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Survival of Native Indonesian Orchid Seeds in Conventional Seed Banking and Its Relationship with Micro-Morphometry

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ABSTRACT: Maintaining orchid seed viability during conventional seed bank storage is essential for ex situ conservation, yet it remains unclear whether seed micro-morphometric characteristics can predict seed longevity. This study evaluated the relationship between seed micro-morphometry and storage longevity in 13 Indonesian orchid species stored at −20 °C for up to nine years. Seed area (SA), embryo area (EA), and seed air space (SAS) were measured from 30 seeds per species using light microscopy and image analysis. Germination tests were conducted periodically throughout storage using asymbiotic culture media, and the data were analyzed using one-way ANOVA, Kruskal–Wallis tests, principal component analysis (PCA), and Spearman's correlation. Significant interspecific differences were observed in all micro-morphometric traits. Dendrobium antennatum had the largest seed and embryo areas, whereas Phalaenopsis amabilis had the smallest. Phaius tankervilleae exhibited the highest SAS, while Vanda tricolor had the lowest. PCA explained 87.8% of the total variation and separated the 13 species into three distinct groups according to their seed morphology. Seed area showed strong positive correlations with both embryo area ($r = 0.885$) and SAS ($r = 0.861$), indicating coordinated allocation of seed structural components. However, storage duration was only weakly correlated with SA, EA, and SAS, suggesting that seed size and internal morphology alone are poor predictors of longevity. The longest viability was recorded in Vandopsis lissochiloides, which retained 98.2% germination after nine years of storage, whereas D. lineale lost viability within one year. These findings demonstrate that orchid seed longevity under conventional storage is species-specific and cannot be reliably predicted solely from seed micro-morphometry. Additional physiological and biochemical factors should be investigated to improve predictions of seed longevity and optimize ex situ conservation strategies for orchids.

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1. INTRODUCTION

Orchidaceae is one of the world's largest plant families, with over 27,800 species across 880 genera found on all continents except Antarctica [1]. Indonesia has 5,000 species, but only a small fraction has been developed [2]. The Bogor Botanical Garden, as an ex-situ conservation site, houses a curated collection of approximately 500 orchid species in the Orchid House [3].

Orchid species such as *Arundina graminifolia*, *Bulbophyllum* spp., *Dendrobium* spp., *Phaius tankervilleae*, and *Vanda* spp. have been used as medicinal plants for along time due to their diverse bioactive compunds [4-7]. Many orchids, especially *Phalaenopsis* spp., are highly prized as ornamental plants because of their visually appealing flowers [6]. The growing economic and ecological significance of orchids underscores the necessity for efficient conservation methods. In situ conservation, which preserves species within their natural habitats and remains the most effective approach for maintaining orchid populations and their ecological interactions, is complemented by ex situ conservation. Seed banks stands out as a practical and cost-effective ex situ approach for orchid conservation, enabling the long-term preservation of genetic diversity and ensuring future access to germplasm for restoration, research, and other conservation efforts [8].

Orchid seeds, which are typically stored at -20°C in accordance with conventional seed banking protocols, suffer a decline in viability for some species under these conditions [9]. Storage at room temperature also leads to rapid loss of viability, indicating that orchid seeds require carefully controlled storage conditions in order to maintain their longevity [10]. Fresh orchid seeds generally have a short lifespan and are therefore particularly dependent on suitable storage strategies [11]. One of the major challenges in orchid seed conservation is their extremely small size (0.15-6 mm), which has earned them the designation “dust seeds” and complicates the determination of seed moisture content and volume [12].

Orchid seeds have distinct morphological and anatomical features that differentiate them from the majority of flowering plant seeds. A seed is formed of a minute embryo which is encased within a thin testa and is encompassed by an internal air cavity known as the seed air space (SAS) [13]. Research has shown significant variation in orchid species regarding seed size, embryo size, testa shape, and the ratio of air space [14, 15]. Seed coat characteristics, such as color, shape, surface texture, and reticulation, as well as embryo color and size, vary systematically among different genera [14, 16]. The structural differences of orchid species are closely linked to their ecological adaptations and conservation biology [14, 17].

The morphology of seeds has also been associated with their germination performance. Species with relatively larger embryos tend to have a greater potential for ex vitro germination, with the ratio between the seed's air space and embryo volume being a key factor in determining germination success [18]. Little is known about whether these morphological traits also affect seed longevity during storage. We therefore hypothesised that interspecific variation in seed size, embryo size, and the proportion of seed air space affects the retention of orchid seed viability during storage.

Seed longevity during storage is primarily determined by seed storage behaviour, which is typically categorised into two groups: orthodox and recalcitrant. Recalcitrant seeds are sensitive to drying and rapidly lose viability during storage, in contrast to orthodox seeds which can tolerate desiccation and maintain viability under dry, low-temperature storage conditions [19]. The majority of orchid species studied so far display orthodox seed behaviour, but their durability differs significantly among different species. Magrini et al. [11] found that seeds of four *Anacamptis* species remained highly viable after being stored for four years, with three species showing germination rates above 90%, indicating robust desiccation tolerance and typical orthodox characteristics. Despite their orthodox nature, orchid seed germination typically decreases with prolonged storage periods. Seeds that have recently been harvested tend to have higher germination rates than seeds that have been stored [20], and this reduction in germination has been attributed, at least in part, to the induction of secondary dormancy during storage [21].

Seed longevity refers to the period of time a seed remains viable and capable of germination. Longevity is a complex trait displaying considerable variation among plant species and even among different seed lots of the same species [22]. The variation is also evident in orchids. Seeds of *Papilionanthe hookeriana* are viable for a lifespan of less than three years [23]. In contrast, seeds of *Paphiopedilum supardii* have a storage life of roughly one year [24]. In contrast, seeds of *Dendrobium macrophyllum* A. Rich. and *D. discolor* Lindl., the viability can be retained for more than ten years when the samples are stored at a temperature of -20°C [25]. Significant interspecific differences in seed longevity have been observed among Indonesian orchids.

The factors underlying the variation in orchid seed longevity are not yet fully understood despite documented differences in this area. No previous study has systematically investigated whether seed micromorphometric characteristics, including seed size, embryo size, or seed air space, are linked to storage longevity. Filling this knowledge gap may provide valuable insights into the mechanisms governing seed persistence and improve strategies for orchid germplasm conservation.

This study sought to categorise the storage longevity of seeds from 13 Indonesian orchids species, representing 7 genera, into 4 storage duration categories: short, medium I, medium II, and long, based on previously established longevity data. We also examined the connection between seed micromorphometric traits and storage longevity to determine structural features that may cause variations in seed lifespan among orchid species.

2. MATERIALS AND METHODS

2.1 Study Material

The 13 orchid species included in this study were *Arundina graminifolia*, *Bulbophyllum graveolens* (F. M. Bailey) J.J.Sm., and *Dendrobium aphyllum* (Roxb.). C.E.C.Fisch, *D. lineale*, *D. mirbelianum* Gaudich., *D. crumenatum* Sw., *D. anosmum* Lindl., *D. antennatum* Lindl., *Phalaenopsis tankervilleae*, *P. amabilis*, *P. cornu-cervi* (Breda), Blume & Rchb.f., *Vanda tricolor*, and *Vandopsis lissochiloides*.

2.2 Sample Collection

Materials used in this study were sourced from routine orchid conservation and research activities undertaken between 2008 and 2024 at the Tissue Culture Laboratory of the National Research and Innovation Agency (BRIN), Indonesia. Seeds of 13 Indonesian orchid species were collected from the living orchid collection at the Bogor Botanic Gardens, maintained by BRIN. Capsules were collected once they had reached full maturity, marked by complete ripening and, in certain cases, the beginning of natural capsule opening.

The orchid capsules were produced either through controlled hand pollination or natural insect pollination within the Orchid Glass House at the Bogor Botanic Gardens. Seeds were aseptically extracted from the capsules upon receipt at the Tissue Culture Laboratory and were then either stored in a conventional seed bank at -20°C as part of the ex situ conservation program or used for propagation and conservation purposes. All observations and measurements were recorded systematically using standardized protocols, despite the capsules being collected at different times over the study period. In most cases, only a single fruit was available for analysis due to the fact that many orchid species produce a limited number of mature capsules.

2.3 Seed Storage at -20 °C Procedure

Mature orchid capsules were carefully opened using a sterile spatula, and any adhering fibrous tissue was then removed, followed by the aseptic extraction of the seeds. Cleaned seeds were evenly distributed in sterile Petri dishes and conditioned in a desiccator for five days at approximately 50% relative humidity to decrease their moisture content before storage.

The seeds were then placed in 2-mL cryogenic vials, which were sealed inside 300-mL glass jars containing orange silica gel sachets to act as a desiccant. The sealed jars were then stored in a conventional seed bank at a temperature of -20 °C 20. Monitoring of relative humidity within each storage jar was carried out using a digital hygrometer (Tinytag View 2, UK), with changes in silica gel colour also serving as an indicator of moisture conditions [26]. The silica gel was replaced whenever its colour changed from orange to green, indicating moisture saturation. The freezer temperature was continuously checked using a thermometer to guarantee consistent storage conditions.

The extremely small size of orchid seeds meant that seed moisture content could not be measured directly by gravimetric methods, so it was estimated indirectly from the equilibrium relative humidity within the sealed storage system. This indirect method of orchid seed conservation is commonly employed, but slight variations in moisture content may arise due to differences in silica gel efficiency and the airtightness of storage containers. The viability of seeds was evaluated through in vitro germination tests, which were carried out in triplicate using both newly harvested seeds and seeds stored for the specified period [26].

2.4 Seed Micro-morphometry Measurement

Micromorphometric measurements were carried out on 30 seeds chosen at random from a single mature capsule (one seed lot) for each species, owing to the limited availability of orchid material. Seeds with a visible embryo were the only ones included in the analysis. The seeds were first surface-sterilised in a 5% Chlorox solution for 5 minutes, then rinsed with sterile distilled water according to the protocol established by Aprilianti et al. [27].

The seeds from each species were then observed and photographed using an Olympus BX53 light microscope at either 10x or 40x magnification, depending on their size. The CellSens software, integrated with the microscope, was used to perform seed micromorphometric analysis, which includes line and area measurement functions (Fig. 1). Orchid seeds, being three-dimensional structures that are challenging to measure accurately in terms of volume, were represented by their two-dimensional projected areas, namely seed area and embryo area, which acted as proxies for their relative dimensions.

The percentage of seed air space (SAS) and the embryo-to-seed area ratio were calculated using the equations listed below.

$$\text{SAS (\%)} = \frac{\text{Seed area-embryo area}}{\text{Seed area}} \times 100\%$$

$$\text{Embrio/seed ratio (\%)} = \frac{\text{Embryo area}}{\text{Seed Area}} \times 100\%$$

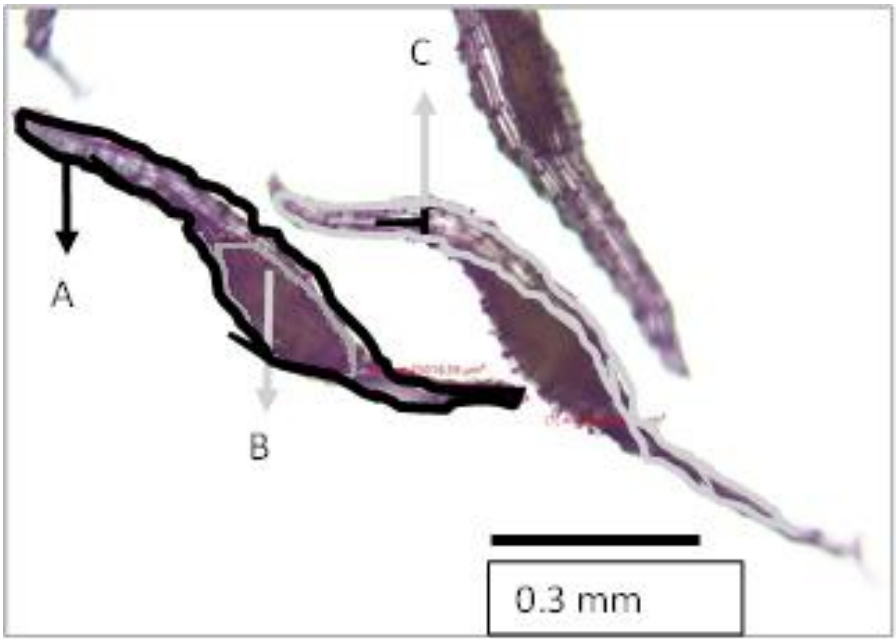

Fig. 1. The measurement of micro-morphometry of a representative orchid (*Arundina graminifolia*): (A) Seed Area, (B) Embryo Area, and (C) Seed Air Space.

2.5 Seed Germination Test

The viability of seeds was assessed through in vitro germination at the start of storage (0 months), at regular intervals over the first 18 months, and then after 2, 3, 5, 6, 7, 8, and 9 years of storage, until all seeds from a single lot had been used up. The initial germination test was conducted using three different culture media to identify the most suitable germination medium for each species. Subsequent viability assessments for that species were then performed using only the identified optimum medium.

The three germination media examined were: (1) modified Knudson C medium supplemented with 150 g L-1 of bean sprout extract, 150 mL L-1 of coconut water, and 1 g L-1 of activated charcoal; (2) modified Vacin and Went medium supplemented with 100 g L-1 of bean sprout extract, 100 g L-1 of tomato extract, 150 mL L-1 of coconut water, and 1 g L-1 of activated

charcoal; and (3) modified Hyponex medium containing Hyponex fertilizer (NPK 25–5–20) as the basal nutrient source, supplemented with 2 g L-1 of peptone, 40 g L-1 of potato extract, and 1 g L-1 of activated charcoal. The media contained 20 g L-1 sucrose and 7 g L-1 agar as the gelling agent. The medium's pH was adjusted to 5.6 prior to sterilisation by autoclaving at 121°C for 25 minutes.

Cryopreserved seeds were first removed from storage at -20°C, then allowed to reach room temperature (20-25 °C) while still sealed in their containers to prevent moisture condensation. All subsequent procedures were carried out aseptically within a laminar airflow cabinet (LAFC). Seeds were first immersed in a sterile distilled water containing 3 drops of Tween 80 and then vacuum-infiltrated for 1 hour using a MEDI-PUMP Model 1132 RV vacuum pump. The surface was then sterilised through immersion in a 10% sodium hypochlorite solution (0.525% NaOCl) for 10 minutes, and then treated with a 5% sodium hypochlorite solution (0.2625% NaOCl) for a further 5 minutes. Seeds were subsequently rinsed three times with sterile distilled water, as per Puspitaningtyas et al. [26], before being evenly distributed onto the culture media using a sterile glass pipette. Seeds of each orchid species were sown on three replicate Petri dishes per culture medium.

The cultures were incubated at 25±2°C under a 12-h light/12-h dark photoperiod with a light intensity of 700-1200 lux.

2.6 Observation Procedure

The developmental stages of the embryo following sowing were used to evaluate seed germination. Successful germination occurred when the embryo ruptured the testa, enlarged and formed a protocorm-like body (PLB), then initiated rhizoids and shoot development, generally taking place within one to two months after sowing [26]. Embryos that did not progress through these developmental stages were classified as ungerminated.

The cultures were monitored at intervals of daily to weekly frequency, depending on the stage of development, until no further germination or seedling development was observed. At least 100–200 seeds per Petri dish were examined under a stereomicroscope (Fig. 2), with the numbers of ungerminated, swollen, and germinated seeds being recorded.

Due to the extremely small size of orchid seeds and their production in large quantities, direct counting of every seed in a Petri dish was impractical. The total number of seeds was estimated by extrapolating the average seed count from several representative microscopic fields of view to the entire culture area. The germination percentage was subsequently calculated using the estimated total number of seeds.

Each treatment comprised three separate replicates, with one Petri dish serving as a single experimental unit. Throughout the incubation period, all Petri dishes were sealed with plastic wrap to maintain aseptic conditions and minimise contamination [26].

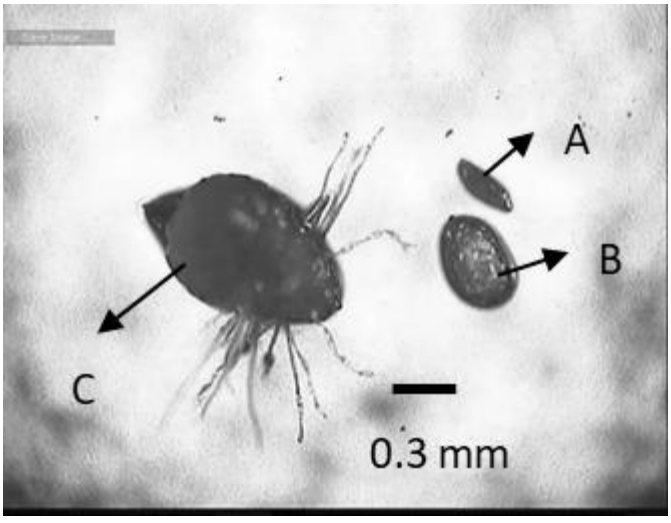

Fig. 2. Variation in seed development of the representative orchid (*Phalaenopsis amabilis*) in KC medium. (A) Ungerminated seed; (B) Swollen seed; (C) Protocorm/germinated seed

Seed germination was counted as the percentage of protocorms developed by using the formula as follows:

$$\text{Germination (\%)} = \frac{\text{Number of germination seeds}}{\text{Total number of seeds observed}} \times 100\%$$

The 13 Indonesian orchid species examined in this study exhibited considerable variation in seed longevity, ranging from less than 1 year to more than 9 years under conventional storage conditions. According to Gupta [28], seed storage can generally be classified into short-term (<1 year) and long-term (>1 year) categories. However, because the present study encompassed a broad range of storage durations, a more detailed classification was developed to facilitate comparisons among species and to investigate the relationship between seed micromorphometric characteristics and longevity. Accordingly, seed longevity was categorized into four classes: Class 1 (short-term, 0-1 year), Class 2 (medium-term I, >1-3 years), Class 3 (medium-term II, >3-6 years), and Class 4 (long-term, >6-9 years).

2.5 Statistical Analysis

Seed micromorphometric measurements were obtained from 30 seeds per species, all originating from a single mature capsule (seed lot). Prior to statistical analysis, the normality of each variable was evaluated using the Shapiro–Wilk test. Seed area (SA) satisfied the assumption of normality and was analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test at a significance level of $P < 0.05$ to compare differences among species. Because only one mature capsule was available for each species, the 30 measured seeds represented subsamples from a single seed lot rather than independent biological replicates. Consequently, a nested ANOVA design could not be applied.

In contrast, embryo area (EA), seed air space (SAS), and the embryo-to-seed area ratio did not satisfy the assumption of normality. These variables were therefore analyzed using the non-parametric Kruskal–Wallis test, followed by Dunn's multiple comparison test to identify significant pairwise differences among species.

To explore relationships among seed micromorphometric variables and seed longevity classes, Principal Component Analysis (PCA) was performed [29]. The suitability of the dataset for PCA was assessed using the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity. The overall KMO value was 0.72, indicating good sampling adequacy for PCA, while Bartlett's test was highly significant ($\chi^2 = 11.21$, $df = 3$, $P < 0.01$), confirming that the correlation matrix was appropriate for multivariate analysis.

Furthermore, the relationships among storage period, seed area, embryo area, seed air space, embryo-to-seed area ratio, and initial germination percentage were examined using Spearman's rank correlation (Spearman's rho). A non-parametric correlation analysis was conducted as several variables failed to meet the normal distribution requirement, thus precluding the use of Pearson's correlation [30]. The strength and direction of the relationships among the measured variables were evaluated using correlation coefficients (r_s) and their corresponding P-values.

3. RESULTS AND DISCUSSION

3.1 Seed Morphology

The seed morphology of 13 examined orchid species is shown in Fig. 3. Observations using a light microscope showed significant differences in seed shape among species, especially in testa size, embryo size, and the space within the seed (SAS). The embryo, visible as the dark central structure enclosed by the testa, showed considerable variation in size relative to the surrounding air space among different species. Seed shape varied from elongated seeds with large seed attachment surfaces to shorter, more compact seeds with relatively small seed attachment surfaces. The morphological differences among the studied orchid species suggest considerable diversity in seed micromorphometric characteristics, which may influence their storage behaviour and longevity.

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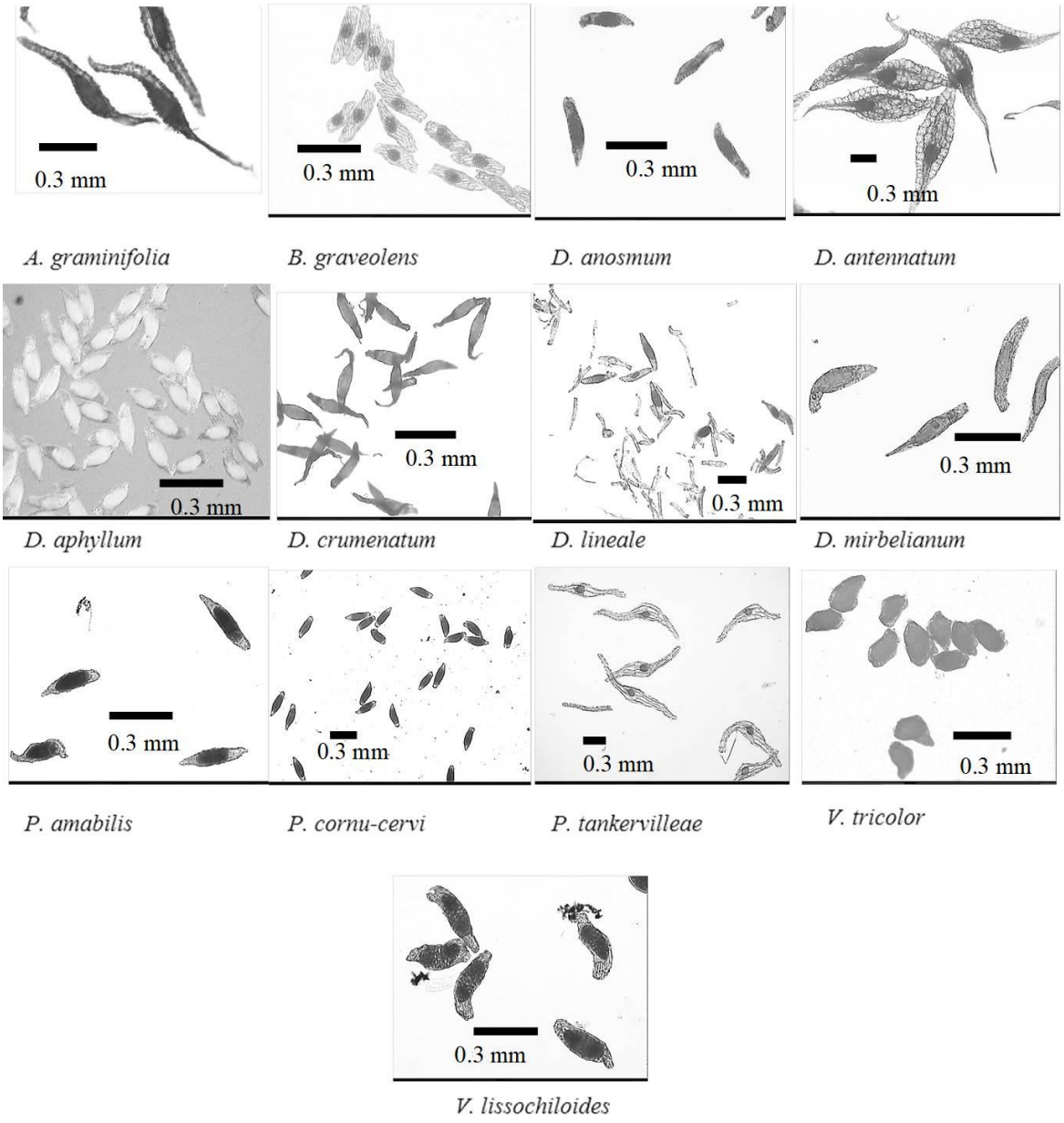

Fig. 3. Seed micro-morphometry diversity among 13 indigenous orchid species in Indonesia.

3.2 Seed Micro-morphometry Measurement

The morphometric analysis showed considerable differences in seed characteristics among the 13 orchid species. A one-way ANOVA revealed a statistically significant difference in seed area among species at a P-value less than 0.05. The species were classified into seven statistically distinct groups based on Tukey's HSD test at an alpha level of 0.05. D. antennatum showed the largest mean seed area at 415,071.72 μm^2, far surpassing that of all other species, whereas P. amabilis had the smallest mean seed area at 5,042.74 μm^2 (Tab. 1).

Similarly, the Kruskal–Wallis test, followed by Dunn's post hoc test, showed significant interspecific differences ($P < 0.05$) in embryo area, seed air space (SAS), and embryo-to-seed area ratio. The species *D. antennatum* had the largest embryo area at 375.5 μm^2, whereas *P. amabilis* had the smallest at 36.08 μm^2. *P. tankervilleae* exhibited the highest SAS at 372.18%, whereas *V. tricolor* displayed the lowest SAS at 45.07%. *V. tricolor* had the highest embryo-to-seed area ratio at 345.93%, whereas *P. tankervilleae* had the lowest at 18.8% (Tab. 1).

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Table 1. The seed area, embryo area, and seed air space of 13 indigenous Indonesian orchid species (with a total sample of each species =30).

No.	Species	Mean (ANOVA) Seeds area µm²	Mean Rank (Kruskal-Wallis H)		
			Embryo area µm²	SAS percentage with area (%)	Embryo/seed ratio (%)
1.	*P. amabilis*	**5,042.74±645^{a}**	**36.08^{a}**	119.38^{c}	271.63^{h}
2.	*V. tricolor*	5,804.59±602^{a}	91.75^{b}	**45.07^{a}**	**345.93^{j}**
3.	*V. lissochiloides*	6,666.22±719^{a}	111.07^{bc}	81.73^{b}	309.30^{i}
4.	*P. cornu-cervi*	7,294.23±643^{a}	121.57^{bc}	106.38^{bc}	284.57^{hi}
5.	*D. aphyllum*	8,389.22±1318^{ab}	150.57^{cd}	104.28^{bc}	286.70^{hi}
6.	*B. graveolens*	12,315.22±1745^{abc}	41.10^{a}	291.35^{gh}	99.63^{c}
7.	*D. crumenatum*	12,781.94±1280^{abc}	206.10^{de}	147.23^{d}	243.77^{g}
8.	*D. lineale*	19,603.30±3611^{bcd}	261.63^{e}	176.17^{de}	214.87^{f}
9.	*D. anosmum*	20,427.89±1972^{cd}	249.90^{e}	234.98^{f}	156.00^{de}
10.	*D. mirbelianum*	26,125.89±3001^{d}	307.77^{f}	208.75^{e}	182.27^{e}
11.	*A. graminifolia*	80,132.98±12815^{e}	344.57^{g}	291.35^{g}	99.63^{cd}
12	*P. tankervilleae*	120,518.49±13708^{f}	243.90^{e}	**372.18^{i}**	**18.80^{a}**
13.	*D. antennatum*	**415,071.72±45,933^{g}**	**375.50^{h}**	347.10^{h}	43.90^{b}

Note: Data of seed area were analyzed using One-Way ANOVA followed by Tukey's HSD post hoc test at $\alpha = 0.05$. Data of embryo area, SAS percentage, and embryo/seed ratio (%) were analyzed using the Kruskal-Wallis H test followed by Dunn's post hoc test with Bonferroni correction at $\alpha = 0.05$. Values followed by different letters within a column are significantly different. Total n = 390.

The principal component analysis (PCA) was conducted using seed area (SA), embryo area (EA), and seed air space (SAS). The embryo-to-seed area ratio was excluded from analysis because it is mathematically derived from the relationship between embryo area and seed area, making it highly collinear with SAS and potentially introducing redundancy into the multivariate analysis.

The PCA biplot showed distinct relationships among the three micromorphometric variables (Fig. 4a). A positive correlation was observed between seed area and SAS, as evidenced by the close alignment of their vectors, indicating that larger seeds typically have more internal air space. In contrast, the embryo area was oriented in a different direction, suggesting that embryo size was independent of overall seed size and SAS. The pattern indicates that embryo development and seed air space constitute separate features of orchid seed morphology.

The first two principal components accounted for 87.8% of the total variation, with PC1 explaining 74.2% and PC2 explaining 13.6% (Fig. 4). The high cumulative variance suggests that these three variables provide a sufficient summary of the micromorphometric variation among the 13 orchid species.

The PCA scores were used to separate the species into three distinct groups through cluster analysis (Fig. 4b). Cluster 1 consisted of seven orchid species: *P. amabilis*, *P. cornu-cervi*, *V. tricolor*, *V. lissochiloides*, *D. aphyllum*, *D. crumenatum*, and *B. graveolens*, which were characterised by relatively small seed, embryo, and SAS areas. Cluster 2 comprised solely *D. antennatum*, which displayed exceptionally large values for seed and embryo size, thereby distinguishing it from all other species. *D. antennatum*'s distinct position suggests that it has a notably different seed morphology compared to the other *Dendrobium*

species studied. The cluster 3 comprised *D. lineale*, *D. anosmum*, *D. mirbelianum*, *A. graminifolia*, and *P. tankervilleae*, which exhibited intermediate values for the measured micromorphometric characteristics.

The distinct separation among the three clusters indicates that orchid seed morphology differs significantly among genera and species, reflecting diverse reproductive and ecological strategies. Species with small seeds and limited embryo size may be well-suited for efficient long-distance wind dispersal, whereas species with larger seeds and embryos may devote more resources to embryo development, potentially enhancing seedling establishment after dispersal. The unique position of *D. antennatum* underscores the considerable morphological diversity within a single genus, implying that seed micromorphometry could offer significant traits for elucidating variation in orchid seed longevity and storage behaviour.

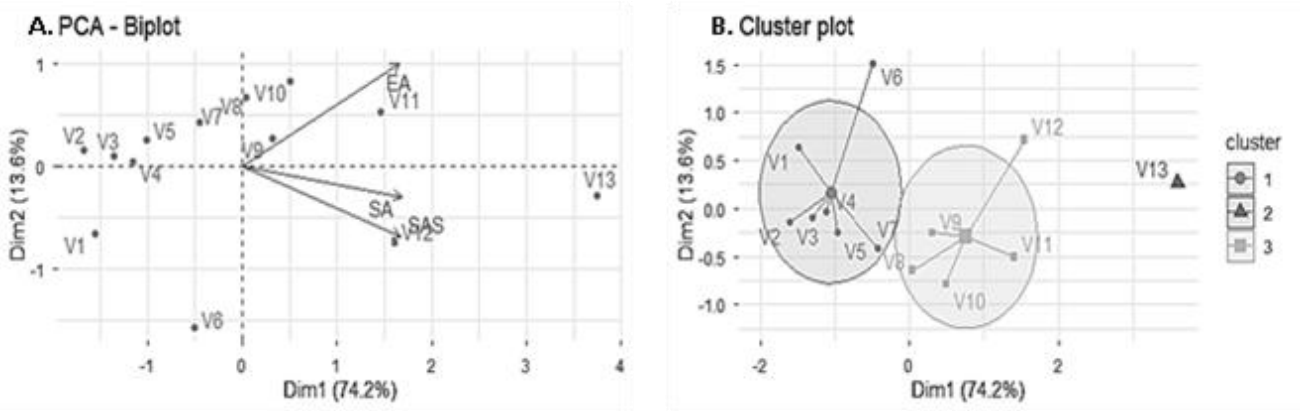

Fig. 4. The results of the PCA using R Studio can be seen in both graphs above: A. PCA - Biplot with 4 arrows (SA = Seed Area, EA = Embryo Area, and SAS = Seed Air Space) and B. Cluster plot, with V1= *P. amabilis*, V2= *V. tricolor*, V3 = *V. lissochiloides*, V4= *P. cornu-cervi*, V5= *D. aphyllum*, V6= *B. graveolens*, V7= *D. crumenatum*, V8= *D. lineale*, V9= *D. anosmum*, V10= *D. mirbelianum*, V11= *A. graminifolia*, V12= *P. tankervilleae*, and V13= *D. antennatum*

3.3 Seed Germination Test from Conventional Storage

The same seed lot was used to evaluate seed germination throughout the storage period until viability fell to negligible levels or the seed stock was depleted. The 13 orchid species were categorised into four storage classifications based on storage longevity under conventional seed bank conditions of -20 °C, which comprised Class 1 (Short-term, 0–1 year), Class 2 (Medium-term I, >1–3 years), Class 3 (Medium-term II, >3–6 years), and Class 4 (Long-term, >6–9 years) (Fig. 5).

Seed longevity showed considerable variation between different species. Species in Class 1, such as *D. lineale* and *D. aphyllum*, showed a rapid decrease in germination within the first year of storage, suggesting a limited ability to withstand long-term conventional storage. *D. anosmum*, *D. antennatum*, and *D. mirbelianum* maintained relatively high germination rates in Class 2 during the initial storage period but experienced a marked reduction after approximately three years. Species such as *P. amabilis*, *D. crumenatum*, and *B. graveolens* retained viability for up to six years before germination significantly decreased. Species in Class 4 (*A. graminifolia*, *V. lissochiloides*, *P. tankervilleae*, and *V. tricolor*) maintained relatively high germination rates for over six years, with viability remaining stable or fluctuating until roughly the ninth year of storage. The results show significant variations in seed durability across different species, even when stored under the same conditions, indicating that inherent seed properties have a substantial impact on storage outcomes.

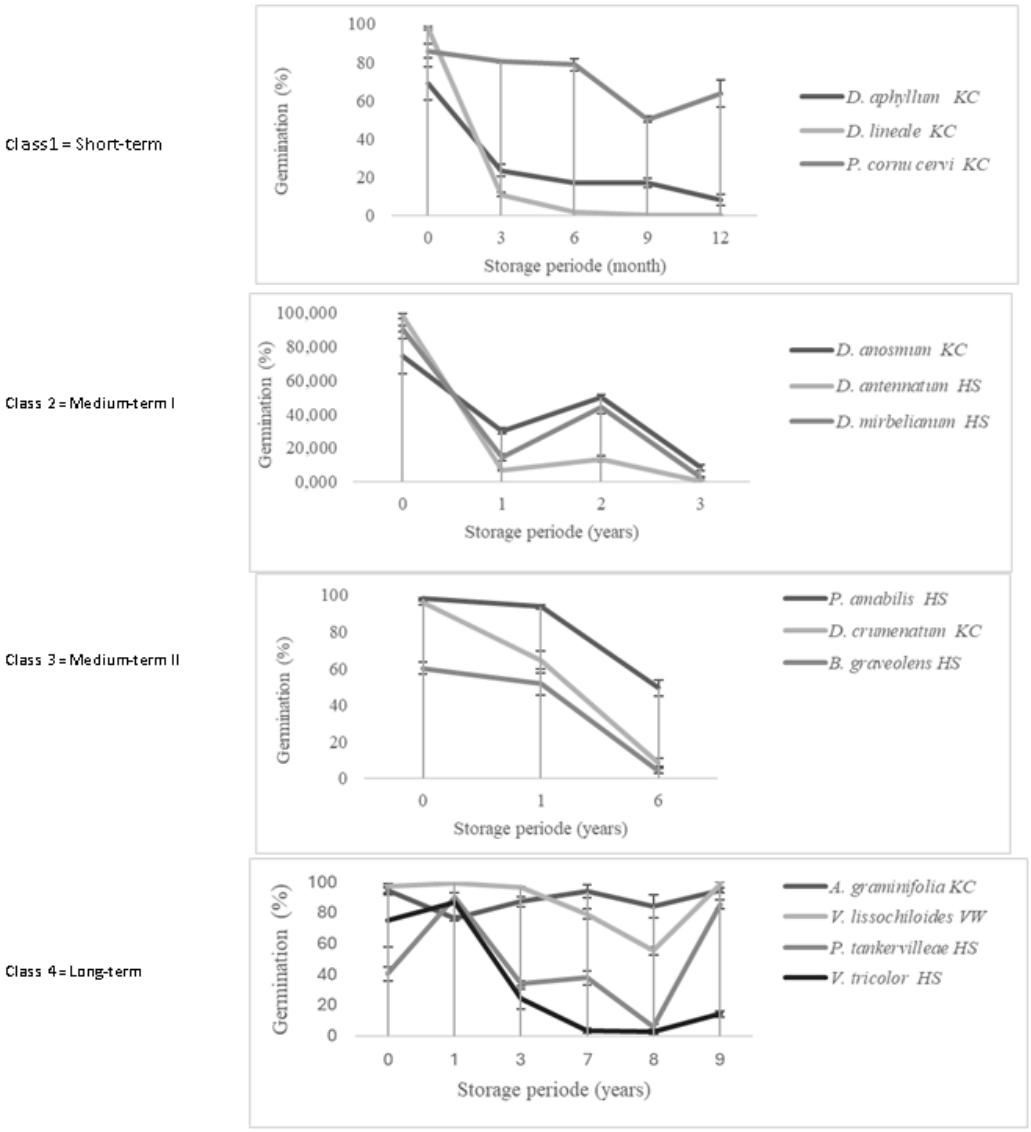

Fig. 5. Germination test results on the best medium (KC=Knudson'C, HS=Hyponex, VW=Vacin and Went) for each orchid seed species in this study under conventional storage, grouped into 4 classes.

The rate of seed germination differed depending on the culture medium employed. The modified Knudson C and Hyponex media supported the highest germination across most species, whereas the modified Vacin and Went medium was particularly suitable only for *V. lissochiloides*. *V. tricolor* germinated most successfully on HS medium, whereas *D. lineale* showed the highest germination on modified KC medium supplemented with 15% coconut water and 150 grams per litre bean sprout extract.

The marked differences in storage longevity among the 13 orchid species suggest that conventional storage at -20 °C does not offer uniform preservation success across different species. Seed longevity appears to depend on species-specific physiological and micromorphometric characteristics, which supports the hypothesis that intrinsic seed traits contribute to long-term viability during ex situ conservation. The significance of developing species-specific seed banking protocols is highlighted by these findings, rather than applying a single storage strategy to all orchid species.

3.4 Relationship Between Seed Storage Period, Germination, and Micro-morphometry

Germination tests were conducted consistently throughout the study by employing the same seed lot, culture medium, and experimental protocol from the start of storage until seed viability decreased or the seed stock was depleted. The 13 orchid species were categorised into four storage groups: Short-term, Medium-term I, Medium-term II, and Long-term, based on the

germination data, as detailed in the Materials and Methods. The data in Table 2 details the initial and final germination percentages for each species at the end of its respective storage period. Data on germination were subsequently combined with measurements of seed micromorphometrics to assess the link between seed morphology and storage durability.

Substantial variation in seed micromorphometry was observed among the six *Dendrobium* species examined. The seed area ranged from 8,389.22 ± 1,318 µm² in *D. aphyllum* to 415,071.72 ± 45,933 µm² in *D. antennatum*, with embryo area spanning from 150.57 to 375.50 µm² and seed air space varying between 104.28% and 347.10%, respectively. These species, despite belonging to the same genus, were distributed across three storage classes. *D. aphyllum* and *D. lineale* had the shortest storage longevity, while *D. anosmum*, *D. antennatum*, and *D. mirbelianum* were classified as Mid Storage I. *D. crumenatum* had the longest storage longevity among the *Dendrobium* species, despite having neither the largest seeds nor the largest embryos. *D. antennatum*, despite having the largest seed and embryo dimensions, showed a germination rate of only 0.323 ± 0.229% after three years of storage (Table 2). Patterns across different genera showed similarities. Among all species, *P. amabilis*, with the smallest seed and embryo dimensions, remained viable for up to six years, whereas *V. lissochiloides* and *V. tricolor* maintained viability for more than nine years despite their relatively small seeds. *V. lissochiloides* showed the highest long-term viability among the species examined, with a retained germination rate of 98.202 ± 2.155% after nine years of storage.

Table 2. Seed germination and storage period with Shapiro-Wilk for normality test of 13 orchid species

No.	Class of storage period	Species	Media	Storage period (years)	Initial germination	Sig	End-of-shelf germination	Sig
1.	**Class 1**	*D. aphyllum*	KC	1	68.973±8.592	0.587*	7.889±2.924	0.724*
2.	Short storage	*D. lineale*	KC	1	97.877±0.879	0.08*	0	0
3.	(0 – 1 years)	*P. cornu-cervi*	KC	1	85.771±3.650	0.78*	63.626±7.018	0.74*
4.		*D. anosmum*	KC	3	74.298±10.148	0.532*	8.397±1.787	0.748*
5.	**Class 2**	*D. antennatum*	HS	3	97.863±1.600	0.641*	0.323±0.229	0.845*
6.	Mid storage I	*D. mirbelianum*	HS	3	90.611±1.583	0.455*	2.668±0.386	0.616*
7.	(>1 – 3 years)	*P. amabilis*	HS	6	97.998±0.383	0.091*	49.313±4.244	0.401*
8.	**Class 3**	*D. crumenatum*	KC	6	95.833±0.962	0.647*	8.714±2.181	0.535*
9.	Mid storage II	*B. graveolens*	HS	6	59.978±3.275	0.007	4.130±1.449	0.375*
10.	(>3– 6 years)	*A. graminifolia*	KC	9	94.660±1.856	0.324*	94.565±1.864	0.123*
11.	**Class 4**	*V. lissochiloides*	VW	9	97.328±1.296	0.058*	98.202±2.155	0.205*
12.	Long storage	*P. tankervilleae*	HS	9	40.34±4.589	0.731*	85.671±2.914	0.064*
13.	(>6-9 years)	*V. tricolor*	HS	9	74.807±17.002	0.819*	14.153±2.102	0.893*

*Sig (p-value) > 0.05 = Normal distribution data

Sig (p-value)< 0.05 = abnormal distribution data

Spearman's rank correlation analysis was conducted to further examine these relationships, incorporating seed storage period, initial germination percentage, and the three seed micromorphometric variables listed in Table 3. Correlations were discovered between seed area and embryo area ($r = 0.885$, $P < 0.01$) and between seed area and internal air space (SAS), ($r = 0.861$, $P < 0.01$), showing that bigger orchid seeds usually have both larger embryos and more internal air space. The embryo area showed a moderate correlation with SAS ($r = 0.578$, $P < 0.05$), indicating coordinated structural development during

seed formation. The results suggest that seed size, embryo size, and internal air space are interconnected developmental characteristics rather than separate morphological features.

Storage longevity showed weak correlations with seed area (r = –0.051), embryo area (r = –0.224), and SAS (r = 0.072). Initial germination percentage displayed weak correlations with seed area (r = –0.137), embryo area (r = 0.143), and SAS (r = –0.187). Seed micromorphometric traits are closely related to one another, but they are not reliable predictors of storage longevity under standard seed bank conditions. The current findings do not confirm the idea that bigger seeds or embryos always have longer storage shelf life.

Table 3. Spearman's rho correlation analysis with -1 < r < 1 based on storage period, Initial germination, SA, EA, and SAS

Variable		Storage period (year)	Initial germination (%)	SA (µm²)	EA (µm²)	SAS (%)
Storage period (year)	Correlation Coefficient	1	-0.096	0.051	-0.224	0.072
	Sig (2-tailed	.	0.754	0.869	0.462	0.698
	N	13	13	13	13	13
Initial germination (%)	Correlation Coefficient	-0.096	1	-0.137	0.143	-0.187
	Sig (2-tailed	0.754	.	0.655	0.642	0.541
	N	13	13	13	13	13
SA (µm²)	Correlation Coefficient	0.051	-0.137	1	0.885**	0.861**
	Sig (2-tailed	0.869	0.655	.	0	0
	N	13	13	13	13	13
EA (µm²)	Correlation Coefficient	-0.224	0.143	0.885**	1	0.578*
	Sig (2-tailed	0.462	0.642	0	.	0.039
	N	13	13	13	13	13
SAS (%)	Correlation Coefficient	0.072	-0.187	0.861**	0.578*	1
	Sig (2-tailed	0.698	0.541	0	0.039	.
	N	13	13	13	13	13

** Correlation is significant at the 0,01 level (2-tailed)

* Correlation is significant at the 0,05 level (1-tailed)

3.5 Discussion

The present study demonstrated considerable variation in the micromorphometric characteristics of orchid seeds among the 13 species examined, particularly in seed area, embryo area, seed air space (SAS), and overall seed shape. Such variation is consistent with the broad morphological diversity previously reported in orchid seeds. Güler [31] noted that orchid seeds differ considerably among genera and species in testa size, shape, colour, and the relative proportions of the testa, embryo, and internal air space. Diantina et al. [14] similarly reported that orchid seed morphology varies among taxa as a consequence

of evolutionary processes associated with survival and dispersal in different habitats. The diverse seed shapes observed in the present study, ranging from elongated and fusiform forms to relatively short and compact forms, therefore reflect the substantial structural diversity of orchid seeds.

The variation in seed size and shape is likely associated with ecological adaptation and reproductive strategies rather than directly with seed longevity. Prasongsom et al. [18] demonstrated considerable variation in seed size, embryo volume, and seed air space among *Dendrobium* species and suggested that these characteristics represent important ecological adaptations related to seed dispersal and seedling establishment. Orchid seeds have evolved distinctive structures that facilitate dispersal while retaining sufficient embryonic structures for subsequent germination. In particular, the relatively small seeds observed in several *Phalaenopsis* and *Vanda* species are consistent with adaptations for efficient wind dispersal. Approximately 70% of epiphytic plant species use anemochory, producing lightweight seeds with thin, net-like testa structures that facilitate aerial dispersal toward suitable host trees [32]. Thus, the relatively small seed areas observed in *P. amabilis* and *Vanda* spp. may represent an adaptation that enhances dispersal efficiency rather than an indication of reduced viability or storage capacity. Conversely, relatively larger seeds may provide greater structural space for embryo development and associated reserves, potentially supporting seedling establishment when environmental conditions are favourable.

The relationships among seed area, embryo area, and SAS observed in this study further support the interpretation that these traits are interconnected aspects of seed development. The strong positive association between seed area and embryo area indicates that larger seeds generally contain larger embryos, while the positive association between seed area and SAS suggests that an increase in overall seed size is also accompanied by greater internal air space. However, these relationships should not be interpreted as evidence that larger seeds necessarily have greater storage longevity. Instead, they may represent coordinated structural characteristics that evolved to balance embryo development, dispersal efficiency, and establishment after dispersal. The PCA results support this interpretation by showing that seed area, embryo area, and SAS collectively explained most of the variation among the studied species and separated them into distinct morphological groups. The clear separation of D. antennatum from the other species, particularly the other *Dendrobium* species, further demonstrates the considerable diversity that can occur even within a single orchid genus.

The testa also plays an important functional role in orchid seed biology. As the outer protective structure, the testa protects the embryo against mechanical damage and environmental stresses, including pathogen attack, ultraviolet radiation, temperature fluctuations, humidity, and oxidative damage [33]. Differences in testa structure and internal organisation may therefore influence physiological properties such as water movement, gas exchange, and the capacity of the embryo to withstand environmental stress. Nevertheless, the results of the present study indicate that these morphological characteristics alone are insufficient to explain differences in seed longevity under conventional storage conditions.

The most important finding of this study is that orchid seed micromorphometric characteristics were not strong predictors of storage longevity. Although seed area, embryo area, and SAS were strongly correlated with one another, their relationships with storage duration were weak. This finding indicates that the structural organisation of orchid seeds is more closely associated with developmental and ecological characteristics than with their capacity to remain viable during prolonged storage at −20 °C. The contrasting storage performance of species with different seed dimensions provides particularly strong support for this interpretation. For example, *D. antennatum* possessed exceptionally large seed and embryo areas but exhibited very low germination after three years of storage, whereas *V. lissochiloides* maintained a very high germination percentage after nine years despite having relatively small seeds. Similarly, *V. tricolor* maintained viability for an extended period despite its relatively small seed and embryo dimensions. These findings demonstrate that large seed or embryo size does not necessarily confer greater longevity under conventional seed bank conditions.

This interpretation agrees with Prasongsom et al. [18], who concluded that differences in seed size, embryo size, and internal air space among orchid species are more closely associated with ecological adaptations for seed dispersal and seedling establishment than with seed longevity. Orchid seeds have evolved to optimise dispersal while retaining sufficient embryo structure for germination, but these evolutionary adaptations do not necessarily determine their resistance to storage-associated deterioration. Consequently, micromorphometric traits may be useful for describing orchid diversity and understanding ecological strategies, but they should not be used independently to predict the duration for which orchid seeds can remain viable in a seed bank.

The substantial variation in storage longevity among the 13 species further indicates that conventional storage at −20 °C does not provide uniform preservation success across orchids. Some species, such as *D. lineale* and *D. aphyllum*, experienced a rapid decline in germination within the first year, whereas *A. graminifolia*, *P. tankervilleae*, *V. tricolor*, and *V. lissochiloides* retained viability for substantially longer periods. Particularly notable was V. lissochiloides, which retained a high germination percentage after nine years of storage. Differences were also evident within the genus *Dendrobium*, where species showed markedly different storage performance despite belonging to the same genus. This interspecific variation suggests that physiological properties specific to each species have a greater influence on storage longevity than seed dimensions alone.

The decline in viability observed during storage is consistent with previous reports on orchid seeds. Franceschi et al. [21] found that seeds of *Grandiphyllum divaricatum*, *Gomesa praetexta*, *G. recurva*, and *G. forbesii* germinated more successfully when freshly harvested than when stored at −20 °C or −80 °C. Prasongsom et al. [20] similarly reported higher germination in fresh orchid seeds than in dry-stored seeds. Freshly matured orchid seeds generally lack primary dormancy, whereas storage may induce secondary dormancy or physiological deterioration, resulting in reduced germination. During seed maturation, embryos undergo reserve accumulation followed by a desiccation phase that prepares the seeds for a period of reduced metabolic activity [34]. However, the ability to tolerate desiccation and subsequent low-temperature storage differs among orchid species. The rapid loss of viability in *D. lineale*, compared with the prolonged viability observed in *A. graminifolia*, *P. tankervilleae*, and *Vanda* spp., therefore suggests substantial species-specific differences in physiological resilience.

The weak relationship between seed morphology and storage longevity suggests that physiological and biochemical characteristics are likely to play a more important role in determining seed survival during prolonged storage. Important factors may include seed moisture content, membrane stability, antioxidant capacity, lipid composition, and resistance to oxidative stress [33]. Although low temperature and reduced moisture content substantially suppress metabolic activity, deterioration processes may still occur during storage. Differences among species in membrane stability, antioxidant defence, lipid composition, and tolerance to desiccation or freezing may therefore explain why some orchid seeds remain viable for many years whereas others deteriorate rapidly under identical storage conditions. This also explains why seed size and embryo dimensions cannot be considered reliable indicators of longevity.

The germination assessment itself was also influenced by sterilisation and culture conditions. Sodium hypochlorite (NaOCl), used in the present study as a surface sterilising treatment, may also enhance seed coat permeability and facilitate water uptake and germination while reducing microbial contamination [35]. The different responses among orchid species to the culture media further indicate that germination percentage reflects not only seed viability but also the suitability of the culture environment. Modified Knudson C and Hyponex media supported relatively high germination in several species, whereas modified Vacin and Went medium was particularly suitable for *V. lissochiloides*. The response of *D. lineale* also demonstrates the importance of medium composition, as previous research led by Utami [36] in 2026 reported improved germination of this species using Vacin and Went medium supplemented with 20% coconut water. The beneficial effects of coconut water may be related to its content of natural phytohormones, amino acids, vitamins, and carbohydrates, which can support orchid protocorm development and partially substitute for synthetic plant growth regulators [37]. Therefore, although the present study used consistent germination procedures throughout the storage assessment, species-specific responses to culture conditions should be considered when interpreting germination as an indicator of viability.

From a conservation perspective, the substantial differences in storage longevity observed among the studied species highlight the importance of species-specific seed banking strategies. Species with rapid viability loss, such as D. lineale and D. aphyllum, require more frequent viability monitoring and potentially more frequent regeneration or recollection of seeds than species capable of maintaining viability for many years. In contrast, species such as *A. graminifolia*, *P. tankervilleae*, *V. tricolor*, and *V. lissochiloides* may tolerate conventional seed bank conditions for longer periods. The storage classifications used in this study therefore provide a practical framework for prioritising monitoring and regeneration schedules, although they should be interpreted as empirical categories rather than universal physiological thresholds.

For species that show poor survival under conventional −20 °C storage, alternative conservation strategies may be necessary. Cryopreservation provides a promising complementary approach for particularly short-lived or threatened orchid species because orchid seeds can tolerate extremely low temperatures and may even show improved germination following cryogenic storage without vitrification [24, 38]. Thus, conventional seed banking and cryopreservation can be integrated as

complementary approaches within broader ex situ conservation programmes, particularly when the objective is to secure orchid genetic resources over very long periods.

Comparisons of longevity among species should nevertheless be made cautiously because initial germination percentages can differ substantially among seed lots. Klepka et al. [39] highlighted the importance of the P50 parameter, defined as the time required for seed viability to decline to 50%, when comparing seed longevity. The storage classifications applied in the present study provide a practical means of describing observed viability patterns under routine seed bank conditions; however, more frequent germination assessments would allow more precise estimation of P50 and other longevity parameters. Such measurements would improve comparisons among species and provide a stronger quantitative basis for predicting storage performance.

Several limitations should be considered when interpreting these findings. Each species was represented by seeds originating from a single fruit, which limits the assessment of intraspecific variation. Storage intervals also differed among species, relative humidity was monitored indirectly, and annual germination assessments were not available for every storage period. These limitations may have obscured subtle relationships between seed micromorphometry and longevity and reduce the extent to which the results can be generalised to entire species or populations. Future studies should therefore include multiple seed lots collected from different individuals and populations and should monitor seed viability at more frequent intervals throughout storage.

Future research should also combine micromorphometric measurements with physiological and biochemical assessments to better explain differences in orchid seed longevity. Parameters such as seed moisture content, membrane integrity, antioxidant activity, lipid composition, and indicators of oxidative deterioration could provide information that cannot be obtained from seed dimensions alone. An integrated approach combining morphological, physiological, and biochemical characteristics may ultimately provide a more reliable basis for predicting seed longevity and developing species-specific storage protocols.

4. CONCLUSION

This study demonstrated substantial micromorphometric diversity among the 13 Indonesian orchid species, with significant interspecific differences in seed area, embryo area, and seed air space (SAS). Seed area was strongly associated with embryo area and SAS, indicating that these characteristics are interconnected components of orchid seed structure. However, these micromorphometric traits showed only weak relationships with storage longevity under conventional seed bank conditions at −20 °C. Species with larger seeds or embryos did not necessarily maintain viability for longer periods, while some species with relatively small seeds, such as *Vandopsis lissochiloides* and *Vanda tricolor*, retained viability for more than nine years.

The findings indicate that orchid seed micromorphometry primarily reflects species-specific structural and ecological adaptations related to seed dispersal and seedling establishment rather than storage longevity. Seed longevity appears to be governed more strongly by physiological and biochemical characteristics that were not measured in the present study. Therefore, seed area, embryo area, and SAS should not be used as standalone predictors of orchid seed longevity. For effective ex situ conservation, species-specific viability monitoring and storage strategies are recommended, particularly for species showing rapid viability loss under conventional storage. Future studies integrating seed micromorphometry with moisture content, membrane integrity, antioxidant capacity, lipid composition, and other physiological and biochemical indicators are needed to improve the prediction of orchid seed longevity and optimise long-term conservation protocols.

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AUTHOR CONTRIBUTIONS

Elizabeth Handini: Investigation, Methodology, Laboratory Analysis, Data Curation, Formal Analysis.

Dwi Murti Puspitaningtys: Methodology, Laboratory Analysis, Data Curation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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