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Chapter in book:

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Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

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Variations in seed micromorphology and morphometry of native Indonesian *Phalaenopsis* and *Paphiopedilum* orchids

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Abstract. Hariyanto S. 2019. Variations in seed micromorphology and morphometry of native Indonesian *Phalaenopsis* and *Paphiopedilum* orchid. *Biodiversitas* 20: 3559-3567. Seeds of ten taxa of the genera *Phalaenopsis* and *Paphiopedilum* orchids were studied using light and scanning electron microscopy (SEM). Qualitative characters (seed and embryo shape, seed and embryo colour, ornamentation of testa cell, testa cell wall shape) as well as quantitative data (seed and embryo length, seed and embryo width, seed and embryo volume, seed length/seed width, embryo length/embryo width, seed volume/embryo volume and air space) were analysed. Seeds of all studied taxa were fusiform in shape and had smooth testa surfaces. *Phalaenopsis* testa cells were elongated with cylindrical cell walls, while *Paphiopedilum* testa cells were polygonal with thin and flat rectangular cell walls. The shape of the embryos was generally ovoid in *Phalaenopsis* and prolate in *Paphiopedilum*. Seed colors ranged from brown to dark brown. Embryo colors varied from light yellow, yellowish-brown, dark brown, black and white. Based on our investigation, there are variations in seed and embryo length, seed and embryo width, seed and embryo volume as well as the percentage of the air space, both in *Phalaenopsis* and *Paphiopedilum*. In general, the values of seed volume, embryo volume and air space in *Paphiopedilum* are higher than in *Phalaenopsis*. Together, the results of the study indicate that morphological and morphometric features can serve to identify live forms and distinguish between species.

Keywords: Epiphytic versus terrestrial habitats, testa cell shapes, testa cell wall shapes, seed air spaces, seed volume

INTRODUCTION

Phalaenopsis Blume and *Paphiopedilum* Pfitzer are orchids whose members are very popular and are commercialized as the most traded potted and cut flower plants in the world. Wild populations are under threat of extinction due to high rates of habitat modification, deforestation, forest fires, illegal harvesting and trade as a consequence of rapid economic development, high population growth and corrupt institutions (Sodhi et al. 2004). All *Paphiopedilum* species are listed by the Conservation on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Appendix I and *Phalaenopsis* are listed in Appendix II. This is in spite of the fact that, according to the CITES, all species from the genus *Paphiopedilum* are prohibited for trade.

Seeds occupy the first rank in the life history of plants. Orchid seeds disperse, germinate and grow into mature plants, and reproduce in a suitable place on certain parts of the tree or on the ground. In general, seeds are responsible for the regeneration and distribution of a species, even playing an important role in the conservation of orchids. Verma et al. (2014) and Tsutsumi et al. (2007) explain that seed dispersal mode and seed size are important factors in regulating the growth of new populations.

Seed morphology varies greatly in shape, color, size, volume of the embryo and testa structure, and some of these characters have been used to establish the phylogeny of species in the genus (Gamarra et al. 2008, 2010, 2015; Cela et al. 2014; Guler 2016). In addition, some seed characters can be related to germination and dispersal, especially in their ecological adaptation (Chaudhary et al. 2014; Zhang et al. 2015). According to research by Chaudhary et al. (2014) in the *Dendrobium* orchid, the volume of the embryo and the percentage of the air space are directly related to the climate and this is reflected in seed ultrastructure.

Previous studies on the morphology of orchid seeds in tropical areas have demonstrated the importance of the seed coat, related to the taxonomy and ecology (Chase and Phippen 1988; Tsutsumi et al. 2007; Akçin et al. 2009; Verma et al. 2012; Molvray and Chase 1999), as demonstrated with scanning electron microscopy (SEM).

Not much is known about how certain seed characteristics may correlate with ecological adaptations. The aim of this study is to reveal the qualitative and quantitative characteristics of the seeds of several species of epiphytic and terrestrial orchids native to Indonesia from different genera, thus contributing to a better understanding of the differences and similarities in their adaptation strategies for seeds in the tropics.

MATERIALS AND METHODS

Plant materials and seed collection

Ten different orchid species were collected from Simanis and DD Orchids Nursery, East Java, Indonesia. The collection for the present study included *Phal. amabilis*, *Phal. amboinensis*, *Phal. bellina*, *Phal. gigantea*, *Phal. tetraspis*, *Phal. venosa*, *Paph. bacchanum*, *Paph. kolopakingii*, *Paph. liemianum*, *Paph. primulinum*; these plants were then hand pollinated during their normal period of flowering. The seeds of mature capsules were collected from 2016 to 2019. The seed samples were taken from 1-2 capsule (s) for each species. The matured capsules that are 3.5-4 months old for *Phalaenopsis* and 8.5-9 months for *Paphiopedilum* were used in this study. The capsules were washed using 10% sunlight detergent solution for 3 minutes to eliminate dust particles, then rinsed 3 times with sterile distilled water. The surface of the capsules was sprayed with 70% alcohol, put on a Petri dish, placed into a laminar flow cupboard and passed over a Bunsen flame; this was repeated 3 times. The capsule was cut into four parts transversally and longitudinally using a sterile scalpel in a sterile Petri dish. The mature seeds were released from the capsules and collected with the help of a sterile spatula. The specimens used for SEM were dried for at least 4 weeks at room temperature and stored in small paper envelopes. All species within *Phalaenopsis* in this study are epiphytic orchids while all species within *Paphiopedilum* are terrestrial orchids.

Morphological and statistical analyses

Seed samples were observed and photographed under a Light Microscope (LM) and Scanning Electron Microscope (SEM). Our morphological parameters included seed shape (SS), seed colour (SC), seed length (SL), seed width (SW), seed length/seed width (SL/SW), seed volume (SV). For embryos, the parameters included embryo shape (ES), embryo colour (EC), embryo length (EL), embryo width (EW), embryo length/embryo width (EL/EW), embryo volume (EV), seed volume/embryo volume (SV/EV), and air space (AS). The SV ($\text{mm}^3 \times 10^{-3}$) was calculated using the formula $2 \left[\left(\frac{L}{2}\right) \left(\frac{W}{2}\right)^2 \left(\frac{\pi}{3}\right) \right]$, where L = length, W = width, $\pi = 22/7$ and embryo volume ($\text{mm}^3 \times 10^{-3}$) with the formula $\frac{4}{3} \pi \left(\frac{L}{2}\right) \left(\frac{W}{2}\right)^2$, where L = length, W = width (adapted from Arditti et al. 1980). The SV/EV values were calculated following the described method (Arditti et al. 1979). For calculating AS (%), the following formula was applied: $(\text{seed volume} - \text{embryo volume}) / \text{seed volume} \times 100\%$ (adapted from Arditti and Ghani 2000). To analyze each species statistically, we used the mean for each quantitative character.

LM study

Seeds were spread on a slide with a drop of water and covered with a cover glass. Values for SL, SW, EL, and EW (at the longest and widest axis) were obtained from an average of thirty seeds and were observed for each species and measurement using a light microscope (Olympus CH 20, Olympus Japan) and standardized ocular meter. Characteristics such as SC, SS, EC, and ES were observed

under Tension stereomicroscope (Nikon SMZ-1, Japan). The SC and EC were described in subjective terms.

SEM study

For SEM preparations, the samples (seeds) were mounted on SEM stubs. The samples were then sputter-coated with palladium/gold (SEM coating system Q150R S mini sputter Coater). Detailed seed coat (testa cells) surface studies were conducted by observing under a Hitachi TM3000, with a filament voltage of 15 kV. The parameters considered were seed coat sculpturing and thickenings.

RESULTS AND DISCUSSION

Qualitative character of seed and embryos

The characteristics of *Phalaenopsis* seeds distinguished from *Paphiopedilum* seeds are elongated testa cell shape, cylindrical testa cell wall shape, ovoid embryo shape, and brown transparent seeds color are showed in Table 1.

Phal. amabilis, *Phal. amboinensis*, *Phal. bellina*, *Phal. gigantea*, *Phal. tetraspis* and *Phal. venosa* show similar character i.e fusiform seed shape, brown and transparent seed color, smooth ornamentation of the periclinal walls, elongated testa cell shape, cylindrical testa cell walls shape and ovoid embryo shape (Table 1).

In the present work, the embryo color of *Phal. amabilis* was yellowish-brown. The testa cells were elongated, longitudinally oriented, parallel to the long seed axis and irregular. The testa cell walls were cylindrical shape and exhibited overlap (Figure 2.1B arrow), becoming circle and rising at the end of the meeting between two testa cells (Figure 2.1D arrow). Certain parts of the testa formed deep furrows (Figure 2.1C arrow).

The embryo color of *Phal. amboinensis* was yellow. The testa cells were elongated, longitudinally oriented and parallel to long axis and irregular. The testa cells walls were cylindrical. Part of the meeting at the end of the two cells was rounded and slightly raised (Figure 2.2B arrow). The end of the basal pole was blunt (Figure 2.2C), while the end of the apical pole became pointed (Figure 2.2D).

Phal. bellina has brown embryo color. The testa cells were elongated, longitudinally oriented, parallel to the long seed axis and irregular. The testa cells walls were cylindrical and the distance between two cells formed a slit in a row (Figure 3.1B). Testa cells were curved, thickened and raised to form a bulge at the meeting point between the two ends (Figure 3. 1B arrow). There was a difference in the shape of the testa cells at the two poles. Cells of the basal pole were bigger and rounder than cells of the apical pole (Figure 3.1C and D).

In *Phal. gigantea*, the embryo color was brown. The testa cells were longitudinally oriented, parallel to the long seed axis and highly irregular. Ridges were elevated with a deep groove (Figure 3. 2B black arrow). Two ends of the testa cells were curved and raised at the meeting point (Figure 3.2B white arrow). Cells of the basal pole were different from cells at the apical pole; cells at the basal pole had an appendage at the end and become pointed (Figure 3. 2C arrow).

Table 1. Morphological characteristics of the genus *Phalaenopsis* and *Paphiopedilum*

Species name	Seed shape	Seed Colour	Ornamentation of the periclinal walls	Testa cell shape	Testa cell walls shape	Embryo Shape	Embryo Colour
<i>Phalaenopsis</i>							
<i>Phal. amabilis</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellowish-brown
<i>Phal. amboinensis</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellow
<i>Phal. bellina</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Brown
<i>Phal. gigantea</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Brown
<i>Phal. tetraspis</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Yellowish-brown
<i>Phal. venosa</i>	Fusiform	Brown and transparent	Smooth	Elongated	Cylindrical	Ovoid	Light yellow
<i>Paphiopedilum</i>							
<i>Paph. baccanum</i>	Fusiform	Brown	Smooth	Polygonal	Thin rectangular	Prolate	Dark brown
<i>Paph. kolopakingii</i>	Fusiform	Dark brown	Smooth	Polygonal	Flat rectangular	Prolate	Brown
<i>Paph. liemianum</i>	Fusiform	Dark brown	Smooth	Polygonal	Thin rectangular	Prolate	White
<i>Paph. primulinum</i>	Fusiform	Brown	Smooth	Polygonal	Flat rectangular	Prolate	Black

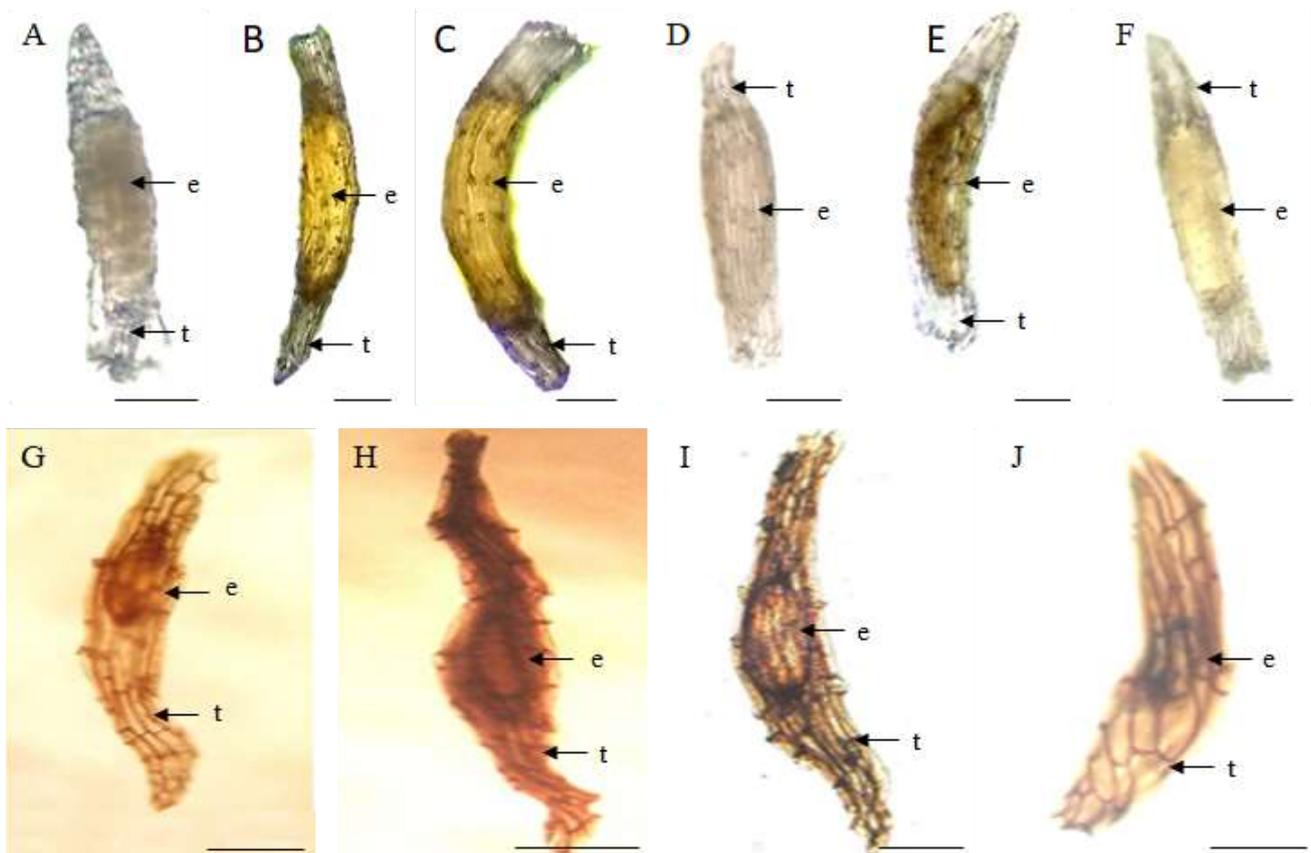


Figure 1. LM photographs. A. *Phal. amabilis* (scale bar=72 µm), B. *Phal. amboinensis* (scale bar=59 µm), C. *Phal. bellina* (scale bar=65 µm), D. *Phal. gigantea* (scale bar=51 µm), E. *Phal. tetraspis* (scale bar=49 µm), F. *Phal. venosa* (scale bar=55 µm), G. *Paph. baccanum* (scale bar=185 µm), H. *Paph. kolopakingii* (scale bar=206 µm), I. *Paph. liemianum* (scale bar=162 µm), J. *Paph. primulinum* (scale bar=161 µm). e= embryo, t=testa.

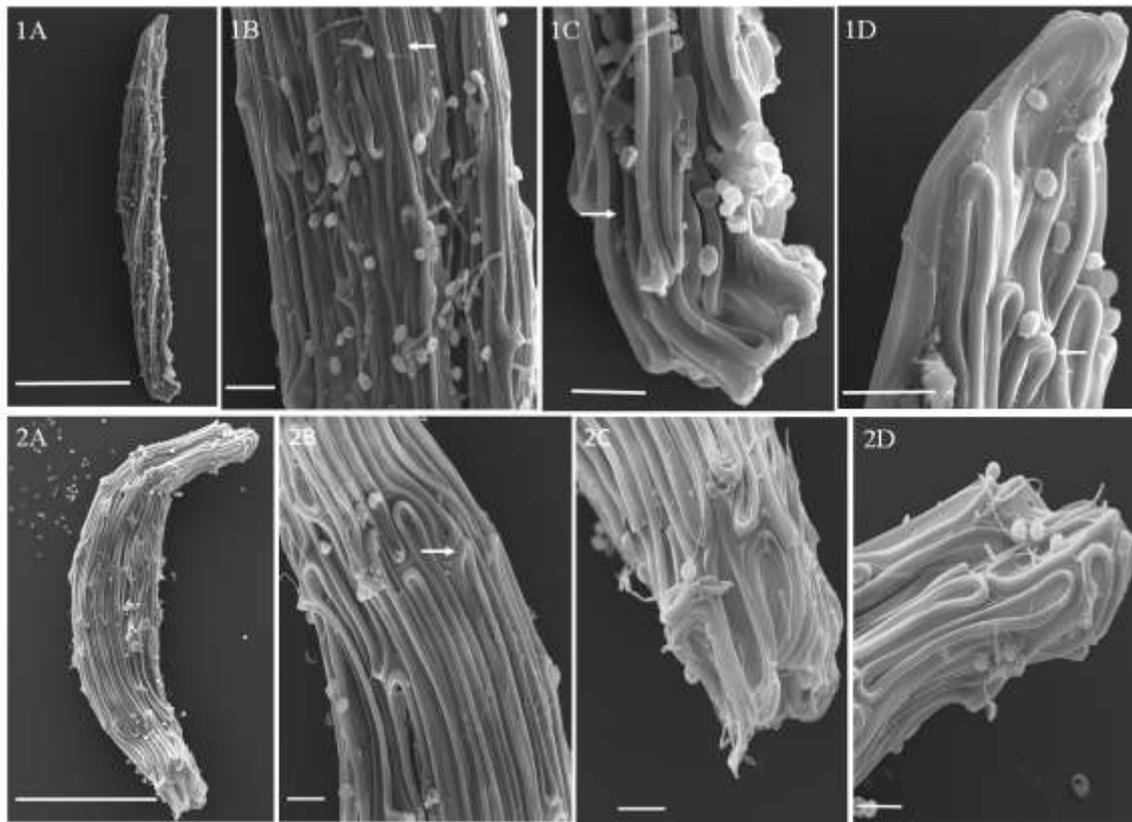


Figure 2. SEM photographs. 1. *Phal. amabilis*, 2. *Phal. amboinensis*, A. Seed shape, B. Testa cells of the medial, (1B. arrow indicate the testa cell overlap, 2B. arrow indicate the end of two cells was rounded and slightly raised) C. Basal pole, (1C. arrow indicates the testa formed deep furrow) D. Apical pole (1D. arrow indicate the end of the meeting between two testa cells becoming round and rising). Scale bars: A=100 μ m, B-D=10 μ m.

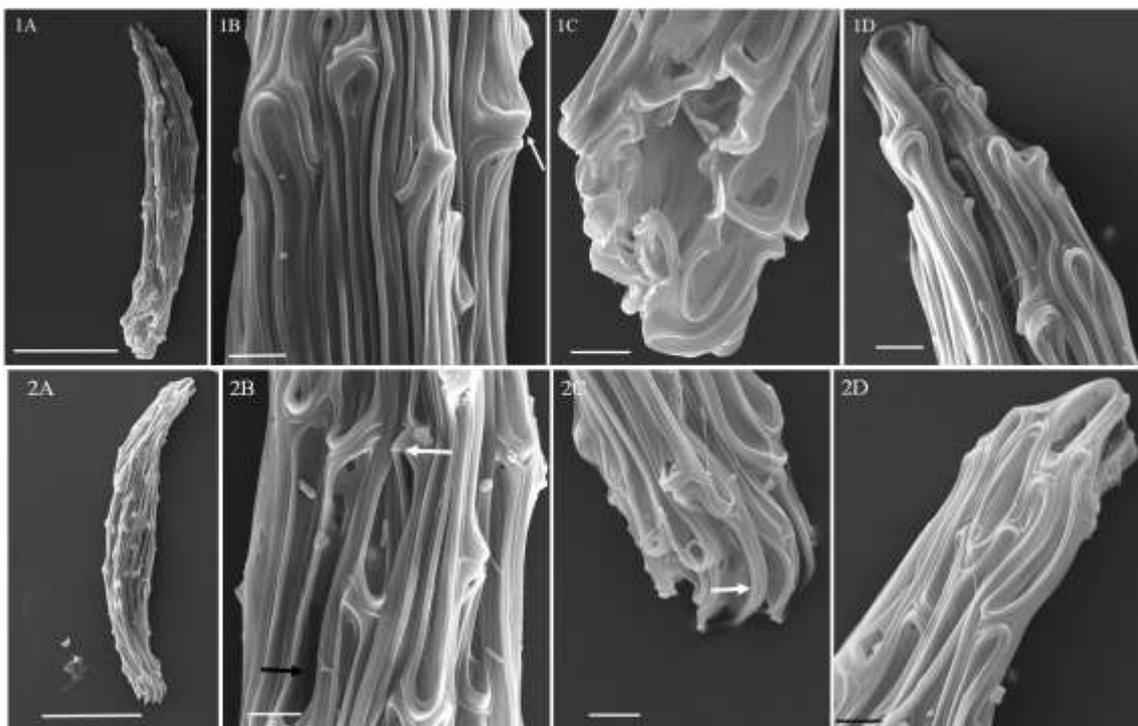


Figure 3. SEM photographs. 1. *Phal. bellina*, 2. *Phal. gigantea*, A. Seed shape, B. Testa cells of the medial (1B. arrow indicate cells are rounded and slightly raised, 2B white arrow indicates the testa cell are curved and raised at the end of two testa cells, black arrow indicates ridge with deep groove), C. Basal pole (2C. arrow indicate the end testa cells at the basal pole had appendage with point of the end), D. Apical pole. Scale bars: A=100 μ m, B-D=10 μ m.

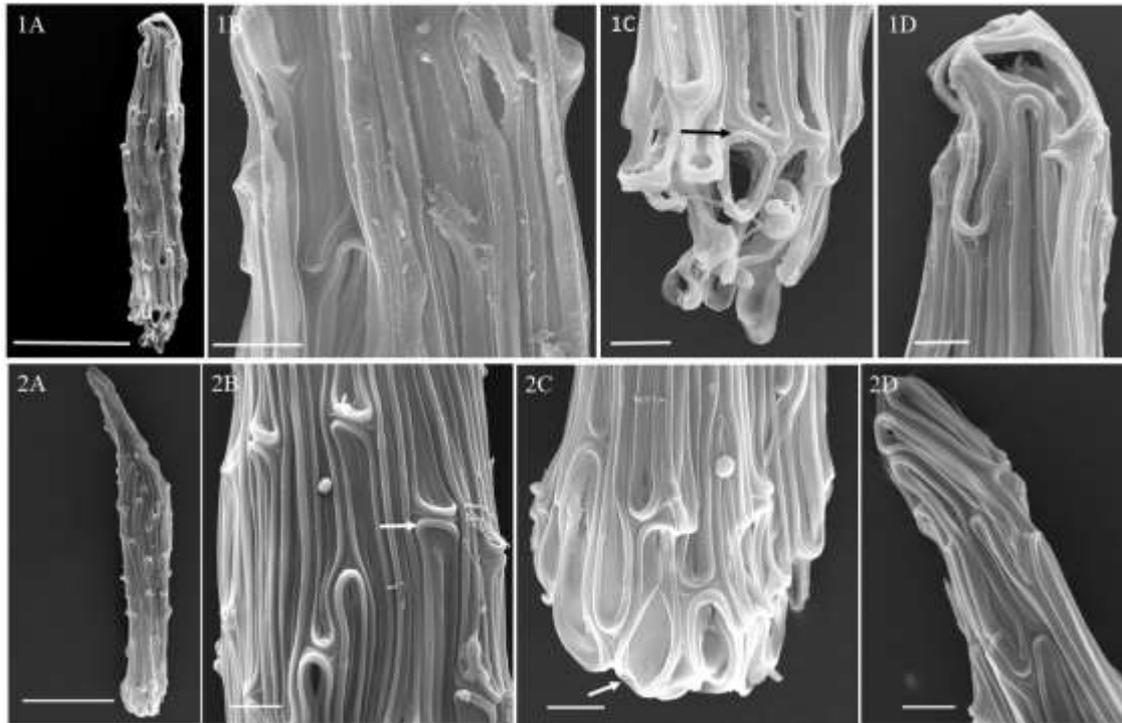


Figure 4. SEM photographs. 1. *Phal. tetraspis*. 2. *Phal. venosa*. A. seed shape, B. Testa cells of the medial (2B. arrow indicates the testa cell walls are raised at the end of the meeting between two cells), C. Basal pole (1C. black arrow indicates the testa cell become wider, rounder, and rose up, 2C. arrow indicates the testa cells at the end of basal pole were widened and rounded), D. Apical pole. Scale bars: A=100 μm ; B-D = 10 μm .

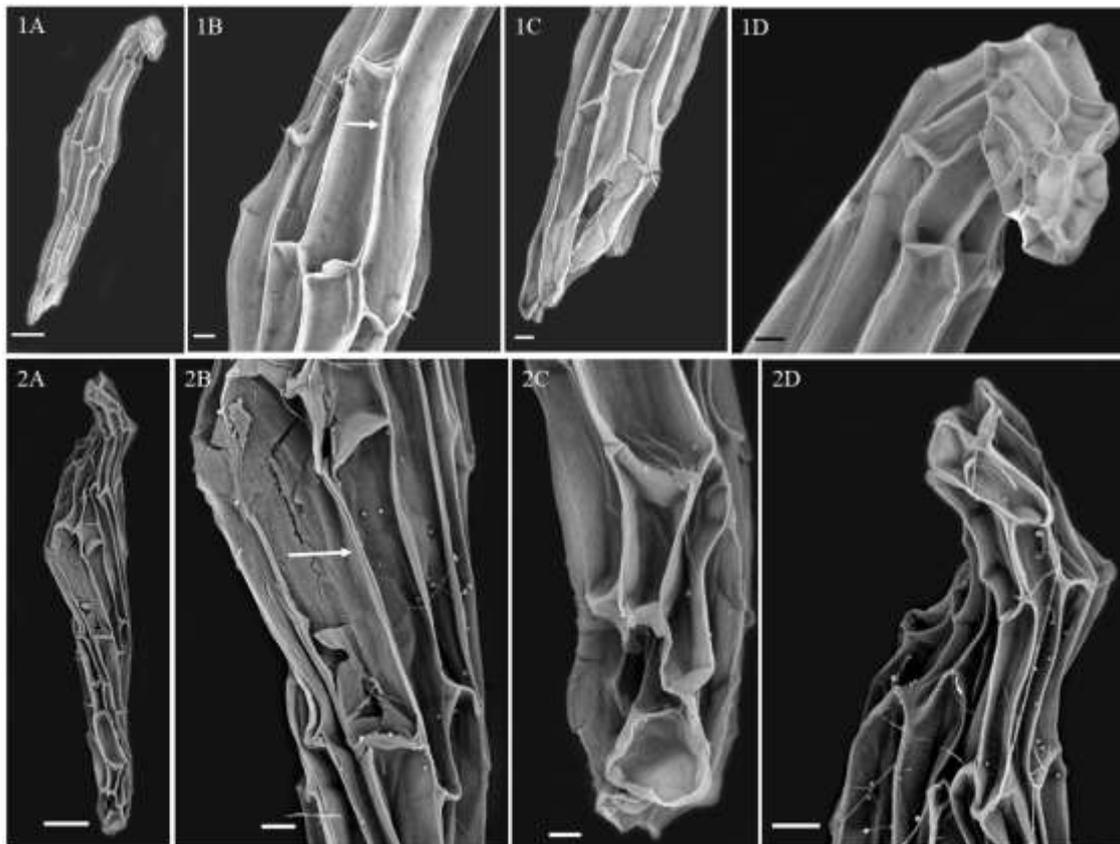


Figure 5. SEM photographs. 1. *Paph. baccanum*. 2. *Paph. kolopakingii*. A. Fusiform seed shape, B. Testa cells of the medial (1B. arrow indicate the testa cell walls are thin, 2B. arrow indicates the cell walls were flat), C. Basal pole, D. Apical pole. Scale bars: A=80 μm , B-D = 20 μm .



Figure 6. SEM photographs. 1. *Paph. liemianum*. 2. *Paph. primulinum*. A. seed shape, B. Testa cells of the medial. (1B. arrow indicates the testa cell walls are thin. 2B. arrow indicates the testa cell walls were flat), C. Basal pole (1C arrow indicates the shape of testa cells at basal pole are polygonal), D. Apical pole. Scale bars: A=80 μ m, B-D = 20 μ m

The embryo color of *Phal. tetraspis* was yellowish-brown. The testa cells were elongated, longitudinally oriented and parallel to the long axis and irregular. The testa cell walls were cylindrical and close proximity (Figure 4.1). At the end of the meeting between two testa cells, the cell walls became wider, rounder and rose up (Figure 4.1C arrow). There was a difference in the shape of the testa cells at the two poles. Cells at the basal pole were smaller than cells at the apical pole.

In the case of *Phal. venosa*, the embryo color was light yellow. The testa cells were longitudinally oriented, parallel to the long axis, irregular, and raised at the end of the meeting between two cells (Figure 4.2B arrow). Testa cells at the end of the basal pole were widened and rounded (Figure 4.2C arrow), but testa cells at the apical pole were more elongated and pointed (Figure 4.2D).

Paphiopedilum seeds can be differentiated with seeds of *Phalaenopsis* in terms of polygonal testa cell shape, rectangular testa cell walls and prolate embryo shape. *Paph. baccanum*, *Paph. kolopakingii*, *Paph. liemianum* and

Paph. primulinum have same character i.e fusiform seed shape, smooth ornamentation of the periclinal walls, polygonal testa cell shape, rectangular testa cell walls shape and prolate embryo shape (Table 1).

In the case of *Paph. baccanum*, the seed color was brown and embryo color was dark brown. Generally, the testa cells were longitudinally oriented with a deep groove, parallel to the long axis and irregular (Figure 5.1). The testa cell walls were thin (Figure 5.1B arrow). Cells at the basal pole were longer compared to cells at the apical pole. The end of the basal pole became pointed, while the end of the apical pole became rounded (Figures 5.1C and D).

In *Paph. kolopakingii*, the seed color was dark brown and embryo color was brown. Testa cells were longitudinally oriented with a deep groove, parallel to the long axis and irregular. The testa cell walls were flat (Figure 5.2B arrow). The basal cell pole was larger and more dented than the cells of the apical pole (Figures 5.2C and D).

Table 2. Micro-morphometric data of seeds related characters (mean \pm SD) from species of the genus *Phalaenopsis* and *Paphiopedilum*

Species name	SL (mm)	SW (mm)	SL/SW (mm)	SV ($\text{mm}^3 \times 10^{-3}$)
<i>Phalaenopsis</i>				
<i>Phal. amabilis</i>	0.433 \pm 0.008	0.072 \pm 0.003	6.029 \pm 0.285	0.589 \pm 0.050
<i>Phal. ambonensis</i>	0.349 \pm 0.006	0.059 \pm 0.002	5.915 \pm 0.221	0.318 \pm 0.020
<i>Phal. bellina</i>	0.302 \pm 0.008	0.065 \pm 0.002	4.646 \pm 0.200	0.333 \pm 0.027
<i>Phal. gigantea</i>	0.348 \pm 0.009	0.051 \pm 0.003	6.823 \pm 0.487	0.240 \pm 0.033
<i>Phal. tetraspis</i>	0.370 \pm 0.009	0.049 \pm 0.004	7.472 \pm 0.657	0.242 \pm 0.036
<i>Phal. venosa</i>	0.345 \pm 0.007	0.055 \pm 0.003	6.305 \pm 0.395	0.272 \pm 0.032
<i>Paphiopedilum</i>				
<i>Paph. baccanum</i>	0.815 \pm 0.002	0.185 \pm 0.002	4.401 \pm 0.043	7.324 \pm 0.128
<i>Paph. kolopakingii</i>	0.874 \pm 0.008	0.206 \pm 0.002	4.254 \pm 0.049	9.668 \pm 0.177
<i>Paph. liemianum</i>	0.983 \pm 0.003	0.162 \pm 0.003	6.074 \pm 0.129	6.755 \pm 0.267
<i>Paph. primulinum</i>	0.815 \pm 0.043	0.161 \pm 0.009	5.083 \pm 0.388	5.532 \pm 0.640

Note: SL- Seed length, SW-Seed width, SV-Seed volume

Table 3. Micro-morphometric data of embryos related characters (mean \pm SD) from species of the genus *Phalaenopsis* and *Paphiopedilum*

Species name	EL (mm)	EW (mm)	EL/EW (mm)	EV ($\text{mm}^3 \times 10^{-3}$)	SV/EV ($\text{mm}^3 \times 10^{-3}$)	AS (%)
<i>Phalaenopsis</i>						
<i>Phal. amabilis</i>	0.187 \pm 0.004	0.072 \pm 0.003	2.607 \pm 0.132	0.509 \pm 0.043	1.158 \pm 0.033	13.554 \pm 2.469
<i>Phal. ambonensis</i>	0.203 \pm 0.005	0.051 \pm 0.002	3.993 \pm 0.190	0.274 \pm 0.020	1.165 \pm 0.053	14.245 \pm 3.641
<i>Phal. bellina</i>	0.211 \pm 0.013	0.051 \pm 0.002	4.149 \pm 0.361	0.286 \pm 0.024	1.168 \pm 0.030	14.294 \pm 2.238
<i>Phal. gigantea</i>	0.192 \pm 0.007	0.043 \pm 0.302	4.496 \pm 0.302	0.184 \pm 0.024	1.308 \pm 0.074	23.291 \pm 4.359
<i>Phal. tetraspis</i>	0.171 \pm 0.008	0.046 \pm 0.003	3.739 \pm 0.305	0.191 \pm 0.030	1.273 \pm 0.082	21.128 \pm 5.090
<i>Phal. venosa</i>	0.183 \pm 0.003	0.047 \pm 0.002	3.929 \pm 0.192	0.209 \pm 0.014	1.309 \pm 0.172	22.338 \pm 10.028
<i>Paphiopedilum</i>						
<i>Paph. baccanum</i>	0.223 \pm 0.003	0.115 \pm 0.002	1.933 \pm 0.037	1.557 \pm 0.054	4.709 \pm 0.208	78.725 \pm 0.921
<i>Paph. kolopakingii</i>	0.270 \pm 0.003	0.157 \pm 0.004	1.726 \pm 0.042	3.466 \pm 0.186	2.797 \pm 0.150	64.150 \pm 1.936
<i>Paph. liemianum</i>	0.271 \pm 0.004	0.083 \pm 0.004	3.267 \pm 0.202	0.797 \pm 0.103	6.974 \pm 0.765	85.501 \pm 1.528
<i>Paph. primulinum</i>	0.274 \pm 0.011	0.105 \pm 0.009	2.636 \pm 0.216	1.590 \pm 0.309	3.608 \pm 0.845	70.898 \pm 6.344

Note: EL- Embryo length, EW- Embryo width, EV- Embryo volume, AS- Air space

The seed colour of *Paph. liemianum* were dark brown and white embryo. The testa cells were longitudinally oriented with a deep groove, parallel to the long axis and irregular (Figure 6.1). The testa cell walls were thin (Figure 6.1B arrow). The medial region was wider than the two poles. The basal cells were short, polygonal and more numerous than the apical cells (Figure 6.1C arrow).

Paph. primulinum had brown seed color and black embryo color. Generally, testa cells were longitudinally oriented with a deep groove, parallel to the long axis and irregular (Figure 6.2). The testa cell walls were flat (Figure 6.2B arrow). The end of the basal pole was blunt, while the end of the apical pole was suppressed (Figures 6.2C and D).

In this study, we did not observe any sculpturing on the cell walls or smooth in any species of the two different genera. This is probably because all of these species are equally alive and come from the tropics, despite their different living forms (epiphyte and terrestrial). This observation corresponds with the opinion of Chaudhary et al. (2014), who noted that seed ornamentations are directly related to the climatic preference of the species rather than its phylogeny. Similarly, according to Shimizu (2012), seed

coat forms that are independent of plant habitat have a wider seed dispersal. The images we observed with SEM showed that different species in the genus *Phalaenopsis* had the same shape of testa cells and cell walls, namely elongated with cylindrical cell walls. Similarly, different species in the genus *Paphiopedilum* had testa cells and cell walls with the same shape, namely polygonal and with thin/flat rectangular cells walls. However, these results contradict with the results of previous research by Arditti et al. (1979, 1980) and Swamy et al. (2004), who showed that testa cells of different species can vary significantly in shape.

Seeds size, ratio SL/SW and seed volume

Table 2 exhibits the size of seeds from ten species of the genus *Phalaenopsis* and *Paphiopedilum*. In spite of the fact that the seeds are microscopic, the result of the investigation showed great diversity in their size. Seed length ranged between 0.302 \pm 0.008 mm (*Phal. bellina*) and 0.983 \pm 0.003 mm (*Paph. liemianum*), and width ranged from 0.049 \pm 0.004 mm (*Phal. tetraspis*) to 0.206 \pm 0.002 mm (*Paph. kolopakingii*). According to Verma et al. (2014), the SL/SW ratio provides information on the degree of seed

truncation and is a good taxonomic character (Arditti et al. 1979 and Vij et al. 1992). In the present work, the species with seeds that were truncated ($SL/SW < 6$) were *Phal. amboinensis*, *Phal. bellina*, *Paph. baccanum*, *Paph. kolopakingii* and *Paph. primulinum*. The following species had elongated seeds ($SL/SW > 6$): *Phal. amabilis*, *Phal. gigantea*, *Phal. tetraspis*, *Phal. venosa* and *Paph. liemianum* (Table 2). The most truncated seeds ($SL/SW = 4.254 \pm 0.049$ mm) were recorded in *Paph. kolopakingii* and the most elongated seeds ($SL/SW = 7.472 \pm 0.657$ mm) in *Phal. tetraspis*, both of which are members of different genus. The seed volume showed variations in both *Phalaenopsis* and *Paphiopedilum* orchids. On average, low seed volume was found in *Phal. gigantea* (0.240 ± 0.0327 mm³ $\times 10^{-3}$), *Phal. tetraspis* (0.242 ± 0.036 mm³ $\times 10^{-3}$), *Phal. venosa* (0.272 ± 0.032 mm³ $\times 10^{-3}$), *Phal. amboinensis* (0.318 ± 0.020 mm³ $\times 10^{-3}$), *Phal. bellina* (0.333 ± 0.027 mm³ $\times 10^{-3}$) and *Phal. amabilis* (0.589 ± 0.050 mm³ $\times 10^{-3}$). When compared with epiphytic species, the seed volume was found to be higher in the terrestrial species (9.668 ± 0.177 mm³ $\times 10^{-3}$ (the highest) in *Paph. kolopakingii*, 7.324 ± 0.128 mm³ $\times 10^{-3}$ in *Paph. baccanum*, 6.755 ± 0.267 mm³ $\times 10^{-3}$ in *Paph. liemianum* and 5.532 ± 0.640 mm³ $\times 10^{-3}$ in *Paph. primulinum*. Clifford and Smith (1969), Rasmussen (1995), Swamy et al. (2004) and Verma et al. (2012) stated that seed size shows a direct correlation with plant habitat, and terrestrial orchids generally possess larger seeds as compared to epiphytic orchids. Yoder et al. (2010) also stated that the seeds of terrestrial orchids are bigger as compared to epiphytic orchids.

Seed to embryo volume and free air space

According to Verma et al. (2014), EV is an important character as it directly affects the percentage of the procurable air space inside the seed. Like their EL/EV ratios, EV was observed in various *Phalaenopsis* species; it was lowest in *Phal. gigantea* (0.184 ± 0.024 mm³ $\times 10^{-3}$) and highest in *Phal. amabilis* (0.509 ± 0.043 mm³ $\times 10^{-3}$). In *Paphiopedilum*, EV ranged between 0.797 ± 0.103 mm³ $\times 10^{-3}$ (*Paph. liemianum*) and 3.466 ± 0.186 mm³ $\times 10^{-3}$ (*Paph. kolopakingii*).

Our data showed that variation was observed in SV/EV. In the species of *Phalaenopsis*, the SV/EV ratio never exceeded two (Table 3), but in the species of *Paphiopedilum*, measurements peaked at 6.974 ± 0.765 mm³ $\times 10^{-3}$ in *Paph. liemianum*. This was followed by *Paph. baccanum* (4.709 ± 0.208 mm³ $\times 10^{-3}$), *Paph. primulinum* (3.608 ± 0.845 mm³ $\times 10^{-3}$) and *Paph. kolopakingii* (2.797 ± 0.150 mm³ $\times 10^{-3}$). Burgeff (1936) experimentally demonstrated the relationship between SV/EV ratio and seed buoyancy. Seeds with a high SV/EV ratio are more buoyant because they possess a greater air space.

Swamy et al. (2004, 2007) stated that AS is an important character because the seeds of most orchids are wind-dispersed, implying that seeds with a greater AS percentage will float in the air for a longer time and thus spread to more distant places. We observed that terrestrial species possessed a comparatively greater percentage of air space in their seeds as compared with epiphytic species. A

greater percentage of air space for *Paph. kolopakingii*, *Paph. liemianum*, *Paph. baccanum*, and *Paph. primulinum* was shown more than 60% of seed i.e. (64.150 ± 1.936), (85.501 ± 1.528), (78.725 ± 0.921) and (70.898 ± 6.344). Zhang et al. (2015) informed that higher AS values in seeds of terrestrial species can help spread them further along the forest floor where wind speeds are lower. Seeds with a greater percentage of air space in *Paphiopedilum* are expected to be more widely distributed and, in fact, we found it difficult to collect their seeds.

It can be concluded that species within *Paphiopedilum* produce larger seeds with smaller embryos and a bigger percentage of air space (AS) than those of *Phalaenopsis* species. Likewise, the volume of seeds and seed volume/embryo volume in *Paphiopedilum* are larger than in *Phalaenopsis*. Large air spaces will increase seed buoyancy and seeds can be dispersed further. Testa cells are elongated in shape, with cylindrical cells walls, in *Phalaenopsis* but they are polygonal, with thin or flat rectangular cell walls, in *Paphiopedilum*. There are some similarities in character that might be related to adaptation to the tropical area, because in all species studied, both epiphytic and terrestrial orchids have the same form of fusiform seeds and a smooth testa surface. The general results of this study inform us that the morphological and morphometric properties of orchid seeds could be utilized as a means of distinguishing between life forms and habitat similarity and aid in identification.

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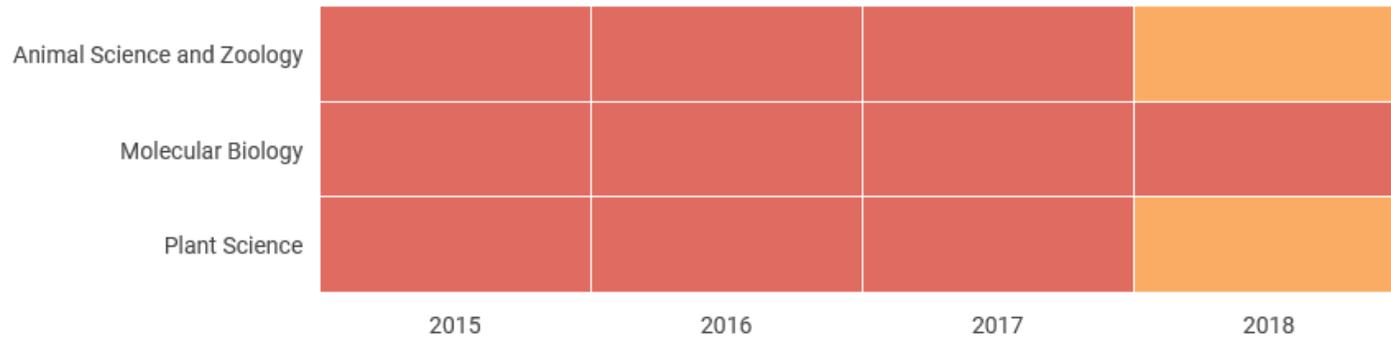
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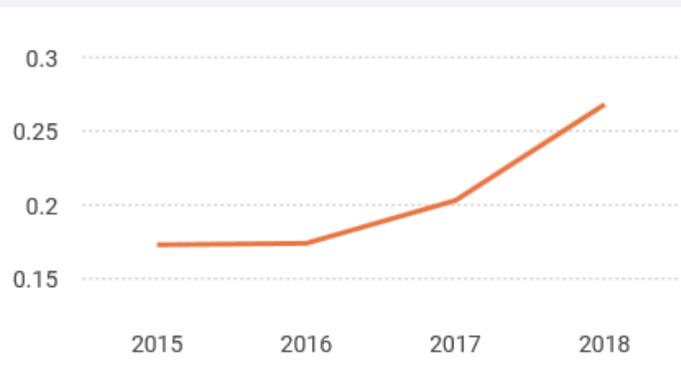


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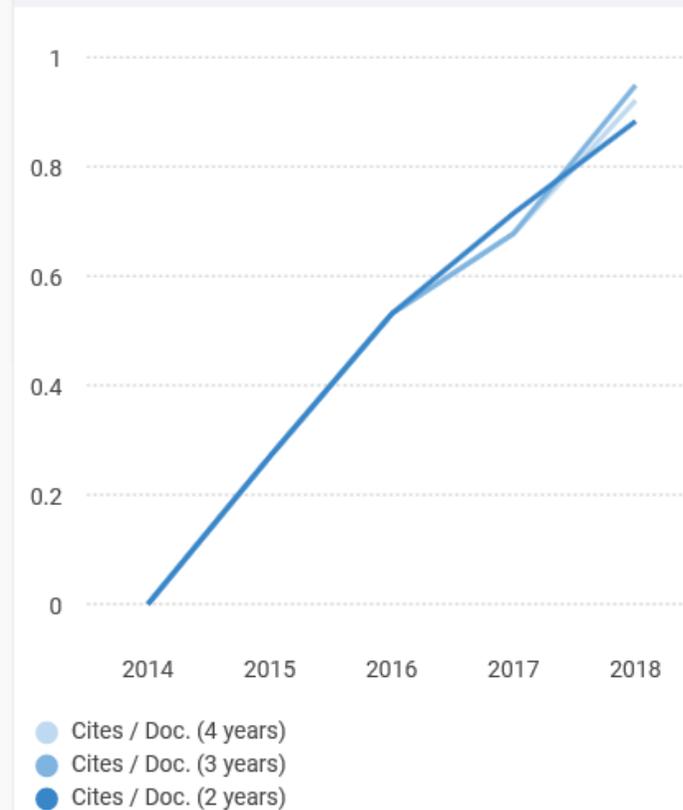
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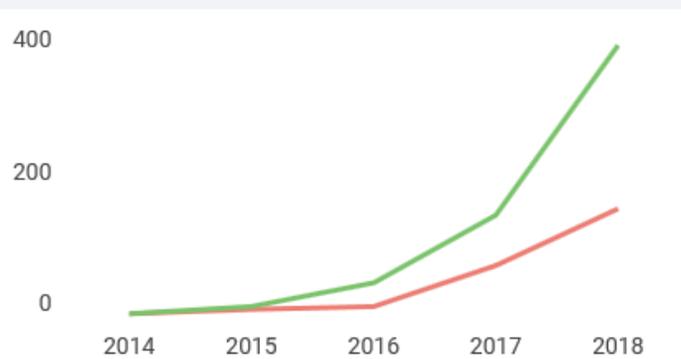
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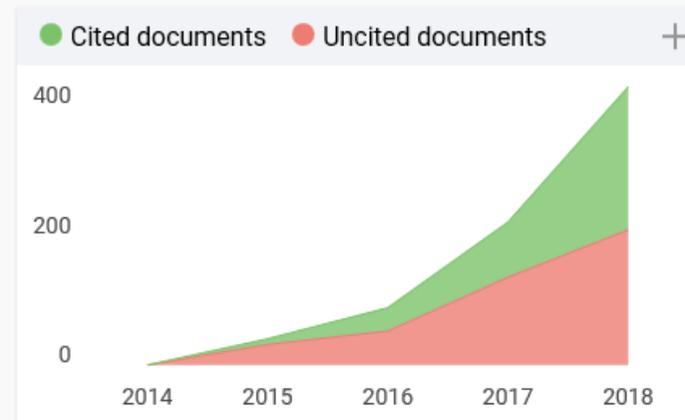
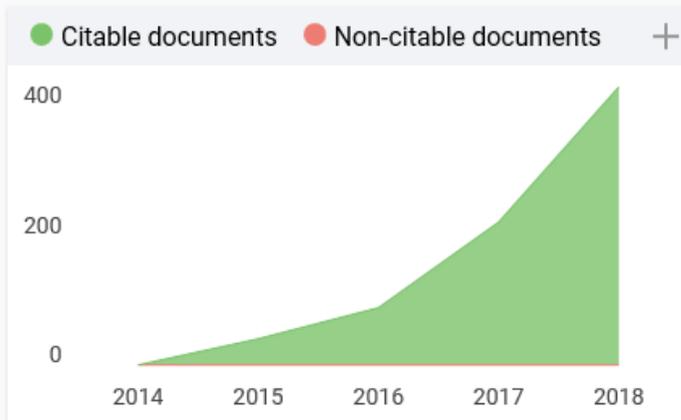
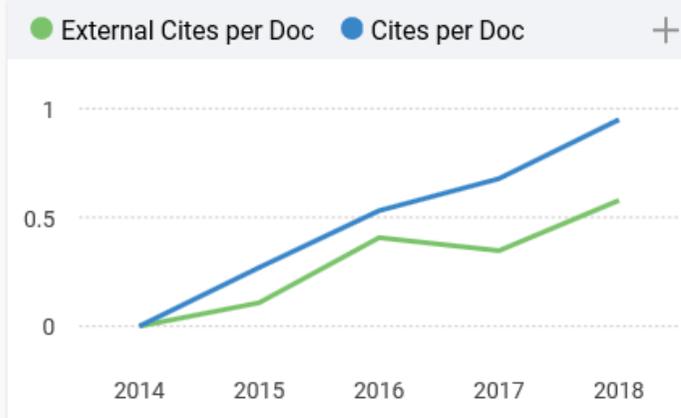


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