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Conservation Comprehensive Approach: Study on Exploration, Habitat Analysis, Propagation, and Reintroduction of the Indonesian Endemic Endangered Titan Arum (*Amorphophallus titanum* Becc.)

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Abstract

Titan arum (Amorphophallus titanum *Becc.*) *is an endemic species of Indonesia that is found naturally only in Sumatra and is classified as endangered, with an estimated 303 mature individuals remaining in their natural habitat. The population is decreasing due to deforestation, tuber exploitation, long flowering times, and protandry. Therefore, both in-situ and ex-situ conservation programs are necessary to preserve this species. The research aims to explore natural populations, analyze habitat conditions, develop propagation methods, and conduct reintroduction efforts to support the conservation of A. titanum. This study was conducted from 2023 to 2024 in Palupuah, Agam Regency, the Agriculture Faculty of Universitas Andalas, and several locations for the reintroduction of A. titanum. The research found that A. titanum habitat consists of forests rich in humus and litter, as well as banana cultivation areas. During the exploration, 7 individuals were identified: one in dormancy, one fruiting, and 5 in the vegetative phase. Seed germination experiments categorized seeds into seven classes based on their weight. The most dominant seed class weighed between 2.9 g and 3.2 g, comprising 38 seeds or 26.9% of the total. The germination rate and seedling growth were high, as all seeds exhibited 100% and produced seedlings of relatively uniform size. Propagation was carried out using in vitro culture techniques with petiole explants. The results showed that 2 mg L^{-1} benzyl aminopurine* (*BAP*) *successfully induced callus formation with a 100% induction rate, while a concentration of 1.5 mg L^{-1} achieved the highest shoot induction rate at 58.3%, with an average of 1.3 shoots and 7.9 roots per explant. The propagated seedlings were subsequently replanted in several locations for reintroduction activities.*

Keywords: *biodiversity, ecotourism, forest, germplasm, redlist IUCN*

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Introduction

One of Indonesia's endemic flora is the *titan arum* (*Amorphophallus titanum* (Becc.)), which is found only in Sumatra and listed as an endangered species (World Checklist of Vascular Plants, 2024). The population of *A. titanum* is estimated to be between 71 toand 999 individuals, with a best estimate of around 303 individuals (Redlist IUCN, 2024). Population decline is due to deforestation, long generative phase, bulb exploitation for trade (Kementerian Lingkungan Hidup dan Kehutanan, 2015), protogynous flowering, and cross-pollination difficulties requiring simultaneous blooms (Korotkova & Barthlott, 2009; Sudarmono et al., 2016; Yudaputra et al., 2021).

A. titanum is highly intolerant to forest disturbance, as it relies on stable microclimatic and ecological conditions that are easily disrupted by deforestation or habitat degradation. This species thrives in humid lowland-highland rainforests of Sumatra, where ideal conditions include temperatures ranging from 2030 C, high relative humidity above 80%, and indirect sunlight provided by a closed forest canopy. It requires moist, well-drained soil rich in organic matter, minimal wind exposure, and a stable, shaded environment to support its complex life cycle. Disturbances to these conditions can significantly hinder its growth, reproduction, and survival (Kementerian Lingkungan Hidup dan Kehutanan, 2015).

Studies on *A. titanum*'s existence are crucial for conservation programs. Research includes germination (Latifah & Purwantoro, 2015), pollination (Sudarmono et al., 2016), genetic diversity analysis (Arianto et al., 2018), bioecology (Nursanti et al., 2019), expedition (Yudaputra et al., 2021), and habitat study (Yudaputra et al., 2022). Exploration and bioecology studies reported several findings. For example, Yudaputra et al. (2021) found 162 individuals of *A. titanum* across Aceh to Lampung. Yusniwati et al. (2024) found 18 individuals of *A. titanum* and one of *A. gigas* at Solok Selatan and 25 individuals of *A. titanum* at Sijunjung, West Sumatra.

Studies on *A. titanum* propagation include seeds (Latifah & Purwantoro, 2015), peculiar callus (Yuzammi et al., 2018),

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petiole cuttings (Setiawan et al., 2023), and in vitro culture (Wati, 2021). Propagation through in vitro culture can accelerate conservation success that provide short-, medium-, and long-term conservation methods. Success in in vitro propagation depends on suitable media, types, and concentrations of plant growth regulators. Cytokinins are typically used for shoot induction and multiplication in various plants. For instance, Gurme et al. (2018) reported 3 mg L^{-1} benzyl amino purine (BAP) induced the best shoots in *A. paeoniifolius*. Combining 2.0 mg L^{-1}BAP, 0.5 mg L^{-1}NAA, and 0.1 mg L^{-1} gibberellic acid (GA_3) also enhanced *A. konjac* K. Koch shoot induction (Li et al., 2021). Additionally, combining 5.0 mg L^{-1} BAP and 0.3 mg L^{-1} NAA increased shoot numbers in *A. muelleri* Blume (Hardjo et al., 2023). Nurfadhilah (2019) reported 1 mg L^{-1} BAP and 1 mg L^{-1} NAA yielded 18.75% explant shoot induction on *A. titanum.* Another study showed 2 mg L^{-1} BAP yielded the highest shoot induction at 55% (Wati, 2021). Information on *A. titanum* in vitro propagation protocols is limited, necessitating ongoing optimization research to support future conservation efforts.

Reintroduction of seedlings to their natural habitat is crucial for *A. titanum* conservation. This process involves transplanting ex-situ propagated seedlings back into the forest to restore wild populations. To date, no reintroduction efforts for *A. titanum* have been reported in the region, indicating a need for further research and implementation. Planned and measured reintroduction efforts are expected to help restore and reintegrate the population into the forest ecosystem. Additionally, reintroduction serves as an educational tool and raises public awareness about conservation. Post-reintroduction monitoring and evaluation are crucial to ensuring the program's success, determining whether the reintroduced seedlings can survive and reproduce in their natural habitat through long-term observation. The potential of *A. titanum* as an ornamental plant for developing ecotourism should not be overlooked. Its unique and striking appearance, along with its infrequent blooming cycle, makes it a significant attraction. Developing ecotourism centered around *A. titanum* can promote conservation efforts and provide economic benefits to local communities, fostering a sustainable relationship between humans and the environment.

The study aimed to develop a comprehensive conservation approach for *A. titanum*, an endangered and endemic plant of Indonesia. It included exploring and documenting wild populations, analyzing habitat characteristics, developing effective propagation methods, and implementing reintroduction programs. The research also evaluated the success of propagation techniques, monitored the survival of reintroduced individuals, and involved local communities in conservation efforts. Ultimately, the study established a replicable model that integrated field and laboratory work to support the long-term preservation of *A. titanum* in its natural habitat.

Methods

Exploration and habitat analysis This study was conducted in June 2023 in Jorong Sitingkai, Kamang District, Palupuah, Agam Regency (Figure 1). The area lies at an elevation of approximately 900 m above sea level (masl), experiences consistently high humidity above 80%, rainfall of about 2,500 mm $year^{-1}$ and temperatures ranging from 22–30 °C. The exploration area around spans 8 km, surveyed using the line transect method. The data was collected from both sides of the line, covering a width of 20 m on each side. Data collection included coordinates of findings, plant height, stem circumference, growth phase, and habitat condition.

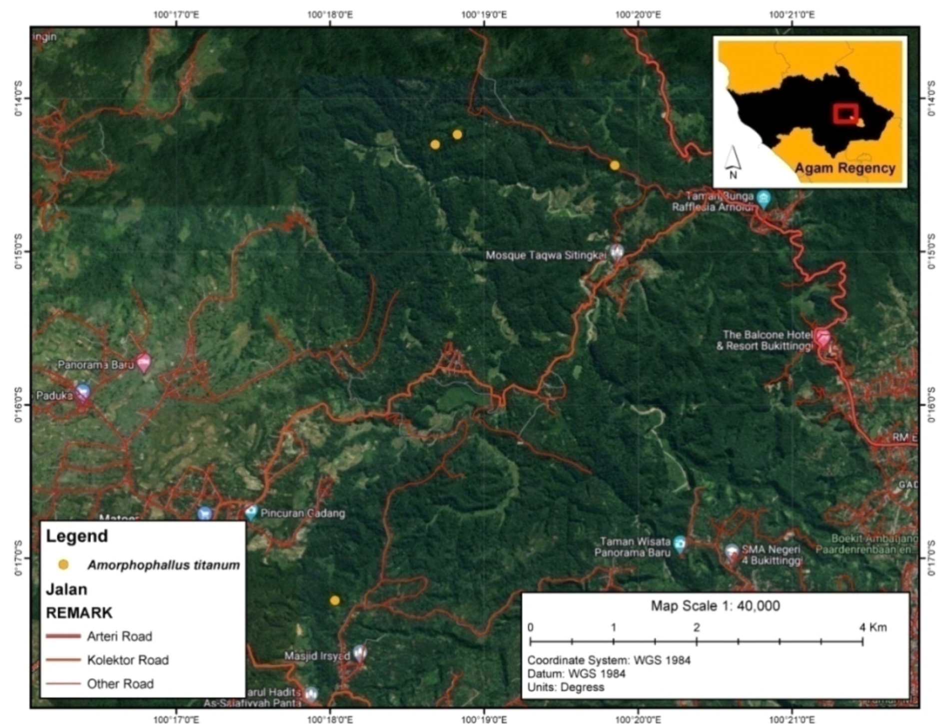

Figure 1 Map of the *Amorphophallus titanum* discovery in Sitingkai village, Agam Regency, West Sumatra.

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Collected data were presented in tables, images, and distribution maps and analyzed descriptively. Additionally, fruit and rachis samples were collected for propagation. This exploration aimed to obtain detailed data on the distribution and condition of *A. titanum* in the study area. Using the line transect method provided a clearer picture of the distribution patterns and habitat conditions of *A. titanum* in Jorong Sitingkai. This research is expected to form the basis for more effective and sustainable conservation strategies, including mapping areas critical for preserving this species.

Seed germination and seedling growth The study was conducted from July 2023 to February 2024 in the greenhouse of the Faculty of Agriculture, Universitas Andalas. Tools and materials used included seedling trays, sprayers, sphagnum moss, compost, water, and *A. titanum* seeds. Observed variables included germination time and percentage. After germination, seedlings were transferred to pots with a 1:1 (v/v) mixture of sphagnum moss and compost. Seedlings were maintained for eight months with periodic watering and NPK fertilization every two months at 1 g pot^{-1}. Observed variables included: seed number, seed number percentage, seedling height, leaf number, leaf length, leaf width, rachis length, and petiole diameter.

Micropropagation of *A. titanum* This research was conducted from January 2023 to January 2024 in the Tissue Culture Laboratory, Faculty of Agriculture, Universitas Andalas. The medium was prepared by weighing 4.4 g L^{-1} MS instant and 30 g L^{-1} sucrose. The pH was adjusted to 5.8 using a pH meter and adjusted with 0.1 N KOH or 0.1 N HCl. Then, 8 g L^{-1} of bacto agar was added, stirred, and heated to boiling. The medium was sterilized using an autoclave at 0.1 MPa and 121 °C for 15 minutes. Petioles were washed with running water, scrubbed with a brush, and detergent added. They were then soaked in bactericide and fungicide solutions at 2 g L^{-1} for 2 hours and soaked in 15% bleach solution for 10 minutes. This process was repeated with a 10% bleach solution for 15 minutes and rinsed with sterile water. Petioles were cut to 1 cm and planted on the medium in a laminar air flow cabinet (LAFC). Culture bottles were stored in an incubation room at 20 °C in the dark for 12 weeks. After callusing, it moved with a light intensity of 1,000 lux for 8 weeks. The study was designed based on a randomized block design with BAP concentration treatments at five levels: 0.5, 1.0, 1.5, 2.0, and 2.5 mg L^{-1}. Each treatment was replicated three times, resulting in 15 experimental units, with each unit containing 10 culture bottles. Observed variables included explant survival percentage, callus formation percentage, callus formation time, callus texture, callus color, shoot formation percentage, shoot formation time, shoot number, shoot height, and root number.

Reintroduction of *A. titanum* Reintroduction involved planting *A. titanum* seedlings aged approximately eight months and around 30 cm in height. Planting locations included Tilatang Kamang District, Agam Regency (100 seedlings); Pelembayan District, Agam Regency (5 seedlings); Ranah Pesisir District, Pesisir Selatan Regency (20 seedlings); and Payo, Tanah Garam District, Solok City (20 seedlings). Tilatang Kamang, located at about 900 masl, has a tropical climate with average temperatures ranging from 20–26 °C, high humidity (88%), and an annual rainfall of 2,500–4,000 mm. The region features hilly terrain covered by dense tropical forests. Pelembayan, situated at elevations around 950 masl, experiences similar climatic conditions with temperatures of 20–26°C and high humidity (88%), with rainfall ranging from 2,000–4.,00 mm annually. In Ranah Pesisir, specifically around Palangai Waterfall, the area is characterized by elevations of 200 masl. The climate is tropical, with daytime temperatures ranging from 2332 °C and nighttime temperatures between 20 °C and 28 °C. This region receives high rainfall throughout the year, averaging 2,000–3,000 mm month^{-1}, supporting its lush forest ecosystem. The Palangai Waterfall area is surrounded by hilly terrain with dense tropical forests, providing an ideal habitat for various endemic species. Lastly, Payo, located at 900 masl, experiences a climate influenced by west winds, with the highest rainfall occurring from October to December and the lowest in May. The region has a temperature range of 20–26 °C and high humidity levels (85–90%). It receives high rainfall, with the average annual precipitation reaching 2,500–3,500 mm (Badan Meteorologi Klimatologi dan Geofisika, 2025). The topography is characterized by hilly land with rich biodiversity, contributing to its potential for agrotourism and the conservation of endemic species, including *A. titanum*.

Data analysis Quantitative data were analyzed using ANOVA and the post hoc test using Duncan's multiple range test. Data analysis used the statistic tool for agricultural research (STAR) software.

Results

Exploration and habitat analysis An exploration discovered 7 individuals of *A. titanum* (Figure 1). There was one individual in dormancy, five in vegetative phase (Table 1 and Figure 3A), and one in fruiting phase (Figure 3B), and

Table 1 Characterization data and coordinates of *Amorphophallus titanum* in Sitingkai Village, Agam Regency

Petiole height (cm)	Petiole circumference (cm)	Growth phase	Habitats	Coordinates	
				Latitude	Longitude
-	-	Dormancy	Forest	-0.24062	100.33082
148*	55**	Fruiting	Forest	-0.23833	100.31137
160	40	Vegetative	Banana field	-0.28796	100.30051
165	52	Vegetative	Banana field	-0.23726	100.31377
160	34	Vegetative	Banana field	-0.23724	100.31374
173	55	Vegeta tive	Banana field	-0.23730	100.31376
45	8	Vegetative	Forest	-0.23837	100.31138

Note: * = flower stalk height, ** = circumference of flower stalks

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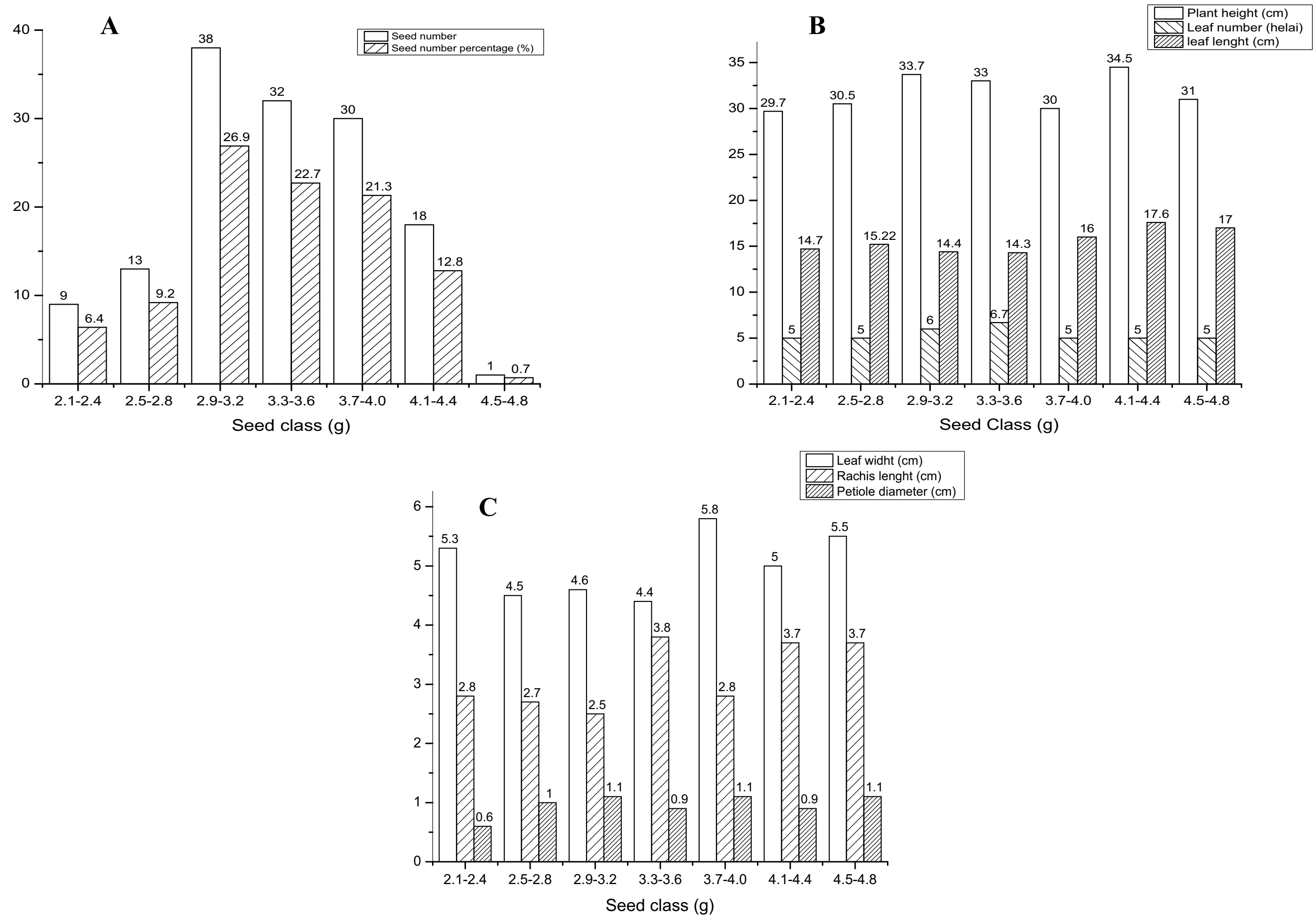

Figure 2 Seedling morphology of *Amorphophallus titanum*. A) Seed number and seed number percentage B) Seedling height, leaf number, leaf length, C) Leaf width, rachis length and petiole diameter of *A. titanum* based on seed weight.

Figure 3 Exploration and propagation of *Amorphophallus titanum*, (a) vegetative phase, (b) fruiting phase, (c) fruits of *A. titanum*, (d) seeds of *A. titanum*, (e) Seed nursery in spagnum moss and charcoal mix medium, (f) eight month old seedlings of *A. titanum*.

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they grow in habitats with moist soil covered by leaf litter, banana cultivation areas, and forest slopes with dense vegetation. The vegetative phase begins with seed germination, followed by the emergence of the petiole (pseudo-stem), rachis, and leaves above the soil surface. The petioles are dark green with irregular white spots. The *A. titanum* in Agam have petioles ranging from 45–173 cm in height and stem circumferences from 8 to 55 cm (Table 1). One individual was found in the fruiting phase, with tightly clustered reddish-orange fruits. Harvested fruits measured 3.1–5.1 cm in length and 2.5–3.5 cm in diameter, each containing 12 dark brown seeds with pointed tips.

Seed germination and seedling growth The exploration activities successfully discovered one *A. titanum* in the fruiting phase that produced over 300 fruits (Figure 3B), with each fruit containing 12 seeds of varying sizes (Figure 3D). Harvesting was conducted on one-third of the total fruits, selecting those with a dark orange-red color (Figure 3C), a length of over 4 cm, and a diameter of over 2.5 cm. A total of 141 *A. titanum* seeds were categorized into seven classes based on their weights, which ranged from 2.1 to 4.7 g. The seed germination used a sphagnum moss and charcoal medium mix (Figure 2E).

Observations showed that medium-sized seeds (2.9–3.2 g) were the most prevalent, accounting for 38 seeds (26.9%), followed by the 3.3–3.6 g with 32 seeds (22.7%), while the least represented was the 4.5–4.8 g, with only one seed (0.7%) (Figure 2A). There were no significant differences in germination time (14–16 days) and germination percentage (100%) across all *A. titanum* seed classes (Table 2). *A. titanum* seedling height ranged from 30 to 34.4 cm, the number of leaves ranged from 14.3 to 17.6, and leaf length ranged from 5 to 6.7 cm (Figure 2B and Figure 4). Similar trends were observed in leaf width, rachis length, and petiole diameter. Leaf width ranged from 4.4 to 5.8 cm, rachis length from 2.5 to 3.8 cm, and petiole diameter from 0.6 to 1.1 cm (Figure 2C).

Micropropagation of *A. titanum* The variance analysis showed that different BAP concentrations significantly affected the callus induction percentage but did not impact the callus formation time. The 2 mg/L BAP concentration resulted in the highest callus induction percentage, reaching 100%, while the lowest percentages were observed at 0.5 and 2.5 mg L^{-1} BAP (66.7%) (Figure 5). Generally, all surviving explants formed callus, and callus formation occurred between 20.7 and 22.5 days after culture (DAC) (Figure 5), beginning with swelling at the explant cut site. The swollen area continued to grow and eventually formed a callus.

Table 2 Germination percentage and germination time of *Amorphophallus titanum* based on the seed weight

Seed weight (g)	Germination percentage (%)	Germination time (days)
2.1–2.4	100	17.0
2.5–2.8	100	16.0
2.9–3.2	100	16.0
3.3–3.6	100	16.0
3.7–4.0	100	16.0
4.1–4.4	100	15.0
4.5–4.8	100	15.0

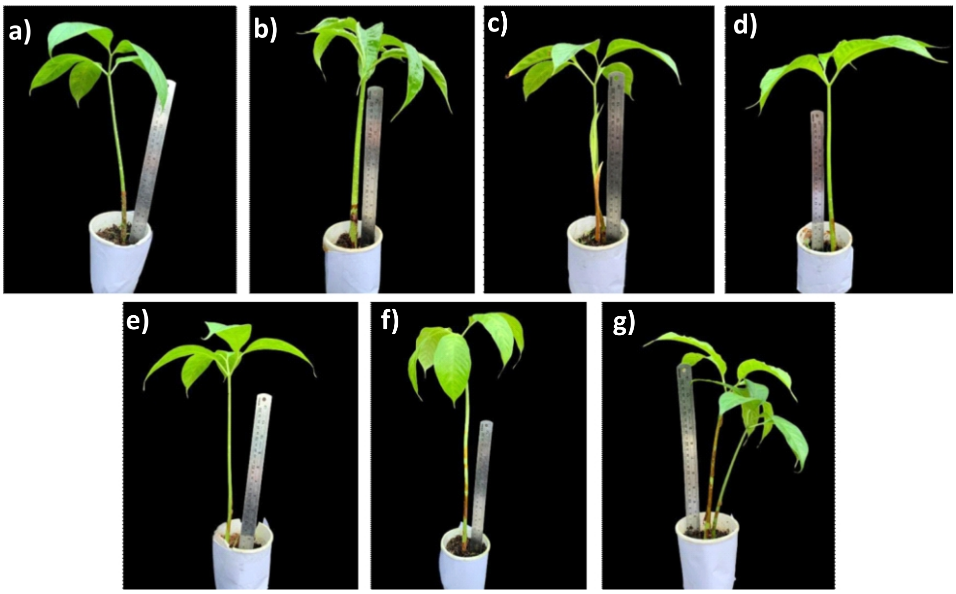

Figure 4 Eight mouth old seedling of *Amorphophallus titanum* based on seed weight. a) 2.1–2.4 g, b) 2.5–2.8 g, c) 2.9–3.2 g, d) 3.33.6, e) 3.7–4.0 g, f) 4.1–4.4, g) 4.5–4.8 g.

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Initially, the callus was translucent white and appeared only at the wound sites on one part of the petiole (Figure 6).

The different BAP concentrations significantly affect the percentage of shoot formation, shoot height, and root

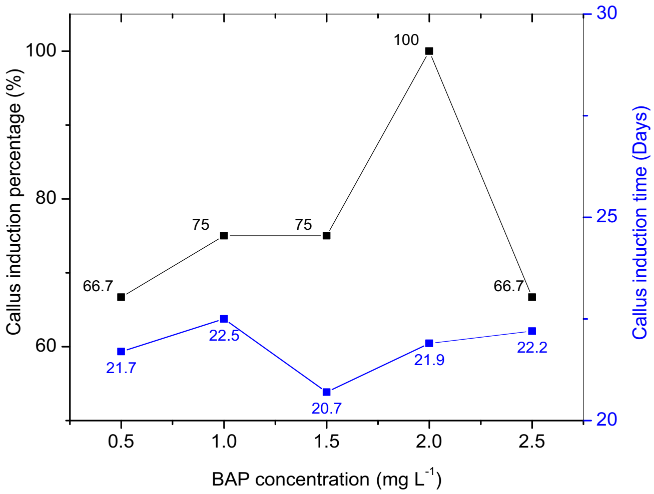

Figure 5 Callus induction percentage and callus induction time from *Amorphophallus titanum* petiole in several BAP concentration. *significant based on Duncan posthoc test.

number in *A. titanum* petiole culture. However, shoot formation time and the number of shoots were not affected. A concentration of 1.5 mg L^{-1} BAP produced the highest percentage of shoot formation at 58.3%, shoot height of 2.1 cm (Table 3), and the number of roots at 7.9 (Figure 7). The data also show that increasing BAP concentration to 2.5 mg L^{-1} inhibited the process of cell differentiation into shoots. Shoots appeared between 66.570 DAC (Table 3), marked by changes in the form of callus undergoing differentiation. Observations showed that the number of shoots ranged between 1.21.5 shoots/explant (Figure 8). Additionally, the number of shoot buds appearing at 18 weeks is relatively high, but only a few of these shoots grow large (Figure 7). This is likely because nutrient translocation is directed only towards the enlargement and growth of a few shoots. Observations on root number show that a concentration of 1.5 mg L^{-1} BAP resulted in the highest number of roots, with 7.9 roots (Figure 8).

Reintroduction of *A. titanum* The planting of *A. titanum* during reintroduction activities took place in several locations: Kamang District, Agam Regency (100 seedlings); Pelembayan District, Agam Regency (5 seedlings); Ranah Pesisir District, Pesisir Selatan Regency (20 seedlings); and Payo, Tanah Garam District, Solok City (20 seedlings). This initiative actively involved local communities, who not only

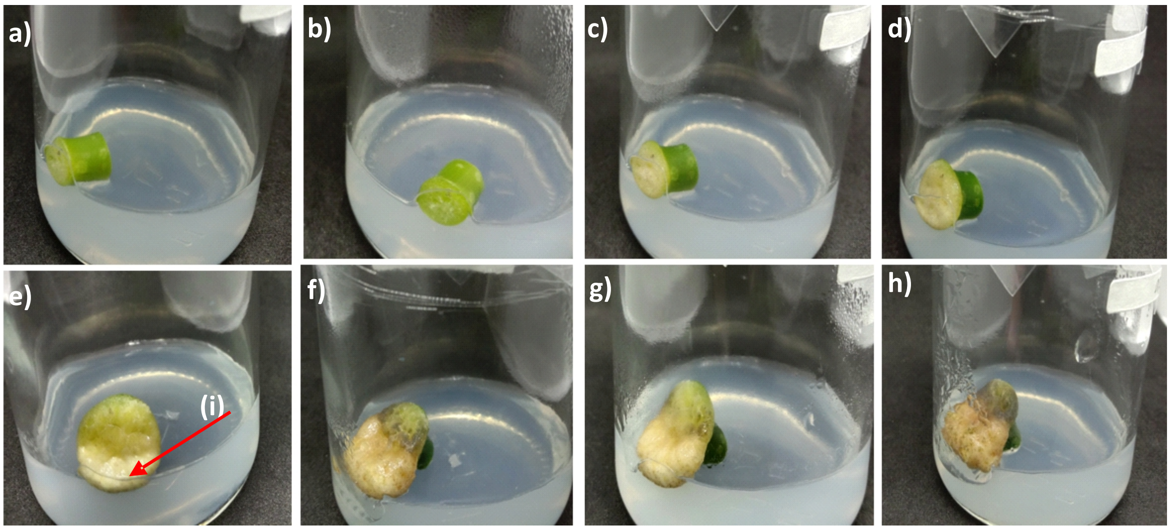

Figure 6 Callus growth of *Amorphophallus titanum* in MS basal medium with addition of 2.0 mg L^{-1} BAP during 8 week after culture (WAC). a) 1 WAC, b) 2 WAC, c) 3 WAC, d) 4 WAC, e) 5 WAC, f) 6 WAC, g) 7 WAC, h) 8 WAC, i) callus.

Table 3 Shoot induction percentage, shoot induction time and shoot height in several BAP concentration

BAP concentration (mg L^{-1})	Shoot induction percentage (%)	Shoot induction time (days)	Shoot height (cm)
0.5	16.7 c	70.0	1.2 b
1.0	25.0 b	66.5	2.2 a
1.5	58.3 a	67.6	2.1 a
2.0	50.0 a	70.0	1.5 b
2.5	0.0 d	-	-

Data followed by different lowercase letters indicate a significant effect based on Duncan posthoc test at the 5% level. – (shoots are not formed).

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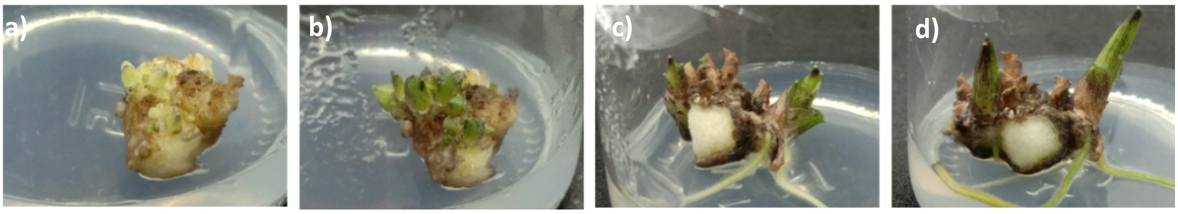

Figure 7 Shoot growth of *Amorphophallus titanum* in MS basal medium with addition 1.5 mg L^{-1} BAP. a) 17 WAC, b) 18 WAC, c) 19 WAC, d) 20 WAC.

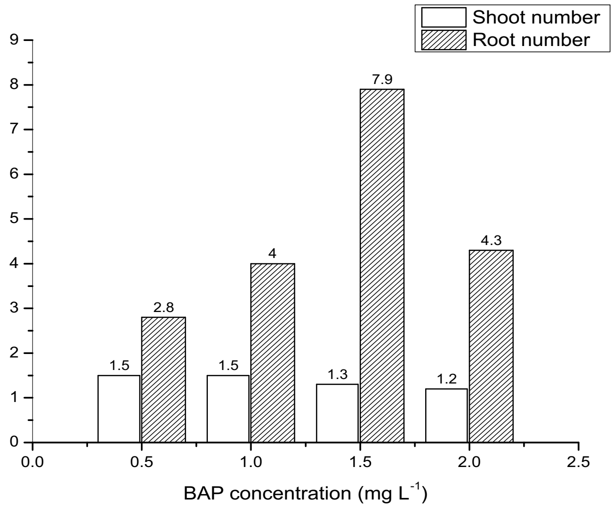

Figure 8 Shoot and root number of *Amorphophallus titanum* in several BAP concentration diring 20 WAC. * Data was indicate a significant effect based on Duncan posthoc test at the 5% level.

participated in the planting process but also attended educational sessions on *A. titanum* conservation.

The results of the reintroduction of *A. titanum* seedlings across all study sites showed promising outcomes. In general, the seedlings demonstrated good growth performance, indicating that the selected habitats provided suitable environmental conditions for the early establishment phase. Although a few seedlings exhibited signs of wilting, new shoots were observed emerging from the base of these plants. This phenomenon aligns with the natural life cycle of *A. titanum*, which includes periods of dormancy followed by the emergence of new vegetative structures. The appearance of new shoots suggests that the tubers remained viable and were actively regenerating, an important sign of adaptability and resilience in their new environments. These early indicators support the potential success of in situ conservation efforts and reflect the ecological suitability of the reintroduction sites for long-term survival and growth of the species.

Discussion

In exploration activity shows that *A. titanum* habitat at soil covered by leaf litter and humid. This is the same with several previous reports; for instance, Nursanti et al. (2019) reported that *A. titanum* are found in humid forests with altitudes of 301–341 masl with a slope of 25–45%, latosol soil type, temperatures of 24–25 °C, and humidity of 80–83%. Yudaputra et al. (2022) also found it in humid soil, forests, and riversides. The fruit size was larger than that reported by Sudarmono et al. (2016), with lengths of 25 cm and diameters of 1.53 cm. Four individuals were found clustered in the same area, while three were separated. Arianto et al. (2018) reported clustered distribution of *A. titanum* due to gravity and water flow dispersing seeds.

A. titanum was discovered growing in a banana plantation, which can be attributed to several ecological and historical factors. It is possible that the land was originally natural forest that was later cleared for agriculture, and some dormant tubers of *A. titanum* remained in the soil. Over time, as the area was transformed into a banana garden, the environmental conditions, such as high humidity, partial shade, fertile soil, and organic litter, remained favorable for the species. These conditions closely mimic its native forest habitat, allowing the dormant tubers to break dormancy and resume growth. This indicates that semi-natural or agroforestry-like landscapes can still support the survival and regeneration of this rare species, especially when minimal soil disturbance occurs.

In a seed germination study, it shows that seed weight is often considered an indicator supporting germination and early plant growth, as larger seeds are thought to contain more endosperm. However, this study showed different results, indicating that even with limited food reserves, the seeds had sufficient energy for germination. These results indicate that the endosperm in the seeds plays a role in supplying energy for germination and seedling growth, even in small amounts. Generally, increased seed weight positively impacted seedling growth, although not significantly. This is consistent with several previous studies. Domic et al. (2019) reported that medium and large seed sizes have the same effect on the germination and seedling survival of *Polylepis tomentella*. Furthermore, Alngiemshy et al. (2020) stated that in *Vicia faba* L., seed size does not affect the germination percentage, which reaches 100% for all seed sizes. However, larger seeds produce optimal root length, leaf number, and plant height. Additionally, Hou et al. (2021) found that size differences still provide the same seed viability, ranging from 69–71% in *Lespedeza davurica* and 97–100% in *Setaria viridis*.

Conversely, there are studies with different results. Tumpa et al. (2021) stated that medium-sized seeds (7 g) produce the best germination percentage of 80% compared to large and small seeds in *Castanea sativa* Mill., with the best plant height also found in medium-sized seeds. Fredrick et al.

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(2021) found that small seeds have the best germination percentage in *Dennettia tripetala*. Yisau et al. (2023) stated that small seeds have the best viability (98%), compared to medium-sized seeds (92.67%) and large seeds (72.33%) in *Anacardium occidentale*.

Additionally, Ambika et al. (2014) reported that large seeds have the highest germination percentage. Iroko et al. (2021) also stated that large seeds have higher germination capacity in *Albizia zygia* (DC.) J. F. Macbr. Aderounmu et al. (2020) found that large *Anacardium occidentale* L. seeds have a high germination percentage (77.33%) compared to medium-sized (73.78%) and small seeds (60.43%). These larger seeds also result in taller plants (26.17 cm), larger stem diameters (7.14 mm), more leaves (11.90), and larger leaf areas (379.21 cm²). Kristó et al. (2023) also reported that in *Triticum aestivum* L., large seeds have higher germination percentages, seed health, and grain yield. In many studies, large seeds typically offer numerous advantages over small seeds and often exhibit a higher percentage of germination. The increased germination rates in large seeds may be attributed to the greater amount of reserves available in larger seeds compared to medium or small seeds. Seed size often influences germination, depending on the seeds' ability to utilize food reserves. The levels of food reserves and glucose vary among different seed sizes and may be a significant factor affecting seed germination.

In micropropagation, living explants were marked by callus formation and not browning. Visual observations showed that callus formed in all treatments was compact and yellowish-white by the end of the observation period. The callus initiation and induction process involves significant changes in the metabolism of explant cells, from the initial callus formation at the cut sites to the callus growing larger and changing color, reflecting various complex metabolic activities and hormonal signals.

The cutting wounds caused physical stress on the explants, activating secondary metabolic pathways that produce compounds such as jasmonate (Li et al., 2022), ethylene (Li et al., 2020), and salicylic acid (Kim & Lim, 2023). These compounds act as signals to initiate the healing and protection process. Plant hormones like endogenous auxin and cytokinins also play crucial roles in callus initiation. Auxin tends to accumulate at the wound site and induces dedifferentiation of the surrounding parenchyma cells. Subsequently, cytokinins interact with auxin to regulate cell division. Dedifferentiation is a process where differentiated cells lose their specific characteristics and revert to a meristematic state, occurring through gene expression changes regulated by auxin and cytokinins (Mostafa et al., 2020).

The process of callus growth, which involves cell proliferation, encompasses various aspects at the molecular, biochemical, and enzymatic levels that are complex. At the molecular level, the cell cycle of synthesis, gap, and mitosis is controlled by Mitogen-activated protein kinase (MAPKs), Cyclin dependent kinase (SDKs), and Cyclin genes (Banerjee et al., 2020). Transcription factors such as the E2F gene play a role in regulating the expression of genes necessary for DNA replication and cytokinesis (Kállai et al., 2020; Sánchez-Camargo et al., 2020). The biochemical aspects of cell proliferation involve an increase in energy processes, including glycolysis and oxidative phosphorylation (Horbay & Bilyy, 2016). Additionally, there is an increase in the biosynthetic activity of macromolecules such as proteins and lipids to support cell growth and division (Storck et al., 2018). The pentose phosphate pathway is also active to provide ribose and NADPH needed for nucleic acid synthesis and other biosynthetic activities (Maruta et al., 2016). Along with the increase in callus size, there is also a change in color that reflects changes in metabolic activity and cell differentiation. At the enzymatic level, there are many key enzymes in cell proliferation, including DNA *Polymerase* and *Topoisomerase* involved in DNA replication (Martinez-Garcia et al., 2021), *Histone Deacetylases* (HDACs) and *Histone Acetyl transferases* (HATs) that modify chromatin structure (Kumar et al., 2021). *Matrix metalloproteinases* (MMPS), *cellulase*, and *pectinase* play a role in cell wall remodeling and cell elongation (Soni & Bacete, 2023).

The initial appearance of shoots was indicated by the formation of white-green and greenish bumps (Figure 7). This is consistent with Nurfadhilah (2019), who stated that shoot formation begins with the swelling of callus forming nodules. Galán-Ávila et al. (2020) mentioned that the bumps are due to tissue differentiation and morphogenesis. According to Raspor et al. (2021), the balance of endogenous auxin and exogenous cytokinin hormones can cause meristematic cells to divide and develop into shoots.

The number of shoots produced is relatively low compared to other studies on the other genus, *Amorphophallus*. Li et al. (2021) reported that the use of 2.0 mg L^{-1} BAP, 0.5 mg L^{-1} NAA, and 0.1 mg L^{-1} GA_3 resulted in 6 shoots/explant in *A. konjac* K. Koch. Similarly, the use of 1 mg L^{-1} BAP and 15% coconut water produced 18 shoots/explant in *A. paeoniifolius* (Dennst.) Nicolson. Likewise, the combination of 2 mg L^{-1} BAP and 4 mg L^{-1} NAA produced 7.7 shoots/explant in *A. konjac* K. Koch (Restanto et al., 2014). This is likely due to the different responses of each species to the types and concentrations of growth regulators applied.

Cell elongation in shoots is influenced by endogenous auxin, which regulates the synthesis of structural proteins to enlarge the cell wall and controls cell elongation at the shoot tip. Additionally, cytokinins play a significant role in cell wall formation during shoot formation (Müller & Leyser, 2011). Various research reported that many genes involved in hormonal responses, cell division, differentiation, and growth are regulated by cytokinins. Cytokinins activate the expression of the Tumorous Shoot Development 1 (TSD1) and Korrigan1 genes, which produce endo-1,4-β-D-glucanase proteins to regulate cellulose synthesis in cell wall formation (Frank et al., 2002; Krupková & Schmülling, 2009). The increased expression of the TSD2 gene encoding S-adenosyl-L-Met–dependent methyltransferase plays a role in pectin biosynthesis during wall assembly and organ formation (Frank et al., 2002; Krupková et al., 2007). The Wuschel (WUS) gene plays a crucial role in shoot meristem formation by maintaining cells in a meristematic state (Jha et al., 2020; Bae et al., 2020; Lopes et al., 2021). CLAVATA (CLV) interacts with the WUS gene to regulate activity of the apical meristem (Xue et al., 2020; Bashyal et al., 2024). Hairy Meristem (HAM) gene is involved in initiating cell proliferation in shoots (Geng & Zhou, 2021).

The increase in root number tends to reduce the number of shoots produced in *A. titanum*. This is consistent with Restanto et al. (2014), who found a decrease in root number as the number of shoots increased in *A. konjac* K. Koch. The balance between auxin and cytokinin is crucial in determining organ development direction. The close relationship between these two hormones is mediated by regulatory mechanisms that control the production and transport of each hormone. Auxin biosynthesis is known to reduce cytokinin biosynthesis and vice versa (Marhavy et al., 2014; Simaskova et al., 2015).

However, some studies also find that the interaction between auxin and cytokinin is not always antagonistic. Exogenous cytokinins can affect endogenous auxin biosynthesis, stimulating rooting processes by increasing the activity of genes related to its biosynthetic pathway (Jones et al., 2010; Choi et al., 2014). Increased cytokinin concentrations are known to be associated with increased expression of the *Tryptophan amino transferase* of *Arabidopsis* 1 (TAA1) gene (Sun et al., 2016) and YUCCA genes (Chen et al., 2016), which are involved in auxin biosynthesis. Cytokinins are also known to cause an increase in auxin at the root meristem (Moubayidin et al., 2013; Tu et al., 2021). Several studies report that auxin can stimulate the expression of many genes involved in root induction. For example, the *Nitrilases* NIT1-4 genes play a role in root formation and development (Olatunji et al., 2017), and the miR156 gene plays a role in adventitious root formation (Ye et al., 2020).

The reintroduction activity aimed to raise awareness and understanding of the importance of preserving this rare species and the community's role in conservation efforts. The planting process was carried out carefully to ensure that the *A. titanum* seedlings received optimal conditions for growth. The planting sites were chosen based on their similarity to the natural habitat of *A. titanum*, which is rich in humus and litter. Additionally, the local community was involved in every stage of the planting process, from land preparation to the planting of seedlings, to ensure the sustainability of this conservation effort.

Monitoring was conducted two months after planting to assess the condition of the plants at the conservation sites. Observations showed that the *A. titanum* seedlings were growing well and had sprouted new shoots. This indicates that the planting environment is suitable for the growth needs of *A. titanum* and that the planting methods used were successful. The emergence of new shoots also suggests that the seedlings are adapting to their new environment and have the potential to grow and develop further. The involvement of the local community in this activity is crucial, not only for the success of the planting but also for the long-term sustainability of *A. titanum* conservation. With increased knowledge and awareness through educational sessions, the community is expected to care for and maintain the planted seedlings and participate in environmental preservation efforts in their area.

Overall, the reintroduction of *A. titanum* was successful not only in the technical aspects of planting and monitoring seedling growth but also in empowering the local community and increasing their awareness of the importance of conserving rare species. This approach is expected to serve as a model for other conservation activities in the future, with a holistic approach that involves various stakeholders to achieve sustainable results.

A. titanum, as an iconic and rare endemic species, offers significant benefits to local communities. Its uniqueness and spectacular flowering event attract both domestic and international tourists, creating opportunities for ecotourism development that can generate income and promote environmental awareness. The species also holds great value for scientific research, particularly in the fields of plant biology, conservation, and climate resilience, making it a living laboratory for students and researchers. Moreover, its conservation can foster local pride and cultural identity, encouraging community involvement in sustainable forest management. Through these avenues, *A. titanum* contributes not only to biodiversity preservation but also to socio-economic development in rural areas.

Conclusion

Through field exploration, seven individuals were discovered in various growth phases: one individual in dormancy, one fruiting, and 5 in the vegetative phase. The habitat is located in forests rich in humus and litter as well as in banana cultivation areas. The research also involved seed germination experiments by dividing the seeds into seven classes based on their weight. The most dominant seed class was the one with a weight of 2.9–3.2 g, consisting of 38 seeds, or 26.9% of the total seeds germinated. The germination results showed that seed size did not affect the germination rate and seedling growth, as all seeds exhibited a 100% germination rate and produced seedlings of relatively uniform size. Additionally, the propagation was carried out using in vitro culture techniques with petiole explants. The results showed that a BAP concentration of 2 mg L^{-1} successfully induced callus formation with a 100% induction rate, while a BAP concentration of 1.5 mg L^{-1} produced the highest shoot induction rate at 58.3%, with an average of 1.3 shoots and 7.9 roots per explant. The propagated seedlings were then replanted in several locations for reintroduction activities, namely in Tilatang Kamang District, Agam Regency (100 seedlings); Pelembayan District, Agam Regency (5 seedlings); Ranah Pesisir District, Pesisir Selatan Regency (20 seedlings); and Payo, Tanah Garam District, Solok City (20 seedlings).

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