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Storage Reserve Composition and Storage Behaviour of *Amorphophallus muelleri* Blume Seed

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ABSTRACT

Amorphophallus muelleri Blume, belonging to the Araceae family, has a high economic value due to the glucomannan content used in both food and non-food industries. Propagation of *A. muelleri* through seed is more efficient than through bulbs and corms because the seed offers higher multiplication and bulking rates. However, limited information on the seed composition and storage behaviour poses challenges for handling and storage. Therefore, this study aimed to determine the seed chemical composition, classify the storage behaviour, and assess the effect of storage temperature on viability. The results showed that the seed contained 51% starch as the primary reserve compound and 15.74% glucomannan. Rapid drying at 35 °C with 40% relative humidity (RH) was found lethal to the seed, while slow drying at 22 °C with 10% RH was harmless for viability. Seed preserved the viability (78-91 %) when the moisture content remained above 50%, but lost viability when the moisture content reduced slowly to 30.9% (MDN1), 21.3 % (MDN2), and 43.25% (PWTA), showing that *A. muelleri* seed is recalcitrant. Moisture content of 60.2% (L1), 62.9% (L2), and 60.2% (L3) led to loss of seed viability with germination average of 5 % during the third month at refrigerator storage (5-7 °C, 40-50 % RH). In contrast, controlled temperature storage of 27-32 °C (>60% RH) maintained seed quality, including water content above 48% and germination above 80% during five months, and for three months.

Keywords: Glucomannan, moisture content, recalcitrant, seed desiccation, starch, viability

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INTRODUCTION

Amorphophallus spp. are among the most widely grown Araceae species in tropical and subtropical regions, including

Indonesia. More specifically, *Amorphophallus muelleri* Blume; sin. *A. oncophyllus* Prain. is highly valued due to the glucomannan content, which is used in both food and non-food industries (Kurniawan et al., 2011). Based on the bulking and multiplication rate, *A. muelleri* propagation using seed is the most effective method compared to bulb and corm. In contrast to bulbs and corms, which require about 300 kg/ha and 1500 kg/ha of planting material, respectively, botanical seed propagation requires only 30-40 kg per ha.

Seed longevity refers to the period during which the seed remains viable and survives while in storage or under natural conditions until germination in the field and production of a normal plant. It is influenced by seed moisture content, storage conditions, temperature, and relative humidity (RH), with the effect varying among species (Nadarajan et al., 2023; Pirredda et al., 2024). Therefore, classification of seed storage behaviour is required to provide appropriate storage methods (Hong et al., 1996). There are three categories for seed storage behaviour, including orthodox, recalcitrant, and intermediate. Orthodox seed tolerates either desiccation (moisture content of 10 %) or sub-zero temperatures without damage, while recalcitrant and intermediate seed are sensitive to desiccation and chilling. Recalcitrant seeds do not survive in dry conditions or low moisture content. This implies that the seeds are viable only for a limited period and cannot withstand moderate and long-term storage. Although less resilient compared to orthodox, intermediate seed can withstand higher degrees of desiccation than recalcitrant seed (Hay et al., 2022; Pirredda et al., 2024; Tchokponhoué et al., 2019). *A. muelleri* is reportedly recalcitrant based on the high moisture content of harvested seed (Sari et al., 2019). However, there is limited evidence regarding the seed tolerance to desiccation and the optimum range of moisture to preserve the viability.

An optimum environment, including temperature and RH, is needed to preserve seed viability during storage. Temperature is one of the crucial factors preventing seed deterioration during storage. The optimum temperature can be determined based on the character of the seed. Orthodox seed requires low temperature and humidity storage conditions, while recalcitrant seed is sensitive to low temperature. For recalcitrant seed, identifying the lowest temperature that can be tolerated without inducing chilling injury is essential to minimise metabolic activity and prolong storage life. Studies on recalcitrant seed storage showed that temperature and period varied across species. For example, *Araucaria angustifolia* stored at 5 °C maintained high viability for up to 12 months and then decreased in 19 months (Gasparin et al., 2020). Environmental conditions also influenced the storage of *Ekebergia capensis*. Seed from the temperate zone maintained viability above 80% after 12 weeks of storage at 1 °C, 3 °C, and 6 °C. In contrast, seed from the sub- tropical zone retained viability of 100% at 6 °C and 16 °C, but lost viability after 38 d at 3 °C. Seed from the tropical zone lost viability at 3 °C (after 9 days) and decreased to 10% and 35% viability at 6 °C and 16 °C, respectively (Bharutha et al., 2020).

Lipids, carbohydrates, and proteins are the main compounds of seeds, and the relative proportions vary among species. Carbohydrates are a major energy reserve and serve as an energy source during seed germination. Compared to lipids, carbohydrates are relatively stable and resistant to oxidation. Therefore, seeds with higher starch content generally have better longevity (Hay et al., 2022). Lipids, specifically those in seeds with high oil content, are stored as energy reserves and are essential for metabolism during germination. However, seeds with high lipid content tend to have shorter longevity compared to starchy ones. This is because lipid or oil content affects the amount of water absorption at a given RH. Lipids are prone to oxidation and changes in fatty acid content during storage, which accelerates damage (Rao et al., 2023). Proteins in seeds function as enzymes, storage proteins, and structural components. During germination, proteins are broken down to provide amino acids for seedling development. This degradation can affect seed vigour and viability due to susceptibility to denaturation, oxidation, and environmental stress, reducing seed life (Shibata et al., 2020).

Limited information on storage reserve composition and storage Behaviour of *A. muelleri* seed has become an obstacle for handling and conservation. Therefore, this study aimed to (i) determine compounds that play important roles in seed handling and conservation, (ii) classify the category of seed storage behaviour by observing the effect of desiccation on moisture content and viability, and (iii) identify the effect of the environment on viability during five months of storage.

MATERIALS AND METHODS

Experiment 1: Determination of Storage Reserves Composition: Starch, Protein, Lipid, and Glucomannan

A single seed lot of *A. muelleri* var Madiun 1 was harvested in June 2022 in Madiun Regency and used to determine seed reserve composition, including starch, protein, lipid, and glucomannan. Total starch seed was measured on a wet weight basis. Samples of 0.5 g seed were ground and dissolved in 2 ml Aquadest, then incubated at 100 °C for 15 minutes. About 5 ml of perchloric acid (9.2N) was added when the suspension was cooled and incubated for 15 minutes. Water was added until the volume of the final suspension reached 10 ml, followed by centrifugation to separate the supernatant and sediment. The supernatant was taken and transferred to another container. The sediment was added with 2.5 ml of perchloric acid of 4.6 N and incubated for 15 minutes. Water was added until the volume of the final suspension reached 10 ml, and the suspension was centrifuged to separate the supernatant and sediment. A 1 ml sample was taken, and 10 ml of anthrone 0.1 % was added, followed by boiling in a water bath for 7.5 minutes. The starch concentration was determined using a spectrometer at ƛ 630 nm, and calculated using a standard curve, constructed with glucose as the standard (Qalsum et al., 2015).

The crude protein content of the seed sample was determined using the Kjeldahl method of AOAC, while crude fat content was measured by extracting the fat using the Soxhlet method. For glucomannan determination, the seed sample was washed and cut into 2-3 mm pieces, then soaked with 1 % (w/v) sodium bisulfite for 1 minute. Subsequently, the sample was dried at 120 °C for 40 minutes and continued at 60 °C until it reached constant weight, followed by grinding into fine flour (Chua et al., 2012). Glucomannan content was analysed using the gravimetry method, measured on a dry basis. A 5-gramme sample was weighed and dissolved in 50 ml of warm water at 75 °C, with 0.5 grammes of aluminium sulfate added, followed by stirring for 35 minutes using a shaker. The sediment was removed from the solvent by centrifugation at 2000 rpm for 30 minutes. The supernatant was collected and added to isopropyl alcohol in a 1:1 ratio while stirring until a clot formed. The sample was filtered using filter paper, and the residue/clot was dried at 60 °C for 24 hours, then weighed.

Experiment 2: Effect of Desiccation on Seed Moisture Content and Viability

Different desiccation methods to reduce seed moisture content may affect viability. Therefore, various types of desiccation methods need to be evaluated to assess the effect on the reduction of viability. Seed desiccation tolerance must be determined to classify the *A. muelleri* category of storage behaviour. Based on the protocol from Hong and Ellis (1996), seed should dry to 10-12% moisture content to determine viability. Seed are classified as orthodox or intermediate when most survive at this moisture level, while seed are considered recalcitrant when most lose viability.

Three seed lots of *A. muelleri* Blume var. Madiun 1 from Central Java, Indonesia were used for experiment 2. MDN1 and MDN2 were harvested in Madiun Regency from June, 9-11th 2022, and June, 6-9th 2022, respectively, while PWTA was harvested in Purwokerto Regency from June, 22-24th. This experiment was conducted from August 2022 until February 2023. Before and after the desiccation test, seed lots were tested for moisture content and germination. The moisture content was tested using the low-temperature method at 103 °C for 17 h and reported as a percentage of fresh weight.

Experiment 2 was performed using a randomised complete design with two factors, namely seed lots (MDN1, MDN2, PWTA) and rate of seed desiccation. Three different rates of desiccation test were designed, namely rapid, moderate, and slow. To reduce seed moisture content, 250 g of seed were evenly spread on trays and subjected to three different desiccation conditions: (i) rapid desiccation using a dehydrator with warm free airflow at 35 °C and 40% RH, (ii) moderate desiccation in an incubator at 35 °C and 50% RH, and (iii) slow desiccation using silica gel placed in a sealed plastic box beneath the samples at 22 °C and 10% RH. The temperature and RH were measured by using a thermohygrometer. To estimate the reduction of seed moisture content, the samples were weighed regularly

and calculated using the following Equation 1 (International Seed Testing Association [ISTA], 2021):

$$\text{Weight of seed lot at a certain \% MC} = (\text{initial weight}) \times [(100\text{-initial MC}) / (100\text{-desired MC})] \quad [1]$$

where MC: Moisture content

Experiment 3: Effects of Storage Environment on Seed Germination

Three lots of *A. muelleri* Blume var Madiun 2 (L1, L2, L3) were used to ascertain the optimum environment for storing seed. L1 was harvested from Klangon Regency (East Java) on 17th July 2023, while L2 and L3 were harvested from Tapos Regency (West Java) on 10th and 25th July 2023, respectively, with 60-63 % moisture content. The experiment was organised using a nested completely randomised design of two factors and three replications, namely storage environment and periods.

About 300 g of seed sample was stored in three different environments of (S1) ambient temperature at 27-32 °C (>60% RH), (S2) controlled temperature at 20-23 °C (50-60% RH), and (S3) refrigerator at 5-7 °C (40- 50% RH) during 5 months (M0-M5). The temperature and RH were measured by using a thermohygrometer. Seed samples were stored in paper bags and tested for moisture content, germination, and T_{50} for each month. The moisture content was tested using the low-temperature method at 103 °C for 17 h and reported as a percentage of fresh weight.

Seed Moisture Content Determination

Two replicates, which were drawn from a seed sample, were cut into pieces no thicker than 7 mm for a maximum of 4 minutes. Each replicate was weighed to four decimal places for 4.5-5 g. Subsequently, containers and covers (porcelain cups) were weighed before and after filling with the sample. The containers were then placed quickly next to the cover in an oven maintained at 103 °C for 17 h. After heating, it cooled down in a desiccator for 30 minutes. The containers with the cover were weighed, and the moisture content was calculated using Equation 2 (ISTA, 2021).

$$\text{Seed MC (\%)} = [(M2\text{-}M3) / (M2\text{-}M1)] \times 100\% \quad [2]$$

where: M1: The weight of the container and cover (g)
M2: The weight of the container, cover, and content before drying (g)
M3: The weight of the container, cover, and content after drying (g)

Germination Test

Three replicates of 50 seeds were sown, using the between-paper method, and incubated at 25 °C. The germination test results are expressed as the percentage by number of normal seedlings (ISTA, 2021). Total germination was calculated using Equation 3:

$$\text{Germination (\%)} = (\text{number of normal seedlings/number of seed}) \times 100 \quad [3]$$

Seedling evaluation was conducted at three-day intervals until germination reached 50% of the final germination value (T_{50}). The germination period was recorded until the seed sample reached the maximum rate of T50 calculated using Equation 4:

$$T50 = Ti + (((N+1/2)-Ni)\ (Tj-Ti))/(Nj-Ni) \quad [4]$$

where T50 is the median germination time, N is the final number of germinated seed, while Ni and Nj are the total number of seed germinated in adjacent counts at time Ti and Tj, respectively, when $Ni < ((N+1)/2) < Nj$ (Kumar et al., 2021).

Normal seedlings were defined as intact seedlings that had all healthy essential structures, including a shoot and root system. The cotyledon types (bifacial hypophyll and unifacial hyperphyll) and the presence or absence of endosperm were used to categorise araceous seedlings. *A. muelleri* had a large, green storage organ of cotyledonary hyperphyll, with no or only inconspicuous traces of endosperm. Before the leaf appeared, it had one or more cataphylls. The first shoot-born root pierced the cotyledon body at the base of the shoot. The hypocotyl and primary roots were absent. It had one or several cataphylls before the leaf appeared. Hypocotyl and primary roots were missing, then the first shoot-born roots broke through the body of the cotyledon at the base of the shoot (Figure 1) (Fadhilah et al., 2025; Tillich, 2014).

Abnormal seedlings showed rot symptoms due to primary infection. The internal condition of the ungerminated seed was assessed by lightly pushing with the thumb. Seeds were classified as dead when fungal growth was visible or when internal tissues leaked or appeared rotten (Figure 2).

Data Analysis

The effect of desiccation on seed moisture content and viability was determined by decreasing the level of moisture content and germination. Seed desiccation tolerance was determined by the lowest moisture content at which either the seed was preserved or lost its viability. Seeds were determined orthodox or intermediate when the germination rate was more than 50 % with 10-12 % moisture content. On the other hand, the seed was determined to be recalcitrant when the germination rate was less than 50 % with 10-12 % moisture content.

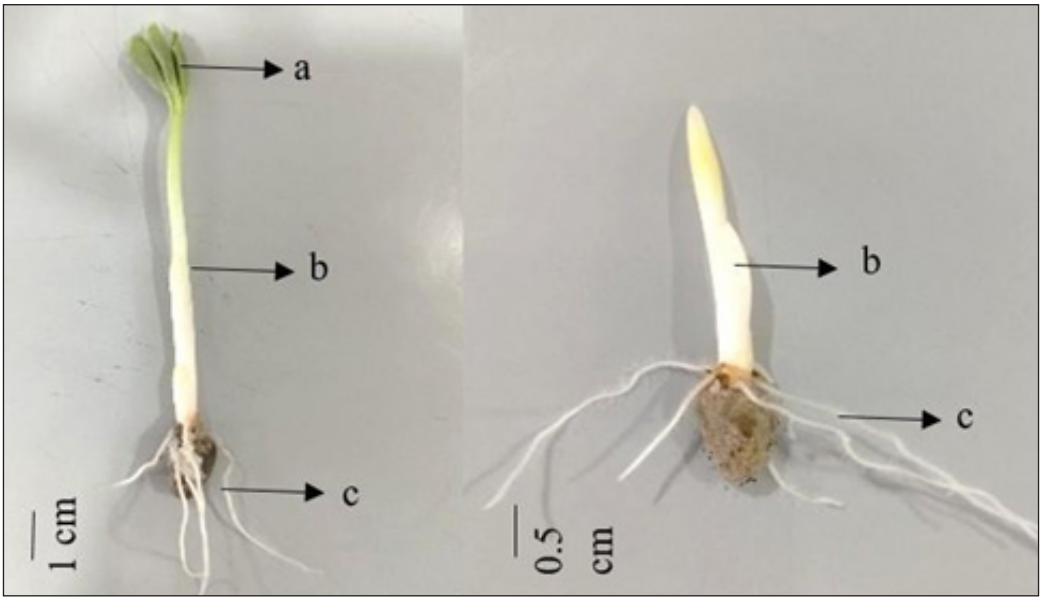

Figure 1. The normal seedling structures of *A. muelleri* (a) leaf; (b) cataphyll; (c) shoot-borne root

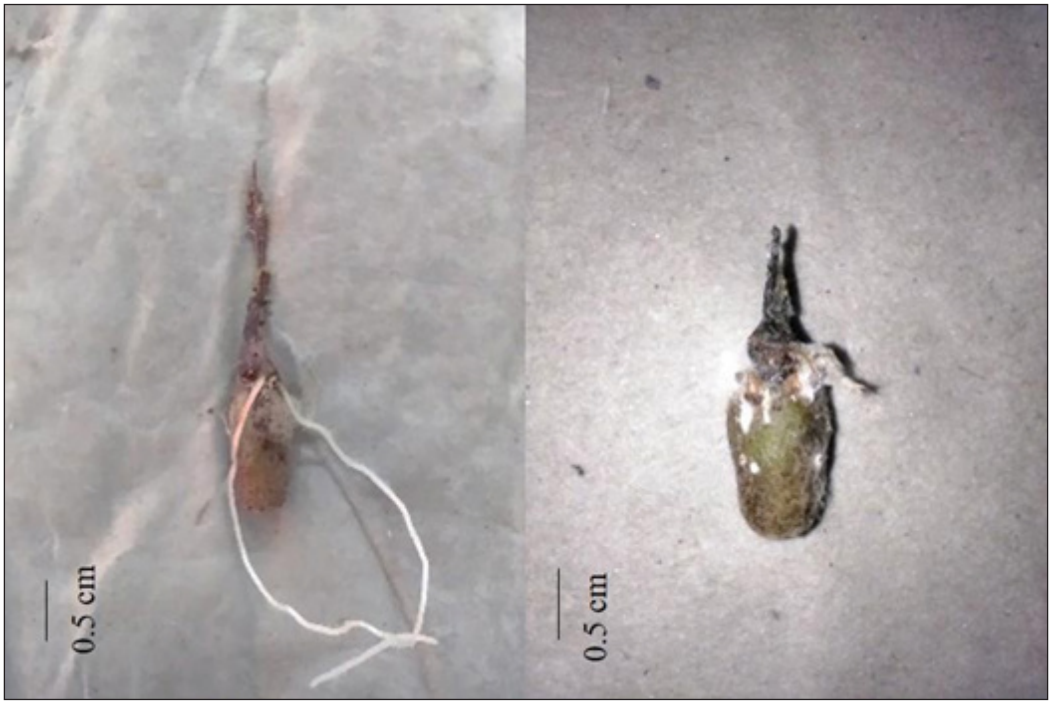

Figure 2. The abnormal seedling caused by primary infection

The optimum storage environment was determined by comparing the moisture content, germination rate, and T50 using ANOVA with a 5 % significance level. When the storage temperature had a significant effect, the analysis continued using DMRT with α=5 %.

RESULTS AND DISCUSSION

Experiment 1: Seed Storage Reserve Composition

Starch was identified as a major component of *A. muelleri* Blume with 51 % content, while protein was 20.76 %, and the lowest content was lipid (1.02 %). Corm of *A. muelleri* Blume is widely used for industrial purposes by extracting the glucomannan content. All part of this plant contains glucomannan, including the seed, which has 15.74 % content (Table 1).

Determination of seed compounds is important due to their role as a reserve source for germination and development. Carbohydrate composition consists of oligosaccharides, including fructans, and polysaccharides such as starch, glucan, and mannan (Aguirre et al., 2018). *A. muelleri* seed not only has starch as a major reserve compound but also glucomannan, which accounts for 15% of the seed reserve. Starch is usually the main polysaccharide stored in seeds, and glucomannan is also a natural macromolecular polysaccharide. The differences in the composition of seed reserves may relate to the germination characteristics. Experiments under stress conditions for Rapeseed cultivars with high oil content showed higher seed germination, vigour, and water uptake than low oil content cultivars (Batool et al., 2022). During seed imbibition, soluble sugars derived from starch are extensively used during the early stages of germination, while soluble proteins are increasingly mobilised as germination progresses. Considering protein contains more hydrophilic radicals than starch, seeds with starch as a significant molecule at the end stage of imbibition had a lower final percentage of water uptake than those in which protein is predominant (Zhao et al., 2018).

Glucomannan has a high viscosity, swelling property, solubility, a good film-forming property, and a good gel property in aqueous solutions (Sun et al*.,* 2023). It can be hydrolysed into monosaccharides using β- mannanase, which catalyses β-D-1,4-mannopyranosyl into mannobiose and mannotriose (Cui et al., 2019).

Table 1
Percentage of A. muelleri *seed reserve components in wet bases*

Seed Reserve Component	%
Starch	51.00
Lipid	1.02
Protein	20.76
Glucomanan	15.74

Experiment 2: Effect of Desiccation On Seed Moisture Content and Viability

Seed desiccation using different rates of drying methods, such as slow (22 °C 10% RH), moderate (35 °C 50% RH), and rapid desiccation (35 ^{O}C 40% RH with warm free airflow) had different effects on moisture content and viability. The duration of desiccation varied from 32 d (moderate drying at 35 °C) to 217 d (slow drying at 22 °C) until it reached a certain moisture content (40-50%; 30-40%; 20-30%). MDN2 and PWTA were dormant since initial germination was lower compared to seed germination after drying treatment.

Seed lots of MDN1, MDN2, and PWTA required 42 days of rapid desiccation method to reduce the seed moisture content from 68.4% to 30.9%, 67.4% to 21.3%, and 67.3% to 43.2 %, respectively. In contrast, seed moisture content only reduced slightly from 68.4% to 55.6%, 67.4% to 62.3%, and 67.3% to 59.8% in 32 days in the moderate desiccation method. Since the seed had germinated, the drying treatment was stopped.

The slow desiccation method required the longest period to decrease the moisture content. The seed moisture content of lots MDN1, MDN2, and PWTA only reduced by 6.2%, 4.8%, and 5.5%, respectively, during 54 days of desiccation. Seed moisture content could not decline to 10-12% using all desiccation methods. Low temperature and RH of slow desiccation inhibited seed germination. The low temperature and RH applied during slow desiccation suppressed seed germination, allowing continued desiccation to identify the lowest moisture content that the seed could tolerate while minimising germinative metabolism. All seed lots reached 38.7-43.8% moisture content after 217 days of slow desiccation treatment (Table 2).

Seed viability before and after desiccation was verified by germination testing. Initial germination of MDN1, MDN2, and PWTA were 73%, 49%, and 79%, respectively. Rapid desiccation treatment using warm free airflow at 35 °C with 40% RH had a lethal effect on the seed. After 42 days of treatment, the seed appeared rotten and mouldy in 10 days of germination testing. Moderate desiccation at 35 °C with 50% RH increased the germination of MDN1, MDN2, and PWTA lots to 87%, 59%, and 89%, respectively, compared to initial germination. Seed responses to slow desiccation differed among seed lots. Germination of MDN2 and PWTA increased to 82.7% and 91.3%, while MDN1 slightly decreased to 78% after 54 days of desiccation treatment. A longer period of rapid desiccation (217 d) reduced seed germination to 48% (MDN1), 59.3% (MDN2), and 68% (PWTA).

The experiment showed that slow desiccation at 22 °C with 10% RH would be suitable for the recalcitrant test for *A. muelleri*. Despite high seed moisture content, low temperature and RH during desiccation prevented seed germination. In contrast, moderate desiccation at 35 °C with 50% RH promoted seed germination after 32 days. These results are consistent with the behaviour of recalcitrant seed, which remains metabolically active and may initiate germinative processes during storage. For example, germination testing of recalcitrant *Trichilia emetica* seed in elevated temperatures up to 6 °C above ambient did

not damage metabolic and ultrastructural integrity in embryonic axes but instead accelerated germinative development and enhanced seedling growth rates (Shersen et al*.,* 2013).

Desiccation treatment affects the germination period. MDN1 and PWTA had shorter germination periods (60 d) after moderate desiccation for 32 d compared to slow desiccation treatment for 52 d (78 d). On the other hand, MDN2 had a shorter germination period (132 d) after 54 d slow desiccation treatment compared to moderate desiccation treatment. A longer period of slow desiccation treatment (217 d) led to a shorter germination period of 45 d (Table 2).

All seed lots preserve the viability (78-91.3%) when the moisture content remains above 50%. Seed germination decreased to 68%, 59.3%, and 48% when the seed moisture content was reduced to 43.8% (PWTA), 42.1% (MDN2), and 38.7% (MDN1), respectively. In addition, viability was lost when the moisture content reduced to 30.9% (MDN1), 21.3% (MDN2), and 43.2% (PWTA) (Table 2).

A different result of experiments was shown, even though both the rapid and moderate desiccation used the same temperature. The use of free airflow in rapid desiccation resulted in a fast loss of seed moisture content and a fall off of seed germination, while moderate desiccation maintained the seed germination. RH had no significant effect on seed viability. Although slow desiccation had the lowest RH, it preserved the seed viability.

A. muelleri seed showed high moisture content at harvest and required a prolonged period to achieve even modest moisture reduction. Seeds are sensitive to desiccation,

Table 2
Seed moisture contents (MC), germination rates and germination periods of A. muelleri *seed after different rates of desiccation*

Dessication Method	Seed Lot	Dessication Period (d)	Initial MC	Final MC	Germination (%)	Germination Period (d)
Slow desiccation at 22 °C 10% RH	MDN 1	54	68.4	62.2	78.0	78
	MDN2		67.4	62.6	82.7	78
	PWTA		67.3	61.8	91.3	78
Moderate desiccation at 35°C 50% RH	MDN 1	32	68.4	38.7	48.0	45
	MDN2		67.4	42.1	59.3	45
	PWTA		67.3	43.8	68.0	45
Rapid desiccation with warm free air flow at 35 °C 40% RH	MDN 1	217	68.4	55.6	87.0	60
	MDN2		67.4	62.3	59.0	132
	PWTA		67.3	59.8	89.0	60
	MDN1	42	68.4	30.9	0	10
	MDN2		67.4	21.3	0	10
	PWTA		67.3	43.2	3.0	10

showing rapid desiccation, but remain viable when the moisture content declines slowly. Viability was preserved when the seed had a minimum water content of 50%. Therefore, *A. muelleri* Blume seed is characterised as a recalcitrant seed.

Similar responses have been reported in previous studies. For example, recalcitrant *Quercus nigra* desiccation at 27 °C for 10 d was suitable, while desiccation at 40 ^{O}C had a detrimental effect (Bonner, 1996). *Embelia tsjeriam-cottam* seed, classified as intermediate and desiccation-sensitive, tolerated moisture content reduction by up to 14%. Sun-drying for three days led to the highest loss of seed germination from 92% to 23.75% during storage at 30 °C after 12 months (Chauhan et al., 2020). In contrast, exposure of recalcitrant seed of *Trichilia emetica* to elevated temperature (5-6 °C above ambient) did not affect germination but enhanced seedling growth rate (Sershen et al., 2013). The desiccation process leads to mechanical, physical membrane, and metabolism-induced damage. Despite high moisture content, seed deterioration occurs due to cell organelles' dysfunction and extensive vacuolization (Lah et al., 2023; Ranganathan & Groot, 2023). Storage of *Ligustrum perrotteiei* seed in the lowest RH (32.4%) and highest rate of desiccation (17.62%) decreased the viability from 100% to 50%, while increasing electrical conductivity from 1.90 dSm^{-1} to 2.67 dSm^{-1} (Aadhavan & Umarani, 2020). Therefore, recalcitrant seeds are sensitive to deterioration either under low or high moisture content.

Experiment 3: Effects of Storage Environment on Seed Germination

Three different environments were applied to determine the optimum temperature and RH for maintaining seed moisture content and viability. Seed moisture content was determined for each month (M0-M5) during seed storage. Initial seed moisture content for L1, L2, L3 were 60.2%, 62.9% and 60.2%, respectively. Each storage environment (S1, S2, and S3) had different effects on moisture content and viability. As the duration of storage increased, there was a rapid decline in moisture content under S1 storage after 3 months, while the decline was more gradual under S2 and S3 conditions (Figure 3).

Storage of S1 showed a significant decrease in seed moisture content to 16.1% (L1), 37.9% (L2), and 24.8% (L3) during 5 months. The moisture content remained above 48%, and germination remained above 80% when stored at S2 (Figure 3). Although seed moisture content remained high (above 46%) under S3 storage, germination sharply decreased after two months, and then viability was lost a month later. The decline in germination and moisture content was in line with S1 storage (Figure 3).

The different storage environments and periods significantly influenced seed germination and T_{50} for all seed lots (Table 3). Initial seed germination of L1, L2, and L3 were 80%, 93%, and 95%, respectively. All seed lots produced a similar pattern of germination and T_{50} result to each different storage environment. Seed germination of S1 storage was steady during three months, then decreased over two months to 50.7% (L1), 30% (L2), and 32% (L3).

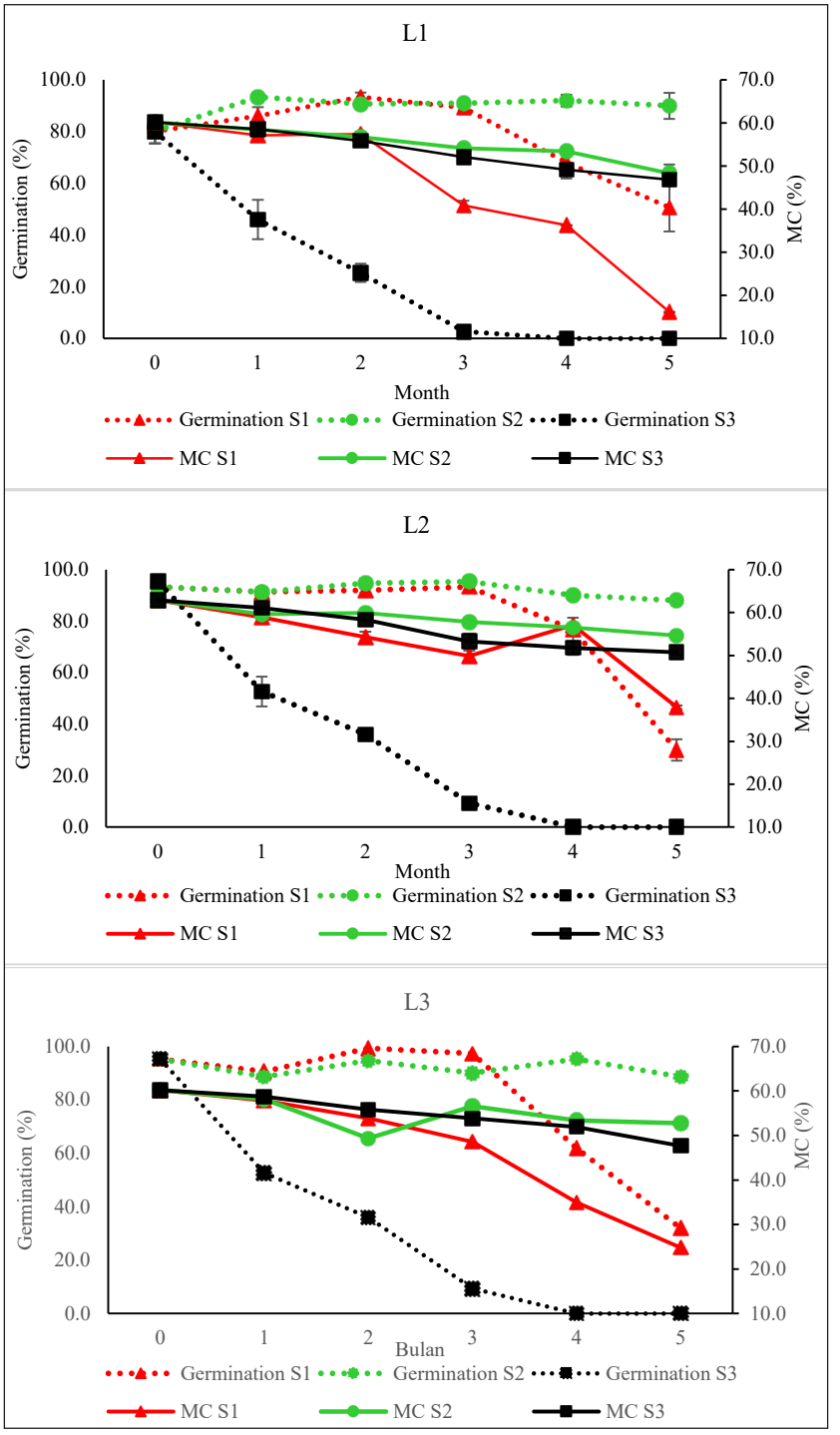

Figure 3. Seed moisture content and germination of lots L1, L2, L3 during five months of storage of S1 (27-32 °C, >60% RH); S2 (20-23 °C, 50-60% RH); S3 (5-7 °C, 40-50% RH)

Meanwhile, germination of S2 storage could preserve seed germination, and there was no significant difference during the 5 months. Germination shortly decreased after a month of storage of S3, then viability was lost after two months of storage (Table 4).

Initial T_{50} showed that L1, L2, and L3 required a long period to obtain a 50% germination rate, namely 92.7 d, 71.4 d, and 86.9 d, respectively. The experiment showed that T_{50} accelerated significantly in the storage of S1 and S2. The lowest T_{50} was reached when L1 (17.0 d), L2 (13.5 d), and L3 (11.8 d) seeds were stored after three months in S1, then increased up to five months. T_{50} reduced steadily during five months of storage in S2 (Table 5).

Table 3
Recapitulation of variance analysis for the effect of seed storage period treatment nested at the storage environment on seed germination and T_{50}

Seed Lot	Source	Germination	T_{50}
L1	S	<0.0001**	<0.0001**
	M(S)	<0.0001**	<0.0001**
	CV	11.9659	12.49667
L2	S	<0.0001**	<0.0001**
	M(S)	<0.0001**	<0.0001**
	CV	6.404195	12.33349
L3	S	<0.0001**	<0.0001**
	M(S)	<0.0001**	<0.0001**
	CV	6.974593	13.80885

Note. S=Storage; M= Storage periods; L= seed lot; CV= coefficient variance; **= values were significantly different according to the anova at $\alpha<0.05$

Table 4
The effect of seed lot and storage environment on seed germination

Seed Lot	Seed Environment	Storage Period (Month)					
		0	1	2	3	4	5
L1	S1 (27-32 °C, >60% RH)	80.0^{ab}	86.0^{ab}	93.3^{a}	89.3^{a}	68.0^{b}	50.7^{c}
	S2 (20-23 °C, 50-60% RH)	80.0^{ab}	93.3^{a}	90.7^{a}	90.7^{ab}	92.0^{a}	90.0^{a}
	S3 (5-7 °C, 40-50% RH)	80.0^{ab}	46.0^{c}	25.3^{d}	2.7^{e}	0.0^{e}	0.0^{e}
L2	S1 (27-32 °C, >60% RH)	93.3^{a}	91.3^{a}	92.0^{a}	93.3^{a}	76.7^{b}	30.0^{d}
	S2 (20-23 °C, 50-60% RH)	93.3^{a}	91.3^{a}	94,7^{a}	95.3^{a}	90.0^{a}	88.0^{a}
	S3 (5-7 °C, 40-50% RH)	93.3^{a}	56.7^{c}	25.3^{d}	0.0^{e}	0.0^{e}	0.0^{e}
L3	S1 (27-32 °C, >60% RH)	95.3^{ab}	90.7^{ab}	99.3^{a}	97.3^{a}	62.0^{c}	32.0^{e}
	S2 (20-23 °C, 50-60% RH)	95.3^{ab}	88.7^{b}	94.7^{ab}	90.0^{ab}	95.3^{ab}	88.7^{b}
	S3 (5-7 °C, 40-50% RH)	95.3^{ab}	52.7^{d}	36.0^{e}	9.3^{f}	0.0^{g}	0.0^{g}

Note. The numbers in the table for each lot, followed by the same letters, show no significant difference at the 5% level DMRT test, respectively

Table 5
The effect of the seed lot and storage environment on T50

Seed Lot	Seed Environment	Storage Period (Month)					
		0	1	2	3	4	5
L1	27-32 °C (>60 % RH)	92.7^{a}	41.4^{e}	20.7^{gh}	17.0^{h}	19.5^{h}	31.6^{f}
	20-23 °C (50-60 % RH)	92.7^{a}	69.1^{c}	41.8^{e}	23.1^{fgh}	22.8^{fgh}	25.6^{fgh}
	5-7 °C (40-50 % RH)	92.7^{a}	53.3^{d}	81.7^{b}	31.0^{gf}	0.0^{i}	0.0^{i}
L2	27-32 °C (±40 % RH)	71.4^{b}	58.2^{c}	17.0^{e}	13.5^{e}	13.8^{e}	37.6^{d}
	20-23 °C (50-60 % RH)	71.4^{b}	71.3^{b}	54.0^{c}	32.8^{d}	51.7^{c}	30.0^{d}
	5-7 °C (40-50 % RH)	71.4^{b}	85.5^{a}	80.2^{ab}	0.0^{f}	0.0^{f}	0.0^{f}
L3	27-32 °C (±40 % RH)	86.9^{a}	32.9^{ef}	14.8^{hi}	11.8^{i}	27.0^{fg}	41.3^{de}
	20-23 °C (50-60 % RH)	86.9^{a}	54.7^{c}	44.5^{d}	30.5^{fg}	27.2^{fg}	22.6^{gh}
	5-7 °C (40-50 % RH)	86.9^{a}	80.0^{a}	66.6^{b}	31.0^{fg}	0.0^{j}	0^{j}

Note. The numbers in the table for each lot, followed by the same letters, show no significant difference at the 5% level DMRT test, respectively

The reduction is consistent with declining moisture content, showing in ambient and controlled storage. This is because embryo development occurred during storage, resulting in faster germination. As a recalcitrant species, *A. muelleri* seeds retain high moisture content at harvest, allowing metabolic activity to continue during storage and contributing to changes in germination behaviour over time.

Different environments of storage affected seed physical appearance. Ambient temperature (S1) allowed seed active metabolism, which resulted in germination. The unavailability of water prevented seedling development. The seed appeared wrinkled and lost the ability to produce normal seedlings. Although the seed in S3 appeared firm, viability was lost after three months due to unbearably low temperatures. Low temperature and high moisture content might lead to seed chilling injury. Storage of S2 was able to suppress seed germination and maintain viability for five months (Figure 4).

This experiment proved that the storage environment is closely related to seed viability. The higher temperature of S1 sped up germination and T50, but only lasted three months. Seed germination started to decline, and T50 increased in the fourth month of storage in S1. Storage of S2 was identified as the most optimum to maintain viability since seed germination was steady and T50 declined in five months. Seed stored under S3 conditions could not maintain viability, showing that low-temperature storage is unsuitable for this species.

Based on the results, temperature affects seed viability and longevity during storage. Recalcitrant seed with high moisture content had an active metabolism of cells. This implies that seed storage at higher temperatures increases several metabolic processes, causing faster deterioration. On the other hand, low storage temperatures with high moisture content also caused a lethal effect. Seed did not tolerate temperatures below 7 °C, while controlled

Figure 4. Seed appearance after a month (A) and five months (B) storage of S1 (27-32°C, >60% RH); S2 (20-23 °C, 50-60% RH); S3 (5-7 °C, 40-50% RH)

storage at 20-23 °C provided the optimum storage condition since it could decrease seed moisture content slightly and preserve viability.

A. muelleri Blume can be stored either up to five months under controlled storage (20-23 °C) or three months under ambient storage (≥27 °C). Other recalcitrant seed such as *Morinda citrifolia,* had short-term storage (2-6 weeks) in temperature of 20-25 °C (Ajongbolo et al. 2025). The germination of *Anonna sylvatica* seed decreased during three-month storage in a cold chamber (16 °C and 40 % RH) (Silva et al. 2024). Seeds with high moisture content (≥ 60 %) need oxygen to maintain metabolism. Therefore, a paper bag container is suitable for seed storage, since it allows gas exchange and a good water vapour transmission rate.

An optimum environment for seed storage is required to maintain quality. Temperature is a crucial factor in preventing seed deterioration. Viability is not only affected by storage temperature but also by seed moisture content. Seed age during storage leads to a decrease in viability when there are no suitable conditions. The metabolic system starts to break down when the seed deteriorates, resulting in slow or failed germination and poor seedling development. Therefore, optimum seed storage slows down the seed metabolism without incurring damage.

Temperature and moisture content are the main factors that affect seed deterioration and viability loss. The viability of recalcitrant seed can be maintained when moisture content decreases slightly below full imbibition but remains above levels that induce chilling injury (Vitis et al., 2020). High humidity and temperature during storage are conducive to the immediate germination of recalcitrant seed. Therefore, optimum temperature and humidity are needed during seed storage to suppress the rate of seed metabolism and respiration, thereby delaying seed germination.

CONCLUSION

In conclusion, *A. muelleri* Blume was found to have high starch seed (51%) with 15.74% glucomannan content. The viability of seed can be maintained at 40-50% moisture content using a slow-degradation method (silica gel at 22 °C and 10% RH). Seed with a moisture content of around 60% may be stored at controlled temperatures (20-23 °C, 50-60% RH) for five months and for three months at embient temperature (26-32 °C, RH >60%). However, the seed cannot withstand storage at low temperatures below 7 °C. These characteristics confirm that the seeds are recalcitrant.

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