 November 2022)
Kenya	World Agroforestry (ICRAF)	Center for International Forestry Research (CIFOR) and World Agroforestry (ICRAF). United Nations Avenue, Gigiri, Nairobi, 00100, Kenya, 30677	https://www.genesys-pgr.org/a/v25KdkkM12R	13 accessions (20 November 2022)
Norway	Svalbard Global Seed Vault	Norwegian Ministry of Agriculture and Food; Nordic Genetic Resource Centre (NordGen); Crop Trust. Svalbard and Jan Mayen, Norwegian island of Spitsbergen, 9170	https://seedvault.nordgen.org/Search	3 accessions (20 November 2022)
Taiwan	World Vegetable Center (WVS)	World Vegetable Center. Shanhua, Tainan, Taiwan 74151, 42	https://genebank.worldveg.org/#/?filter=v2ldmDbgRXZ&p=0	90 accessions (20 November 2022)
UK	Millennium Seed Bank (MSB)	Royal Botanic Gardens, Kew. Richmond, London, TW9 3AE	http://apps.kew.org/seedlist/SeedlistServlet	2 accessions (20 November 2022)
United Arab Emirates	International Center for Biosaline Agriculture (ICBA)	International Center for Biosaline Agriculture, United Arab Emirates. Academic City, Al Ain Road Al Ruwayyah 2, Near Zayed University Dubai, United Arab Emirates, 14660	https://www.genesys-pgr.org/a/v2MBLRREPJy	1 accession (20 November 2022)
USA	International Moringa Seed Bank (IMSB)	Sustainable Bioresources, LLC. Naalehu, Hawaii, 350	http://sustainablebioresources.com	150,000 seeds (09 May 2023)
USA	Germplasm Resources Information Network (GRIN)	USDA Germplasm Collection, U.S. National Plant Germplasm System (NPGS). 10300 Baltimore Blvd. Room 330, Bldg. 003, BARC-West Beltsville, 20705	https://npgsweb.ars-grin.gov/gringlobal/accessiondetail?id=1088343	20 seeds (18 November 2022)

lection contains the parts of a plant that cannot be easily pressed on the herbarium sheet. Seed collections in BO, therefore, were technically purposed as herbarium sheet extensions, but have potential to be germinated.

Conclusions

It can be concluded that the best growing medium for the germination of *M. oleifera* seeds was the mixture medium consisting of sand, soil, manure, and rice husk charcoal with a ratio of 1:1:1:1. The temperature storage of 5 °C was the best storage temperature for

24 months yielding in the highest germination percentage, germination rate, and germination index, and the shortest germination time. A critical key to maintaining seed viability is effective seed storage through seed banking. There were 11 seed banks that published their *M. oleifera* seed collections online recently with one to 99 accession or 20-150,000 seeds to 76 kg collection.

Author contribution

All authors have reviewed the final version of the manuscript and approved it for publication. IR and R designed the research; IR, ADP, and IPGPD performed the research, collected, and analyzed the data; IR, ADP, R, and IPGPD wrote, edited, and reviewed the paper. All authors were the main contributor of this paper.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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