Jurnal Pembelajaran Dan Biologi Nukleus p-ISSN: 2442-9481
Vol 11 (4): 1265 - 1274, December 2025 e-ISSN: 2685-7332

Viability of *Vanda limbata* Orchid Seeds With Diferent Storage Period in Organic Tomato Extract Media

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Submitted September 20 Th 2025, and Accepted December 02 Nd 2025

Abstract

Background: *Orchid propagation using seeds is very difficult to develop conventionally, because orchid seeds do not have endosperm. Research regarding the shelf life of orchid seeds is important as an effort to determine the viability/ability of orchid seeds to germinate. Propagation through tissue culture techniques with shelf life of seeds is one of the conservation and multiplication efforts of horticultural plants. This research aims to determine the germination ability of Vanda limbata orchid seeds stored for different periods of time through the development phases of Vanda limbata orchid embryos.* **Methodology:** *This research used a Completely Randomized Design with three treatments, namely fresh orchid seeds, orchid seeds stored for 1 week and orchid seeds stored for 3 weeks with each treatment having 5 replications. This research using organic tomato extract media as a natural growth regulator.* **Findings:** *The results of the research showed that V. limbata orchids planted fresh (P1) experienced growth and development until they reached phase 6 (Short Apical Meristem (SAM) was detected and were green), and during the 1 week storage period they reached phase 5 growth and development (the size of the embryo was round, enlarged and green), whereas seeds stored for 3 weeks only experienced growth and development reaching phase 3 (the embryo was round or oval, the testa was still white and had no testa). The three treatments orchid seeds that were planted directly without the seed storage process experienced faster seed germination than seeds that were stored for 1 or 3 weeks.* **Contribution:** *This research has an important contribution especially ex situ plant conservation.*

Keywords: *Conservation; Orchids; Shelf Life; Vanda limbata; Viability*

https://doi.org/10.36987/jpbn.v11i4.8199

INTRODUCTION

Indonesia has very high biodiversity (Dewi et al., 2024). One of the biological resources that is found naturally in Indonesia is orchids. Orchids are in great demand among orchid collectors because they have high economic value (Jolman et al., 2022; Heriansyah & Indrawanis, 2020). Apart from being used as ornamental plants, orchids have also begun to be used as medicinal and cosmetic ingredients (Tsutsumi et al., 2020). Orchid plants are often found in Indonesia, especially in East Nusa Tenggara Province. According to Dewi et al., (2022) that orchid plants can also be found in the Timor Island area, namely in the Saenam natural forest with as many as 10 types of orchids. One of the orchids that is often found on Timor Island is the *Vanda limbata* orchid.

Propagation of the *Vanda limbata* orchid is mostly done conventionally by thinning the shoots. However, propagation through seeds is very difficult to do conventionally because orchid seeds do not have endosperm. The endosperm in seeds is an important factor in supplying food reserves for the embryo. The absence of endosperm in orchid seeds is something that needs to be considered for propagation through seeds (Apriliyani & Wahidah, 2021; Darmawati et al., 2021; Franceschi et al., 2019; Levinawati et al., 2024; Warseno, 2015). One of the efforts made to propagate the *Vanda limbata* orchid through seeds is using tissue culture techniques.

Tissue culture technique is the propagation of plants by growing plant body parts into special media under sterile conditions which grow and develop into new individuals that are exactly the same as the parent (Fardilla et al., 2024; Iyah et al., 2024; Kusuma et al., 2023; Sarjani et al., 2023). Saving *Vanda limbata* orchid seeds that can be done is by storing *Vanda limbata* orchid fruit in the refrigerator at a temperature of -10 ^{0}C. Handini et al., (2016) stated that preservation of the *P. supradii* Braem and Loeb orchid can be done by storing the seeds at a temperature of -20 ^{0}C. The results of this research show that there is the ability of *P. supardii* seeds to germinate for 12 months on 4 different types of media.

Orchid research on the shelf life of seeds by Claudia et al., (2013) showed that black orchid seeds (*Coelogyne pandurata*) that were sown after being stored for 4 weeks and 2 weeks experienced a decrease in the ability of the seeds to germinate. A good percentage is found in sowing fresh *Coelogyne pandurata* orchid seeds. With this research, it can be seen that although sowing fresh orchid seeds has the highest germination percentage, storing orchid seeds needs to be done as an effort to conserve and prepare the seeds before in vitro sowing.

The media used for germinating orchid seeds can use organic media derived from natural ingredients (Haris & Mercuriani, 2018). This is because natural ingredients are easy to obtain at relatively affordable prices. Apart from that, the use of natural extracts such as tomatoes is a good alternative as a natural growth regulator (Franceschi et al., 2019).

The use of tomato extract in culture media has good benefits in explant growth because it is included in the cytokinin growth regulator which plays a role in the formation of chlorophyll (Darmawati et al., 2021). Apart from that, the cytokinins contained in tomato extract will stimulate cell division in the meristem tissue

(Anur et al., 2024; Gayatri et al., 2023). According to Agustin & Putra (2020) showed that the best performance was shown by the growth of *Phaius tankervilleae* orchid embryos experiencing a significant increase in media with the addition of 100 grams/L of tomato extract. The addition of tomato extract as a natural growth regulator at this concentration can induce cell growth thereby increasing embryo development in the *Phaius tankervilleae* orchid.

With this background, it is necessary to carry out research on the propagation of *Vanda limbata* orchid seeds using tissue culture using organic tomato extract as a medium with a shelf life of orchid seeds. This research aims to determine the germination ability of *Vanda limbata* orchid seeds through embryonic development phases with different seed shelf life. This research has an important contribution to ex situ conservation.

METHOD

The research was conducted in July 2025 at the Biology Laboratory, Biology Education Study Program, Faculty of Teacher Training and Education, University of Timor. The subject of this research is organic tomato extract media and *Vanda limbata* orchid fruit.

Experimental design

The design of this research was a completely randomized design using organic tomato extract media which was repeated 5 times, so that 15 experimental units were obtained. Each experimental unit contained ±100 seeds of orchid taken from the capsule (Magrini et al., 2019).

Table 1. Treatment of Culture Media on *Vanda limbata* Orchid Seeds

Treatments		Times				
		U1	**U2**	**U3**	**U 4**	**U5**
Organic Medium	Fresh orchid seeds (P1)	P1U1	P1U2	P1U3	P1U4	P1U5
	Orchid seeds stored for 1 week (P2)	P2U1	P2U2	P2U3	P2U4	P2U5
	Orchid seeds stored for 3 weeks (P3)	P3U1	P3U2	P3U3	P3U4	P3U5

Seed Storage Treatments

The sample used in this research was orchid seeds (*Vanda limbata*) obtained from Bansone, Kefamenanu City, North Central Timor. The seeds used for treatment are mature seeds ± 7 months old. The treatments in this study included the shelf life of *Vanda limbata* orchid capsules, namely 3 weeks, 1 week, and fresh (just picked). For storage treatment, orchid seeds were stored for 1 week and 3 weeks in a refrigerator at -10 ^{0}C (Franceschi et al., 2019; Magrini et al., 2019; Puspitaningtyas & Handini, 2021).

Orchid capsules are washed using detergent and bleach then washed using running water and after that it is sterilized first with Soak the capsule in 70% alcohol for 3 minutes. Then capsules washed thoroughly with sterile water 3 times then placed in alcohol 95% for 15 seconds and heated with a Bunsen flame for 2-3 seconds (Franceschi et al., 2019).

Sterile seeds were transferred to treatment media in BSC (Bio Safety Cabinet) sterilized with UV light. After the seeds are moved inside culture bottle, the culture bottle is closed with a plastic bottle cap. After that bottle Cultures were incubated indoors/ the cultivation room. The culture room is cleaned every day and the culture bottles are sprayed with 70% alcohol every two days. The culture room temperature was maintained at 25 ˚C. lighting using fluorescent lamps with a power of 20 watts (Diantina et al., 2022).

Cultural Medium Preparation (Organic Tomato Extract Medium & Sterilization Medium)

Tomatoes are cleaned later weighed 500 grams and cut into thin pieces and put in Beaker glass. Add distilled water in a ratio of 1:1 (100 grams of tomatoes add 100 ml of distilled water), then blend until smooth. Tomato pure filtered into an Erlenmeyer to obtain a stock solution of tomato extract with 100% concentration (Agustin & Putra, 2020). Then add sucrose and distilled water accordingly with the many media that will be created. Next, the pH of the media is measured at range 5.8. If it is too low, add NaOH and if it is too high added HCl. Agar is added to the medium and heated until boiling. The media is then poured into a culture bottle and covered with aluminum foil. Culture bottles that have been filled with media sterilized in an autoclave with a pressure of 1.5 psi at a temperature of 121˚C for 45 minute (Handini et al., 2016).

Observed Parameters

The parameter observed was the germination capacity of *Vanda limbata* orchid seeds seen from the shelf life of the fruit for different periods of time in organic tomato extract media (Agustin & Putra, 2020). Embryo development in orchid seeds can be seen from the embryo phases such as: Phase 1 (F1) is the embryo in the orchid seed before planting. Phase 2 (F2), namely: the embryo swells and appears with brown stripes indicating a ruptured testa. Phase 3 (F3), namely: the embryo does not have a testa, the shape is round or oval, the testa still remains white. Phase 4 (F4), namely: the embryo increases in size, is round and yellow in color, the testa still remains. Phase 5 (F5), namely: the embryo increases in size, is round and green in color. Phase 6 (F6), namely: Short Apical Meristem (SAM) is detected and is green (Agustin & Putra, 2020; Claudia et al., 2013).

Data analysis

This research was analyzed using analysis of variance (ANOVA). The Least Significant Difference Test (BNT) at the 5% level was used to determine the mean difference for each treatment (Fardilla et al., 2024; Kusuma et al., 2023).

RESULT AND DISCUSSION

The results of observing the germination of *Vanda limbata* orchid seeds planted in organic media were measured based on the growth and development phases of *Vanda limbata* orchid seeds during 8 weeks of planting. Based on the ANOVA results, if Fcount is smaller than Ftable 0.05, then each treatment has results that are not significantly different, meaning that the shelf life of the seeds has not affected seed germination in the culture medium. On the other hand, if Fcount is smaller than Ftable 0.05, then each treatment experiences results that are not significantly different, so the results are significantly different in each treatment. LSD test results can be seen in observation tables 1 WAP (week after planting) and 8 WAP. Table 2 describe *Vanda limbata* orchid seeds observed under a microscope 1 week after planting, show that P1 and P2 experience changes in germination from phase 1 to phase 3. In this phase, white oval/round embryos and testa skin will appear. At P3, brown swelling of the embryo is still visible and the testa skin is cracking.

Table 2. Percentage of Growth and Development Phases of *Vanda limbata* Orchid Seeds (1 WAP)

Treatment (n=100)	Growth and Development Stages of *V. Limbata* Orchid Seeds (1 WAP)						
	F0	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
P1	0,00	6,97[a]	72,84[a]	20,20[a]	0,00	0,00	0,00
P2	0,00	35,14[a]	52,16[a]	12,70[a]	0,00	0,00	0,00
P3	0,00	92,28[a]	7,72[a]	0,00[a]	0,00	0,00	0,00

Numbers followed by the same letter in the same column indicate results that are not significantly different in the least significant difference test (BNT) of 5% ($\alpha = 0.05$). Description: P1=fresh orchid seeds; P2=Orchid seeds stored for 1 week; P3= Orchid seeds stored for 3 weeks. Code of F1 - F6 is the embryonic development stage in the orchid seed (attached in observe parameter).

Table 3. Percentage of Growth and Development Phases of *Vanda limbata* Orchid Seeds (8 WAP)

Treatment (n=100)	Growth and Development Stages of *V. Limbata* Orchid Seeds (8 WAP)						
	F0	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
P1	0,00[b]	0,00[bc]	5,97[a]	14,48[ab]	12,63[ab]	17,33[ab]	49,86[a]
P2	0,00[b]	14,53[bc]	13,24[ab]	22,95[a]	28,10[a]	21,18[a]	0,00[ab]
P3	0,81[b]	79,34[a]	19,85[ab]	0,00[bc]	0,00[bc]	0,00[bc]	0,00[ab]

Numbers followed by the same letter in the same column indicate results that are not significantly different in the least significant difference test (BNT) of 5% ($\alpha = 0.05$). Description: P1=fresh orchid seeds; P2=Orchid seeds stored for 1 week; P3= Orchid seeds stored for 3 weeks. Code of F1 - F6 is the embryonic development stage in the orchid seed (attached in observe parameter).

Table 3 shows that P1 is experiencing growth and development with the appearance of a short green apical meristem or what is said to have entered phase 6. P3 shows growth and development up to phase 5 (the embryo is round, enlarged and green). At P3 it only enters phase 2, where the embryo experiences swelling and is still

brown. This is in accordance with Handini et al., (2016) who stated that there was a decrease in the viability of *Paphiopedilum supardii* orchid seeds by 26.75 % after storage for 12 months at -20 ^{0}C. The decrease in orchid seed viability can be seen through the protocorm getting smaller due to the long shelf life of the seeds (Agustin & Putra, 2020; Claudia et al., 2013; Pradhan et al., 2022; Pujasatria et al., 2020).

According to Arli et al., (2023); Franceschi et al., (2019); Handini et al., (2016); Pradhan et al., (2022); Sarjani et al., (2023) stated that newly harvested seeds approximately 2 - 3 weeks after sowing can germinate to form protocorms depending on the type. The germination percentage of *Coelogyne pandurata* seeds decreased to 1 - 7% after storage for 9 months. The longer the storage time, the ability of seeds to germinate will decrease to 0%, while fresh seeds have a percentage of 98 - 99% (Claudia et al., 2013; Franceschi et al., 2019; Kendon et al., 2017; Wahyudiningsih et al., 2018). Comparison of the growth and development of *Vanda limbata* Orchid seeds with different shelf lives observed under a microscope at 1 WAP and 8 WAP (in figure 1).

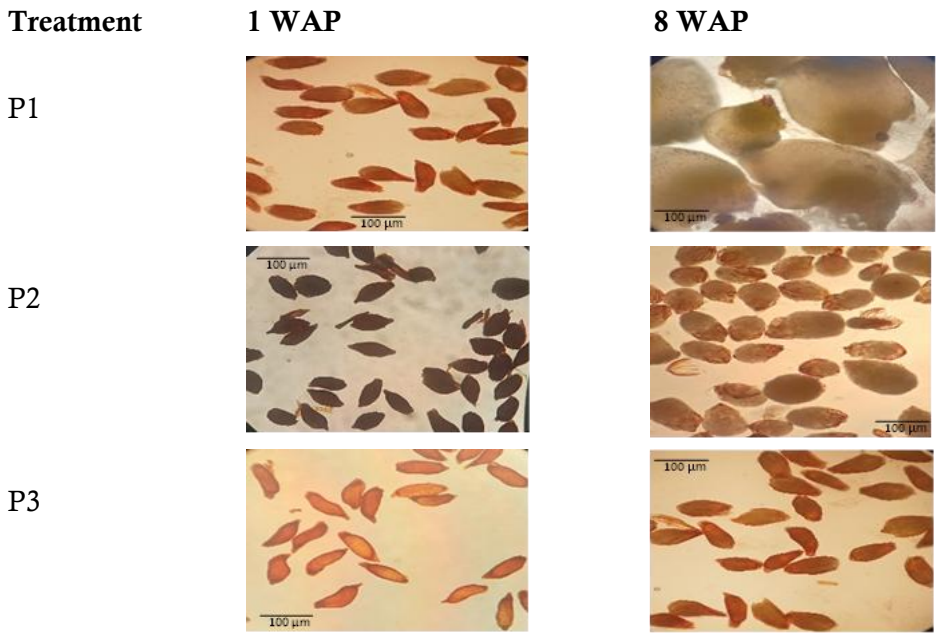

Figure 1. Comparison of *V. limbata* Orchid Seed Germination in each treatment (Microscope magnification 10x10). Description: P1=fresh orchid seeds (bar = 10 µm); P2=Orchid seeds stored for 1 week (bar = 10 µm); P3= Orchid seeds stored for 3 weeks (bar = 100 µm).

Based on Figure 1, it shows that observations of the growth and development phases in the germination of *Vanda limbata* orchid seeds sown in organic tomato extract media. Orchid seeds that germinate more quickly are *Vanda limbata* orchid seeds that are planted fresh (just picked). According to Claudia et al., (2013) stated that seeds sown after being stored for 2 weeks experienced germination of up to 20 %, with storage for 4 weeks only experienced germination of up to 2.5 %, whereas when sowing fresh seeds the percentage of seed germination in the medium increased to 60 %.

This can prove that *Vanda limbata* orchid seeds planted fresh have a higher level of physiological condition and viability so that the percentage of growth is higher.

Meanwhile, orchid seeds were stored for 1 week and seeds were stored for 3 weeks before being planted at a temperature of -10 ^{0}C, germination was slower, because the viability of *Vanda limbata* orchid seeds decreased. The lifespan of *Vanda limbata* orchid seeds stored in the refrigerator can be influenced by humidity and appropriate temperature.

The results of the germination test from year to year can experience fluctuations in the percentage of seed germination (Handini et al., 2016; Puspitaningtyas & Handini, 2021). The success of seed viability over shelf life is very dependent on the seed storage process, the existence of good regulation of the temperature and humidity of the water in the seeds (Jiang et al., 2017; Pradhan et al., 2014; Puspitaningtyas & Handini, 2021; Udomdee & Chin, 2014).

The decline in seed quality is gradual and cumulative and is irreversible or irreversible because it is caused by physiological changes, which is a process of decreasing seed viability. During the storage period, seed viability is influenced by two factors, including internal factors and external factors. Internal factors that influence seed viability are the genetic characteristics of the plant and the availability of water. Meanwhile, external factors that influence are the presence of microorganisms, seed storage space, humidity and temperature (Azad et al., 2024; Diengdoh et al., 2020; Diantina et al., 2022; Magrini et al., 2019).

CONCLUSION

This research can be concluded that *Vanda limbata* orchids stored for different periods of time at a temperature of -10 ^{o}C affect the germination percentage of *Vanda limbata* orchid seeds, therefore, to get good results, *Vanda limbata* orchid seeds must be sown fresh or just picked. This research contributes as information and additional data on the use of natural extracts as growth regulators in vitro to induce germination of fresh orchid seeds. This research still needs to be refined with further research on the storage period of *Vanda limbata* orchids, namely by subculturing *Vanda limbata* orchids in vitro. Regarding the storage period of orchid seeds for 1 week and 3 weeks on embryo growth, it was found that storing orchid seeds for 1 week was more effective in embryo growth than storing orchid seeds for 3 weeks.

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How To Cite This Article, with *APA style* :
Dewi, N. P. Y. A., Sele, Y., & Luruk, T. O. (2025). Viability of *Vanda limbata* Orchid Seeds With Diferent Storage Period in Organic Tomato Extract Media. *Jurnal Pembelajaran dan Biologi Nukleus*, *11*(4), 1265-1274. https://doi.org/10.36987/jpbn.v11i4.8199

Conflict of interest : The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions : All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was submited by [**Ni Putu Yuni Astriani Dewi**]. All authors contributed on previous version and revisions process of the manuscript. All authors read and approved the final manuscript.