International Journal of Environment, Agriculture and Biotechnology
Vol-11, Issue-4; Jul-Aug, 2026
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Seed Morphometry and Viability Assessment of *Dendrobium gibsonii* Paxton by the double-staining technique

Satyam Tamang*[1], Subhas Chandra Roy[2], Bhumika T. Yonzon[3], Jayita Sarkar[4]

[1]Department of Botany, Plant Genetics & Molecular Breeding Laboratory, North Bengal University, Darjeeling, West Bengal, PO-NBU-734013, India; Email: ghising.satyam@gmail.com (ORCID id:0009-0009-4192-0093)
[2]Department of Botany, Plant Genetics & Molecular Breeding Laboratory, North Bengal University, Darjeeling, West Bengal, PO-NBU-734013, India; Email: subhascr2011@gmail.com
[3]Department of Botany, Plant Genetics & Molecular Breeding Laboratory, North Bengal University, Darjeeling, West Bengal, PO-NBU-734013, India; Email: ; bhumikat.yonzon30@gmail.com (ORCID id: 0009-0003-6080-3120)
[4]Department of Botany, Plant Genetics & Molecular Breeding Laboratory, North Bengal University, Darjeeling, West Bengal, PO-NBU-734013, India; Email: jayitasarkar0686@gmail.com (ORCID id: 0009-0006-3302-1846)
Corresponding Author: ghising.satyam@gmail.com

Received: 13 Jul 2026; Received in revised form: 10 Aug 2026; Accepted: 14 Aug 2026; Available online: 19 Aug 2026

Abstract— *Dendrobium gibsonii is an epiphytic ornamental orchid native to Southeast Asia, including Northeast India, with significant conservation value but with inadequate information on seed biology. We examined seed morphometry using light and scanning electron microscopy and measured seed and embryo length, width, volumes, seed length and width (SL/SW), embryo length and width (EL/EW) ratios, and seed volume-to-embryo volume ratio (SV/EV). Viability and permeability were also assessed after scarification with 0.5, 1.5, 3.0, and 5.0% NaOCl for 5, 10, 15, and 30 min (5 mg seeds per treatment), followed by TTC and Trypan Blue staining. The interaction between NaOCl concentrations and time was highly significant for both permeability and viability (all $p < 0.001$). Tukey tests indicated that 3% and 5% produced the higher permeability increases (notably at 15–30 min), whereas 1.5–3% for 10–15 min (3%×10 min = 93.81 ±1.25% viability) was optimal for viability; prolonged 5% exposure was toxic. Morphometry revealed minute, dust-like, fusiform seeds with a prolate-spheroid embryo and <50% air space, implying reduced buoyancy and limited dispersal. These traits are taxonomically informative. Future work should expand morphometric comparisons across other Dendrobium species to determine species-versus genus-specific indicators, perform controlled buoyancy and storage trials, validate TTC outcomes via in vitro germination, and evaluate gentler scarification methods (enzymatic, mechanical, sonication) to enhance permeability without compromising viability, thereby facilitating propagation and conservation strategies.*

Keywords— ***Dendrobium gibsonii, morphometry, viability percentage, permeability percentage, scarification, double-staining, 2,3,5-triphenyltetrazolium chloride (TTC), Trypan blue, light microscopy (LM), scanning electron microscopy (SEM).***

I. INTRODUCTION

Orchids (family Orchidaceae), with approximately 28,000 known species (Vitt *et al.,* 2023), are known for their attractive flowers, and many possess ethnomedicinal and therapeutic properties (Teoh, 2016). In addition, because of their floral beauty, orchids are commercially important in the floriculture market (Khuraijam *et al.,* 2017; Hussain and Eraqui, 2023). Orchids are possibly the most manipulated plants for hybrid production worldwide (Li *et al.,* 2021) and are heavily exploited for aesthetic and floricultural purposes (Maulida *et al.,* 2023; Iiyama *et al.,* 2024). *Dendrobium gibsonii* Paxton is an epiphytic orchid belonging to the subfamily Epidendroideae and tribe Malxideae of Orchidaceae. It is native to Southeast Asia (World Flora Online, 2026), including parts of Northeast India (Sharma and Bhattacharyya, 2023). It is grown as an

ornamental species for its floral beauty and has substantial conservation value (Kesta and Warrington, 2023). However, it lacks comprehensive information on seed biology and germination processes. Like many other orchids, this species faces threats due to habitat loss and unsustainable collection (Kumar *et al.,* 2024). Its intricate reproductive biology complicates and hinders normal propagation (Kesta and Warrington, 2023; Liu *et al.,* 2025). Similar to many orchids, the seeds of *D. gibsonii* are minute, dust-like, without endosperm, and have a tiny embryo enclosed in a thin testa that facilitates their long-distance dispersal. Therefore, seed traits, such as length, width, volume, embryo size, and air space volume, and their quantitative characterisation, are significant for understanding dispersal ecology, viability, germination behaviour, and taxonomic relationships with other *Dendrobium* and allied species (Alomía *et al.,* 2016). However, the assessment of seed morphometry of *D. gibsonii* has not yet been performed in detail, and related information on seed viability is essential for ex situ conservation and propagation programs. Moreover, reliable viability assessment of orchid seeds is practically challenging and contentious because of their small size, inconsistent testa permeability, and often poor correlation between conventional tetrazolium staining and actual germination (Soares *et al.,* 2021; Pradhan *et al.,* 2022). The double-staining technique, which involves the use of both vital and non-vital dyes, is a rapid microscopy-based method for assessing orchid seed viability and germination. It also allows proper visualisation of embryo integrity and testa permeability and reduces the risk of under- or overestimating viability (Magrini *et al.,* 2019). In this context, combining comprehensive seed morphometry with a reliable double-staining procedure can provide a more complete assessment protocol for seed quality and behaviour in orchids with poorly known characteristics. Correspondingly, in the present work, we studied the seed morphometry of *Dendrobium gibsonii* and its viability and permeability were assessed using double-staining techniques. Specifically, it involves (i) quantification of key seed and embryo characteristics using light microscopy (LM) and scanning electron microscopy (SEM), and (ii) assessment of the efficiency of the double-staining method for characterising viable, unviable, and dead seeds in *D. gibsonii* in terms of viability and permeability percentages. The outcomes of this study can establish a strong basis for its ex-situ conservation and propagation.

II. MATERIALS AND METHODS

Plant materials

A healthy *Dendrobium gibsonii* plant (Figure 1a) was collected from Mungpoo, Darjeeling, West Bengal, planted in a nursery, and bloomed in May–June 2024 (Figure 1b). Healthy and suitable-sized flowers were selected and artificially self-pollinated to obtain fruits or capsules. The fruits matured in approximately 120 days. Healthy fruits (Figure 1c and d) were selected, and seeds were collected in small plastic vials.

(a) (b) (c) (d)

Fig.1. Morphological details of Dendrobium gibsonii. (a) an adult plant (b) stem bearing the flowers (c) fruits or capsules (d) a mature capsule.

Observation of seed morphology under the Light Microscope (LM)

An appropriate number of seeds were taken and soaked in distilled water for 10 min, and then the original colour of the entire seed, including the embryo, and shape were observed through a compound light microscope using a 10x objective lens and a 10x eyepiece lens. In addition, the length and width of both seeds and embryos were measured from 50 different seeds using an ocular micrometre. One ocular division was standardised as 14.58µ. The recorded length and width of both seeds and embryos were used to calculate the volume of seed and embryo, air space percentage, seed length and width

(SL/SW), embryo length and width (EL/EW), seed volume and embryo volume ratios. The microphotographs were also taken to reveal the overall dimensions and features of seeds and embryos. Since the shapes of the seed and embryo were fusiform and prolate spheroids, respectively, the following formulae were used (Arditti, 2000) to determine their average volumes:

$$\text{volume of seed} = 2\left(\frac{1}{3}\pi r^2 h\right)$$

Where, $r = \frac{\text{width}}{2}$ and $h = \frac{\text{length}}{2}$

$$volume\ of\ embryo = \frac{4}{3}\pi\left(\frac{L}{2}\right)\left(\frac{W}{2}\right)^2 or\ \frac{\pi}{6}LW^2$$

Where, L = length, W = width

$$volume\ of\ air\ space\ between\ testa\ and\ embryo\ (\%) = \frac{vol.of\ seed - vol.of\ embryo}{vol.of\ seed}\ X\ 100$$

Observation of seed morphology under the Scanning Electron Microscope (SEM)

Mature orchid seeds were collected from dehisced capsules and briefly air-dried on filter paper to remove excess moisture. The seeds were then transferred to a desiccator containing silica gel for 24 h to prevent seed collapse and rehydration. Dried seeds were mounted on aluminium SEM stubs using double-sided carbon adhesive tape, with seeds oriented for lateral, polar, and basal views where possible. Mounted seed samples were examined using a JEOL JSM scanning electron microscope operated at an accelerating voltage of 10 kV, a working distance of 10–12 mm, and in high-vacuum mode. Digital images were captured at magnifications ranging from 100× to 1000× to reveal the seed shape, testa cell sculpturing, wall thickening, and micropylar and chalazal ends (Aytar *et al.,* 2024).

Seed Scarification

Scarification was performed by treating the seeds with four different concentrations (0.5, 1.5, 3, and 5 %) of sodium hypochlorite (NaOCl) solution for 5, 10, 15, and 30 min each. 0.1% (v/v) Tween 20 was also added to each treatment (Magrini and Vitis, 2017; Magrini *et al.,* 2019). The exact proportion used was 3 mL of NaOCl with 2 drops of Tween 20. Tween 20, a non-ionic surfactant, mainly functions as a wetting agent and emulsifier, enhancing the penetration of scarifying solutions into seed coats, improving surface disinfection, and breaking dormancy without damaging the embryos. Thus, considering both the NaOCl concentration and treatment duration, 16 treatment conditions were assessed. The non-scarified seeds kept in sterile distilled H_2O served as experimental controls (four sets for 5, 10, 15, and 30 min). For each set (treatment and control), 5 mg of seeds (1200 – 1500 seeds) were used. Following treatment with NaOCl, each seed set, except the control, was rinsed twice with sterile distilled water for 5 min and centrifuged at 5000 rpm for 5 min following each rinse. Subsequently, the treated seeds were soaked in sterile distilled water for 1 h to remove residual hypochlorite, thereby preventing interference with TTC reduction in viable embryos (Waes and Debergh, 1986).

Double-staining

A 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) was prepared, and the pH was adjusted to 7.0 using 1 M KOH. Both non-scarified and scarified seeds (by the above method) were transferred into a 1.5 mL microtube containing 1 mL of 1% TTC solution. They were incubated in the dark at 30 ± 2°C for 24 h (Waes and Debergh, 1986). The TTC solution was then removed, and the seeds were rinsed twice (3 min each) with distilled water, including centrifugation at 5000 rpm for 5 min between the rinses. Following TTC staining, the seeds were subjected to a second staining procedure using Trypan Blue (TB) dye to assess seed coat permeability. Trypan Blue is a vital dye that is excluded from viable cells with intact plasma membranes and selectively stains only nonviable cells, and impart blue colour to both nonviable and dead cells. Therefore, TB dye provides reliable indications of seed coat permeability. During double staining, the TTC solution was first decanted, and the seeds were washed twice with 1 mL of distilled water for 3 min per wash. Subsequently, seeds were stained with 0.5 mL of 0.4 % TB solution for 10 min, followed by two rinses with sterile distilled water for 5 min each to remove excess dye.

Observation and assessment

Both treated and control seeds underwent double staining and were spread on glass slides (600 – 1000 seeds/slide) and observed under a compound light microscope. Based on the staining pattern, four types of seeds were visible: *[i] blue testa and red embryo (viable), [ii] blue testa and whitish embryo (nonviable), [iii] dead seeds with both testa and embryo blue (nonviable),* and [iv] *both testa and embryo whitish (nonviable and impermeable)*. All seeds were observed and counted to determine the viability and permeability percentages, following the methodology outlined by Magrini et al. (2019), with required modifications.

Viability percentage

Viability percentage was calculated as follows:

$$V_{TTC+TB}(\%) = \frac{v}{t} \text{ X } 100$$

Where, $V_{TTC + TB}$ (%) = Viability percentage scored by double staining (TTC and Trypan blue), v = total number of viable seeds and t = total number of tested seeds, i.e.

all the seeds with a blue-stained testa (viable, nonviable and dead) and impermeable seeds.

Permeability percentage

Seed coat permeability percentage (P) was calculated as follows:

$$P(\%) = \frac{p}{t} \text{ X } 100$$

where p = total number of permeable seeds and t = total number of tested seeds.

Statistical Analysis

Two-way ANOVA, with a post hoc Tukey multiple comparison test to compare all groups, was used to assess the effects of two variables, NaOCl concentration and treatment duration for scarification, on viability and permeability. Jamovi 2.6.44 was used for all the statistical analyses.

III. RESULTS

Seed morphometry

Both light microscopy (LM) and scanning electron microscopy (SEM) revealed the seed structure of *D. gibsonii* as fusiform (Figure 2a to f) with a centrally placed prolate spheroid embryo (Figure 2a, e and f). The length, width, and volume of both the seed and embryo and the air space in the testa were estimated using data obtained through light microscopy (Table 1). The seed colour was observed under a light microscope and was pale yellowish to light greenish (Figure 2f). The mean seed length-width (SL/SW) ratio was 4.82±0.61. Similarly, the mean embryo length-width (EL/EW) was 2.16±0.29. The seed volume-to-embryo volume ratio (SV/EV) predicts buoyancy, which is directly proportional to buoyant capacity. In the present study, the mean SV/EV was 2.04±0.67, indicating that the air-space volume was marginally lower.

Table 1: Seed and embryo morphometric parameters of Dendrobium gibsonii showing length, width, volume, and free air space in the testa (n = 50). Values are expressed as the mean ± standard deviation.

	Seed	Embryo
Length (µm)	400.66 ±39.22	148.72 ±12.15
Width (µm)	83.69 ±7.69	69.70 ±7.39
Volume (µm^3)	7.42×10^5 µm^3 ±$1.60\text{x}10^5$	3.83×10^5 µm^3 ±0.86×10^5
Air space (%)	46.27 ±16.07	
SL/SW	4.82±0.61	
EL/EW	2.16±0.29	
SV/EV	2.04±0.67	

Assessment of Viability

Two-way ANOVA revealed highly significant effects of NaOCl concentration ($F_{4,180} = 14{,}894, p < 0.001$), treatment time ($F_{3,180} = 112, p < 0.001$), and their interaction ($F_{12,180} = 161, p < 0.001$) on seed viability percentage. Furthermore, Tukey's post hoc test showed that 1.5–3% for 10–15 min was the optimal treatment, with 1.5 for 15 min, 3% for 10 min, and 5% for 5 min achieving the highest viability, i.e. 93.16±1.11, 93.81±1.25, and 93.07±1.16%, respectively. The highest viability percentage, i.e. 93.81±1.25%, outperformed the control by more than 64%. Four distinct groups were identified: control (22–32%), toxic treatment (74–78%), moderate treatment (84–91%), and best treatment (92–93 %). Therefore, scarification at 1.5–3 % NaOCl or 10 –15 and 5% for 5 min are reliable for *D. gibsonii* seeds (Table 2).

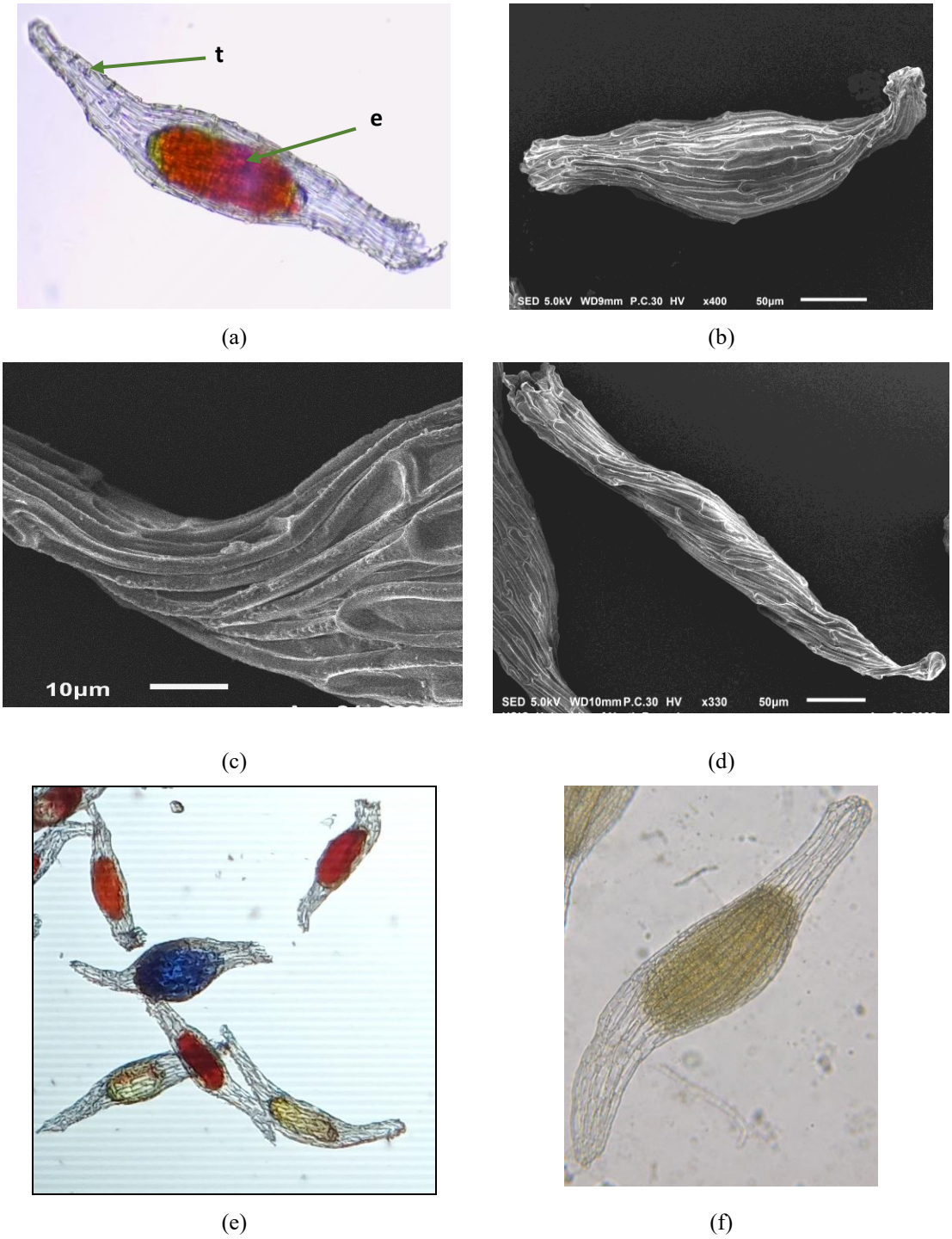


Fig.2. Microscopic observation of seeds of Dendrobium gibsonii. (a) a viable seed observed under a compound LM, t = testa and e = embryo; (b) – (d) seeds observed under a SEM; (e) viable seed (blue testa with red embryo); non-viable seeds (blue testa with whitish embryo, dead seeds with blue testa and blue embryo); (f) permeable seed (both embryo and testa are whitish) observed under LM.

Furthermore, the interaction plot (Figure 3a) shows a gradual increase in viability percentage over time in the control (0% NaOCl), although it remained much lower than that in all sacrificed groups. Scarification with 0.5% and 3% NaOCl resulted in a gradual increase in viability up to 15 min before declining at longer durations. Among these, 1.5% and 3% NaOCl yielded the highest viability, peaking at 15 and 10 min of exposure, respectively. Whereas at 5% NaOCl, the viability percentage steadily decreased from 5 to 30 min.

Table 2. Viability (%) of D. gibsonii seeds after double staining and scarification with different NaOCl concentrations (0.5, 1.5, 3, and 5%) for different times (5, 10, 15, and 30 min). Values denote mean ± SD (n=10). Letters progress from 'a' (lowest viability) to 'j' (highest viability) based on estimated marginal means. Treatments sharing the same letter(s) are not significantly different (Tukey HSD, p > .05).

Double-staining	Scarification by NaOCl solution (%)	Treatment time (min)			
		5	10	15	30
TTC (1%) + Trypan blue (0.4%)	0 (Control)	22.48 ±1.07^{a}	26.27 ±1.41^{b}	29.52 ±1.41^{c}	32.87 ±1.11^{d}
	0.5	80.68 ±0.96^{g}	88.94 ±0.87^{i}	91.31 ±1.75^{i}	87.01 ±2.05^{h}
	1.5	89.41 ±1.35^{i}	92.29 ±2.32^{j}	93.16 ±1.11^{j}	85.81 ±1.64^{h}
	3	91.15 ±1.90^{i}	93.81 ±1.25^{j}	86.73 ±1.49^{h}	84.58 ±1.01^{h}
	5	93.07 ±1.16^{j}	88.72 ±1.09^{i}	78.11 ±0.85^{f}	74.40 ±0.69^{e}

Table 3. Permeability (%) of D. gibsonii seeds after double staining and scarification with different NaOCl concentrations (0.5, 1.5, 3 and 5%) for different times (5, 10, 15, and 30 min). Values denote mean ± SD (n=10). Letters progress from 'a' (lowest permeability) to 'd' (highest permeability) based on estimated marginal means. Treatments sharing the same letter(s) are not significantly different (Tukey HSD, p > .05).

NaOCl (%)	5 min	10 min	15 min	30 min
0 (Control)	42.76 ± 1.72 ^{a}	45.32 ± 2.25 ^{a}	50.64 ± 2.24^{a}	53.09 ± 1.14 ^{a}
0.5	92.81 ± 1.35 ^{b}	93.13 ± 1.23 ^{b}	95.22 ± 1.90 ^{b}	97.03 ± 1.60 ^{c}
1.5	93.64 ±1.67 ^{b}	97.57 ±1.14^{c}	98.04 ± 1.37 ^{c}	99.02 ± 1.05 ^{d}
3.0	95.50 ±2.03 ^{b}	97.55 ± 1.85 ^{c}	98.05 ± 1.08 ^{c}	99.02 ± 1.05 ^{d}
5.0	96.84 ± 1.33 ^{c}	98.02 ± 1.04 ^{c}	99.98 ± 1.11^{d}	99.70 ± 0.49 ^{d}

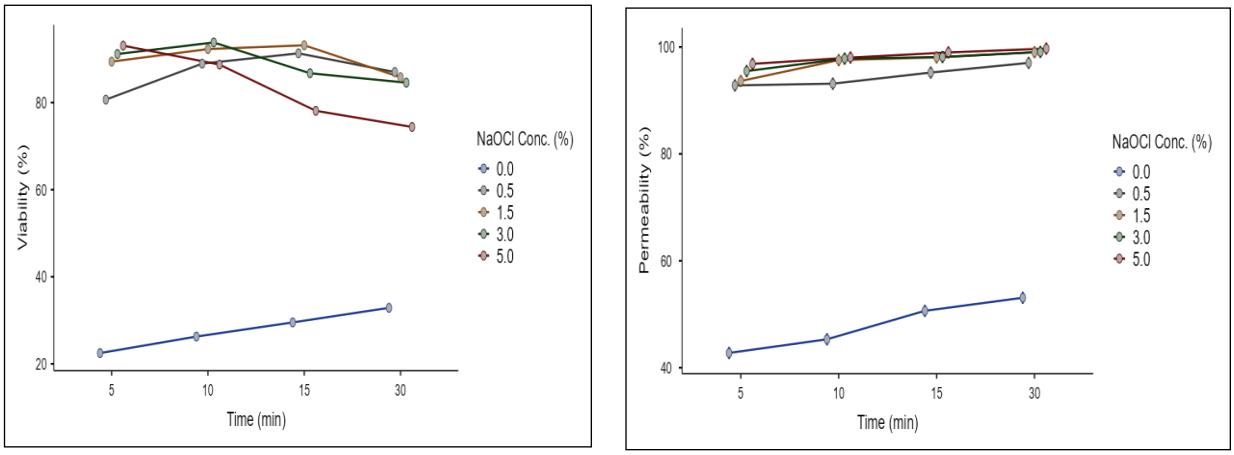
Assessment of Permeability

Similarly, two-way ANOVA of permeability percentage demonstrated a highly significant effect of NaOCl concentration ($F_{4,180}$ = 8,469.0, $p <$ 0.001), treatment duration ($F_{3,180}$ = 115.1, $p < 0.001$), and their interaction ($F_{12,180}$ = 10.6, $p < 0.001$). In addition, Tukey's post hoc analysis revealed numerous significant pairwise differences (p < .001) among the tested combinations. Accordingly, Tukey's post hoc comparisons revealed that the control (0 %) significantly differed from all NaOCl treatment groups at all treatment times, confirming the effectiveness of NaOCl in enhancing seed permeability. Compared to the control at 5 min, all treated groups showed significantly higher permeability (Table 3). The same pattern was evident at 10, 15, and 30 min. Among the treated groups, even at lower concentrations (0.5 and 1.5%) and minimum treatment times (5 and 10 min), the permeability was >90%. The three treatment sets, such as 1.5%, 3%, and 5 % NaOCl, showed higher increases in permeability, mainly at 10, 15 and 30 min. The highest permeability percentage, i.e. 99.70%, outperformed all control values by more than 87% (Table 3).

Furthermore, the interaction plot (Figure 3b) illustrates the effects of NaOCl concentration (0, 0.5, 1.5, 3, and 5%) and scarification duration (5, 10, 15, and 30 min) on seed permeability. Compared to the control (0 %), all scarified treatments showed enhanced permeability, with a general upward trend across different scarification times. The 1.5 % NaOCl treatment exhibited a steep increase from 5 to 10 min, whereas the 3 % and 5 % treatments demonstrated more gradual increases. Overall, 5 % NaOCl treatment yielded the highest permeability across all time points, peaking at 30 min.

IV. DISCUSSION

In the present study, compound light and scanning electron microscopy revealed that the seeds of *Dendrobium gibsonii* are minute, dust-like, and fusiform. The mean length and width of *D. gibsonii* were recorded as 400.66±39.22 µ and 148.72±12.15 µm, respectively.



(a) (b)

Fig.3: Interaction plots showing the effects of sodium hypochlorite (NaOCl) concentration (0%, 0.5%, 1.5%, 3.0%, 5.0%) and scarification time (5, 10, 15, and 30 min) on (a) seed viability percentage and (b) permeability percentage.

When comparing the seed length with the orchid seed classification system (Barthlott *et al.,* 2014), the species falls within the small-seed size class (200–500 µ). The seed testa was ornate and composed of elongated cells arranged longitudinally. The testa cells have raised anticlinal walls with periclinal reticulation joining adjacent cells, giving a uniform reticulate pattern to the entire testa. Both the raised anticlinal and periclinal walls were covered with slightly coarse wax deposits. These waxy deposits facilitate adhesion to wet substrates along streams (Diantina *et al.,* 2020) and impart impermeability owing to their water-repelling properties (Yoder *et al.,* 2000). Since the seed shape is fusiform, the testa cells in the median distended portion are uniformly elongated and appear quasi-polygonal in some sections due to periclinal wall extension, while they are elongated and narrower at either end (micropylar and chalazal ends) of the seeds. The micropylar end is characterised by a funnel-shaped opening with undulation at the edge due to the irregular elongation of the testa cells. The chalazal end is tapered, closed, and roughly rounded, with furrows of the anticlinal and periclinal walls of the testa cells. These features are similar to those of many other *Dendrobium* spp. (Swamy *et al.,* 2007; Aprilianti *et al.,* 2021; Prasongsom *et al.,* 2022; Betageri and Kotresha, 2024). The embryo is yellowish to greenish, prolate-spheroid-shaped, centrally placed, and occupies approximately 53.73% of the total seed volume. Accordingly, the seed was found to possess 46.27±16.07% air space, which is a typical feature of epiphytic or semi-terrestrial orchid species adapted for wind dispersal (Aprilianti *et al.,* 2021). It has been found that seeds with an air space value above 50% are light and optimally buoyant, so they can be dispersed easily by wind and spread to a wider geographical area (Hariyanto *et al.,* 2020). Correspondingly, the seeds of *D. gibsonii* are small, have less than 50 % air space, are less efficient in maintaining optimum buoyancy in the air current, and poorly disperse to spread over an extensive geographical area. Therefore, in *D. gibsonii*, the seed characteristics suggest that the seeds are less buoyant in the air and are less efficiently dispersed over a broad area. This corresponds with the actual distribution pattern of *D. gibsonii* and its restricted population sizes.

Furthermore, viability and permeability tests by scarification with NaOCl and subsequent double staining revealed fascinating results. The double-staining technique was used to assess both viability and permeability using the same treatment sets. These sets included the same seeds, different NaOCl concentrations, and varying scarification or treatment times for both viability and permeability studies. This approach helped minimise the assessment time and made the experiment easier and more accurate (Magrini *et al.,* 2019). The first remarkable result is that, relative to controls (non-scarified), the scarified seeds showed significantly higher viability and permeability. Several previous studies have observed two main reasons why NaOCl scarification increases the viability and permeability of orchid seeds. First, the micro-openings created by scarification facilitate imbibition, and the water entering the seeds initiates metabolic activation (Lee and Yeung, 2023). Second, scarification changes the chemical composition of the testa cell walls and weakens the lignified and waxy layers, facilitating smooth embryo expansion (Pierce *et al.,* 2019). Among the different NaOCl concentrations (0.5, 1.5, 3, and 5%) and scarification times (5, 10, 15, and 30 min), 1.5–3% for 10 –15 min was the optimal treatment, in which 1.5% for 15

min, 3% for 10 min 5% for 5 min exhibited the highest viability (93.16±1.11, 93.81±1.25, 93.07±1.16% respectively). However, scarification with 5% NaOCl for 15–30 min was detrimental, resulting in a decrease in viability relative to other treatments. Moreover, changes in permeability in relation to the control, as well as changes in NaOCl concentrations and scarification times, were also significant. Compared to the control, all scarified seeds showed higher permeability. Seed permeability increased gradually across all scarification sets, and maximum permeability was observed in the 5% NaOCl treatment for 15 and 30 min. In contrast, lower seed viability was observed at shorter scarification times and lower concentrations. Correspondingly, 5% NaOCl for 15–30 min was confirmed to be detrimental. This result may be due to the toxic effects of higher NaOCl concentrations (Vujanovic *et al.,* 2000). This is also evident from the findings that 1.5–3% for 10–15 min demonstrated optimum viability, whereas 5% for 15–30 min resulted in decreased viability.

V. CONCLUSION

The morphometric characterisation of *Dendrobium gibsonii* seeds, comprising their minute, dust-like nature, fusiform shape, and prolate-spheroid embryo, provides a basis for correlative taxonomic studies. Future research should follow the same course of study to analyse other *Dendrobium* species. This can verify whether the morphological traits assessed in the present study are species- or genus-specific. Furthermore, qualitative and quantitative evaluations of seed and embryo lengths and widths, seed-embryo ratio, and air space volume across many other species and genera will enable the development of characteristic markers for proper identification. The finding that *D. gibsonii* has a free air space volume of less than 50% indicates its lower buoyancy in air and water, which provides insight into the ecological constraints on its dispersal capacity. Future studies should assess dispersal potential through controlled buoyancy experiments. Moreover, NaOCl is a corrosive and toxic scarification agent; alternative scarification approaches, including enzymatic treatment, mechanical abrasion, or sonification, should be tested for their ability to increase permeability without impairing viability. Furthermore, the protocol for staining viable embryos with TTC is questionable. Therefore, exact in vitro germination experiments should be conducted to correlate viability and germination percentages. Simultaneously, there is scope for seed-storage studies in *D. gibsonii*. These can be used to examine the viability and longevity of seed viability under different temperature and humidity conditions, which adds to the conservation approaches.

ACKNOWLEDGEMENTS

We sincerely acknowledge the Head, Department of Botany, University of North Bengal, Darjeeling, West Bengal, for kindly permitting access to the departmental laboratory facilities. We also thank the faculty members of the department for their valuable support and cooperation during this study. Finally, we extend our sincere appreciation to the Technical Officers of the University Science and Instrumentation Centre (USIC), University of North Bengal, for providing access to the Scanning Electron Microscope facility and for their assistance in obtaining the SEM images of the seed samples.

REFERENCES

[1] Alomía, J. A., Muñoz, E., Acosta, A. M., & Otero, J. T. (2016). Morphometric analysis of *Vanilla* seeds (Orchidaceae) by microscopic techniques. *Lankesteriana, 16*(1), 21–26. https://doi.org/10.15517/lank.v16i1.23501

[2] Aprilianti, P., Handini, E., & Puspitaningtyas, D. M. (2021). A seed morphometry study of selected species of *Bulbophyllum* and *Dendrobium* (Orchidaceae) in relation to their dispersals. *Biodiversitas, 22*(12), 5564–5571. https://doi.org/10.13057/biodiv/d221241

[3] Arditti, J., & Abdul Ghani, A. K. (2000). Numerical and physical properties of orchid seeds and their biological implications. *New Phytologist, 145*(3), 367–421. https://doi.org/10.1046/j.1469-8137.2000.00587.x

[4] Aytar, E. C., Deniz, I. G., İncedere, D., Kömpe, Y. O., Basili, T., Harzli, I., & Durmaz, A. (2024). Comparison of seed morphology and seed coat chemistry in *Ophrys* (Orchidaceae) species. *ACS Omega, 9*, 33773–33788. https://doi.org/10.1021/acsomega.4c03130

[5] Barthlott, W., Große-Veldmann, B., & Korotkova, N. (2014). *Orchid seed diversity: A scanning electron microscopy survey*. Botanic Garden and Botanical Museum Berlin.

[6] Betageri, S., & Kotresha, K. (2024). Seed morphometrics with embryo diversity of wild orchids in North Karnataka. *Journal of Diversity Studies,* (2),39–50. https://doi.org/10.51470/JOD.2024.03.02.39

[7] Diantina, S., McGill, C., Millner, J., Nadarajan, J., Pritchard, H. W., & McCormick, A. C. (2020). Comparative seed morphology of tropical and temperate orchid species with different growth habits. *Plants, 9*(2), Article 161. https://doi.org/10.3390/plants9020161

[8] Hariyanto, S., Pratiwi, I. A., & Utami, E. S. W. (2020). Seed morphometry of native Indonesian orchids in the genus *Dendrobium*. *Scientifica*, Article 3986369, 1–14. https://doi.org/10.1155/2020/3986369

[9] Hussain, M. F., & Eraqui, S. (2023). Orchids: A wonderful ornamental plant. *Asian Journal of Crop Science, 8*(4), 167–172. https://doi.org/10.9734/ajrcs/2023/v8i4197

[10] Iiyama, C. M., Vilcherrez-Atoche, J. A., Germanà, M. A., Vendrame, W. A., & Cardoso, J. C. (2024). Breeding of ornamental orchids with focus on *Phalaenopsis*: Current

approaches, tools, and challenges for this century. *Heredity, 132*(4), 163–178. https://doi.org/10.1038/s41437-024-00671-8

[11] Ketsa, S., & Warrington, I. J. (2023). The *Dendrobium* orchid: Botany, horticulture, and utilization. *Crop Science, 63*, 1829–1888. https://doi.org/10.1002/csc2.20952

[12] Khuraijam, J. S., Sharma, S. C., & Roy, R. K. (2017). Orchids: Potential ornamental crop in North India. *International Journal of Horticulture & Crop Science Research, 7*(1), 1–18.

[13] Kumar, J., Katoch, D., Thakur, A., Pathania, A., Anand, A., Choudhary, K., & Shelja. (2024). A comprehensive review on threats and conservation status of orchids. *Journal of Applied Biology and Biotechnology, 12*(2), 43–47. https://doi.org/10.7324/JABB.2024.150084

[14] Lee, Y. I., & Yeung, E. C. (2023). The orchid seed coat: A developmental and functional perspective. *Botanical Studies, 64*, Article 27, 1–15. https://doi.org/10.1186/s40529-023-00400-0

[15] Li, C., Dong, N., Zhao, Y., Wu, S., Liu, Z., & Zhai, J. (2021). A review for the breeding of orchids: Current achievements and prospects. *Horticulture, Environment, and Biotechnology, 7*(5), 380–392. https://doi.org/10.1016/j.hpj.2021.02.006

[16] Liu, M., Yuan, L., Wu, L., Lu, Z., Yang, C., & Jin, C. (2025). The establishment of a micropropagation system for *Dendrobium kingianum* via somatic embryogenesis. *Agrobiodiversity, 2*(3), 56–58. https://doi.org/10.48130/abd-0025-0007

[17] Magrini, S., & de Vitis, M. (2017). In vitro reproduction of three *Limodorum* species (Orchidaceae): Impacts of scarification methods and nitrogen sources on mature seed germination and seedling development. *Plant Biosystems, 151*(3), 419–428. https://doi.org/10.1080/11263504.2016.1179698

[18] Magrini, S., Barreca, D., & Zucconi, L. (2019). A rapid double-staining technique to improve seed viability testing in terrestrial orchids. *Plant Biosystems, 153*(6), 877–882. https://doi.org/10.1080/11263504.2019.1587541

[19] Maulida, D., Yusnita, Y., Hapsoro, D., Agustiansyah, A., & Karyanto, A. (2023). Interspecific hybridization of *Dendrobium mirbelianum* × *D. nindii* or *D. discolor*, in vitro seed germination, seedling growth and plantlet acclimatization. *Biodiversitas, 24*(5), 3004–3011. https://doi.org/10.13057/biodiv/d240555

[20] Pierce, S., Spada, A., Caporali, E., Ceriani, R. M., & Buffa, G. (2019). Enzymatic scarification of *Anacamptis morio* (Orchidaceae) seed facilitates lignin degradation, water uptake and germination. *Plant Biology, 21*, 409–414. https://doi.org/10.1111/plb.12694

[21] Pradhan, N., Fan, X., Martini, F., Chen, H., Liu, H., Gao, J., & Goodale, U. M. (2022). Seed viability testing for research and conservation of epiphytic and terrestrial orchids. *Botanical Studies, 63*(1), Article 3. https://doi.org/10.1186/s40529-022-00333-0

[22] Prasongsom, S., Thammasiri, K., & Pritchard, H. W. (2022). Seed dormancy concepts in orchids: *Dendrobium cruentum* as a model species. *Seed Science Research, 32*, 175–186. https://doi.org/10.1017/S0960258522000198

[23] Sharma, H. B., & Bhattacharyya, D. (2023). Checklist of the genus *Dendrobium* Sw. (Orchidaceae) in Manipur, India. *Journal of Threatened Taxa, 15*(7), 23567–23574. https://doi.org/10.11609/jott.8272.15.7.23567-23574

[24] Soares, J. S., Sorgato, J. C., Ribeiro, L. M., & Ramos, J. M. C. (2021). Seed viability test of orchids native to the Brazilian savanna. *Pesquisa Agropecuária Tropical, 51*, 1–7. https://doi.org/10.1590/1983-40632021v5167069

[25] Swamy, K. K., Kumar, H. N. K., & Ramaswamy, S. N. (2007). Studies on seed morphometry of *Dendrobium* species. *Phytomorphology, 57*(1–2), 33–43.

[26] Teoh, E. S. (2016). *Medicinal orchids of Asia*. Springer International Publishing.

[27] Vitt, P., Taylor, A., Rakosy, D., Kreft, H., Meyer, A., Weigelt, P., & Knight, T. M. (2023). Global conservation prioritization for the Orchidaceae. *Scientific Reports, 13*(1), Article 6718. https://doi.org/10.1038/s41598-023-30177-y

[28] Vujanovic, V., St-Arnaud, M., Barabé, D., & Thibeault, G. (2000). Viability testing of orchid seed and the promotion of colouration and germination. *Annals of Botany, 86*, 79–86. https://doi.org/10.1006/anbo.2000.1162

[29] Waes, J. M., & Debergh, P. C. (1986). Adaptation of the tetrazolium method for testing seed viability and scanning electron microscopy study of some Western European orchids. *Physiologia Plantarum, 66*(3), 435–442. https://doi.org/10.1111/j.1399-3054.1986.tb05947.x

[30] World Flora Online. (n.d.). *World Flora Online taxon WFO-0000939265*. Retrieved May 4, 2026, from https://www.worldfloraonline.org/taxon/wfo-0000939265

[31] Yoder, J. A., Zettler, L. W., & Stewart, S. L. (2000). Water requirements of terrestrial and epiphytic orchid seeds and seedlings, and evidence for water uptake by means of mycotrophy. *Plant Science, 156*, 145–150. https://doi.org/10.1016/S0168-9452(00)00246-6