SEED GERMINATION OF THE CORPSE GIANT FLOWER *Amorphophallus titanum* (Becc.) Becc. Ex Arcang: THE INFLUENCE OF TESTA [Perkecambahan Biji Bunga Bangkai Raksasa *Amorphophallus titanum* (Becc.) Becc. ex Arcang: Pengaruh Testa]

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ABSTRACT

Amorphophallus titanum is famous as the gigantic inflorescense and economically prospective due to its 20% glucomannan contents. Various cultivation techniques including germination have been conducted due to the delay in the seed germination of *Amorphophallus titanum*. Previous studies revealed that *A. titanum* seeds has not produced faster and better germination rate. Therefore this research was aimed to test the following hypotheses: (1) Fruit pericarp and the pericarp inhibited the germination, (2) testa/seed coat inhibited germination, (3) GA_3hormone promoted the germination rate. The germination pattern was also monitored. The experiments consisted of: Experiment 1: sowing the fruit with the seeds inside and Experiment 2 with two treatments: testa peeling and GA_3 hormone treatments. The results of Experiment 1 showed that the fruit pericarp and the pericarp inhibited the germination for 124 days. Experiment 2 resulted in: (1) the delay of the germination for 7-35 days caused by the testa/seed coat, (2) GA_3 hormone promoted the germination rate 2.19 coefficient of germination rate; and higher GA_3 (1000 ppm) may enhance the seedling growth (reached the highest 23.6 ± 1.3).We also recorded developmental stages from the seed germination, first-leaf emergence and tuber development in series of photographs overtime during the experimental period.

Key words: Germination, *Amorphophallus titanum*, GA_3 hormone.

ABSTRAK

Amorphophallus titanum dikenal sebagai bunga bangkai raksasa yang prospektif secara ekonomi untuk dikembangkan mengingat kandungan glukomanannya yang mencapai 20%. Berbagai teknik budidaya dalam hal ini teknik perkecambahan menjadi topik yang dipilih dalam studi ini mengingat rendahnya kecepatan perkecambahan biji *Amorphophallus titanum*. Dari penelitian-penelitian sebelumnya belum ditemukan metode perkecambahan biji *A. titanum* yang dapat menghasilkan daya kecambah dan kecepatan perkecambahan yang baik. Oleh karena itu, penelitian ini bertujuan untuk menguji hipotesis: (1) Perikarpa dan daging buah menghambat perkecambahan, (2) testa/kulit biji menghambat perkecambahan, (3) Hormon GA_3 meningkatkan kecepatan perkecambahan. Pola perkecambahan juga diamati. Percobaan terdiri dari: (1) Percobaan 1: Studi perkecambahan biji utuh dalam buah dan (2) Percobaan 2: Studi perkecambahan dengan dua perlakuan: tanpa testa dan aplikasi hormon GA_3. Hasil percobaan 1 menunjukkan kulit dan daging buah menghambat perkecambahan selama 124 hari. Percobaan 2 menghasilkan: (1) Testa menghambat perkecambahan selama 7-35 hari, (2) Hormon GA_3 meningkatkan koefisien kecepatan perkecambahan sebesar 2.19; dan semakin tinggi GA_3 (1000 ppm) dapat memacu pertumbuhan semai (mencapai 23.6 ± 1.3). Penelitian ini juga menghasilkan pola perkecambahan dalam serangkaian tahapan perkembangan dari biji yang berkecambah hingga semai dengan satu daun pertama serta perkembangan umbinya.

Kata Kunci: Perkecambahan, *Amorphophallus titanum*, hormon GA_3.

INTRODUCTION

Amorphophallus titanum (Becc.) Becc. Ex Arcang is well known worlwide as the corpse gigantic inflorescense. It is also an economically prospective plant with 20% glucomannan contents (Ananto, 2000 *unpublished*; Sugiyama and Santosa, 2008) higher than those in *A. konjac* which is 10.34% (Harijati *et al*., 2011). The other industrial potency of the glucomannan in *Amorphophallus* spp is an absorbent for water and aqueous solutions (Greve *et al*., 1996). Unfortunately, the habitats of *Amorphophallus titanum* (Becc.) Becc. Ex Arcang. are mostly characterized by fertile soils which is usually located near plantation. Therefore, this condition may threaten the population of *A. titanum*.

The decreasing number of *A. titanum* individuals was also caused by the slow rate of germination. The seeds of *A. Titanum* germinated after 42-56 days (Roemantyo, 1991) or 12-91 days (Latifah *et al*., 2001) with their germination capacity of 80%. This may suggest that the dormancy period was varied from 42 to 91 days caused by the presence of testa and remaining-fruit flesh on the seed surface (Latifah *et al*., 2001). Their seed germination may be inhibited by the

hard endocarp that was degraded slowly by either soil microbes or disperser agents. Their shiny-red fruit pericarps were not enough to attract the fruit/ seed dispersers because the fruit pericarp may contain the oxalate crystals. Humans and animals can be sensitive to these oxalate crystals. These oxalate-crystal contents may also inhibit degradation process in nature (Bewley and Black, 1994a). Normally, the degradation of the fruit pericarp and seed coats involved either soil organisms or other environmental factors such as the heat of sunlight, rains or interface with rough-surface of soils or rocks (Bewley and Black, 1994b). Furthermore, their thick, hard endocarps and hard testa also degraded slowly. Therefore, some techniques of propagation and cultivation can be applied and improved to save this species. For example, the application of a growth hormone GA_3 (gibberelic acid) stimulates germination through carbohydrate mobilization in seeds (Atwell *et al*., 1999).

This research was aimed to test the following hypotheses: (1) Fruit pericarp and the pericarp inhibited the germination, (2) testa/seed coat inhibited germination, (3) GA_3 hormone promoted the germination rate. The germination pattern was also monitored. The experiments consisted of: Experiment 1: sowing the fruit with the seeds inside and Experiment 2 with two treatments: testa peeling and GA_3 hormone treatments.

MATERIALS AND METHOD

The seeds were collected from Kerinci Seblat National Park, Batang Suliti Resort, Solok District, West Sumatera Province, Indonesia. Currently, the parent plant has been cultivated in Cibodas Botanic Gardens (vak I.B.28), West Java Province, Indonesia (accession number : C2000070025). The seeds were sown in potting mix containing sand and moss placed in 41 x 32.5 x 14.5 cm plastic trays. These trays were covered by transparent plastic to keep the media moist. Mosses were made from the roots of bird-nest ferns (*Asplenium nidus*) which were chopped and dried under sunlight for 1-2 days, then they were meshed (through 5-mm mesh) as many as 3 liter per tray. Before sowing, the seeds were disinfected by a pesticide and the media were fumed by Furadan 3G.

This research was located in a green house of Cibodas Botanic Gardens in Juli – November 2000 at 1,400 m elevation. The climatic data as follows: 3405 mm/year rainfall total; 26.8 °C/year maximum temperature, 10.1 °C/year minimum temperature, 87%/year maximum humidity and 92%/year minimum humidity in average. The microclimate of the glass house was: 28.5 °C/year maximum temperature, 15.8 °C/year minimum temperature, 87%/year maximum humidity, 92 % / year minimum humidity and 43%/year light intensity in average.

The hypotheses were tested by undertaking two series of experiments. These two experiments used a completely-randomized research design (Steel and Torrie, 1993). Germination capacity, germination rate and simultaneity and seedling height were analysed by one-way analysis of variance using GLM (General Linear Model) Procedure to determine if there were significant treatment effects.

Experiment 1

The germination capacity of *A. titanum* seeds sown in intact fruit was studied to manipulate natural condition. The intact fruit (with the seeds inside) were directly planted in sand or moss as treatments for 3 months. Eighty intact ripe fruit were used (115 seeds in total). The ripe fruit (Figure 1) were harvested from the parent plant after 24 days (including period of transportation and a-few-day room storage). After harvesting, the fruit were stored in a plastic bag for 7 days; this was an after ripening treatment in order to give more homogenous in the ripening level for all fruit. Before sowing, all fruit were mixed randomly. The sowing media treatments consisted of sand and moss with two replicates (four experimental units;

Figure 1. Ripe infructescence/fruit were harvested and collected from Kerinci Seblat National Park, Batang Suliti Resort, Solok District, West Sumatera Province, Indonesia

Experiment 2

This experiment used 69 seeds from fruit that was previously used in Experiment 1. As many as 69 seeds were extracted from the fruit that had been used in Experiment 1 previously This experiment involved three treatments with two replicates:1) with-testa seed samples, without GA_3 application (T0), 2) without-testa seed samples, without GA_3 application (T1), 3) without-testa seed samples, soaked in by 1000 ppm GA_3 for 48 hours (T2) referred to previous experiment with best results by Latifah *et al*. (2001). Sand was used as the sowing media as according to Latifah *et al*. (2001) the sand increased the germination capacity of *A. titanum* seeds. Ten seeds per experimental unit were used for total 6 experimental units. After the seeds were soaked in water containing fungicide Dithane N45 for 24 hours, the seeds were dried in room temperature; then some seed samples were soaked in 1000 ppm GA_3 for 48 hours (T2).

Observation on the Developmental Stages of *A. titanum* from Seed to Seedling

During the period of Experiments 1 and 2, after the seeds germinated and grew, five seedling samples from different develomental stages were taken out for growth observation for 19 weeks (133 days after sowing) and the tuber was observed after 24 weeks (168 days) to avoid growth interruption. The variables measured and observed were the total height of the seedling, the number of cataphylls, the number of roots (the roots observed qualitatively without differenciating between primary, secondary and tersier roots; the main/prominent roots were counted), the height of leaf, the length of petiole and the diameter of lamina (leaf blade). When the seedling stage was 'leaf bud stage', the total height referred to the height from above sowing/growing media to the highest tip of the the highest cataphyll; and when the seedling had petioles and laminas, the total height of the seedling was measured from above media tothe highest tip of the lamina. The fully open leaf (100% open) had its height measurement less than the less-than 100% open leafas the height total of the leaf was measured from the base/above sowing media surface to the the highest tip of thelamina. For statistical analysis of seedling growth during the experimental period, the data used were those until the highest total height measured to avoid bias. Regression analyses were used to estimate the growth pattern of the leaf at the 95% confidence level or $p<0.05$ (Steel and Torrie, 1980; Zar, 1999; Miles and Shelvin, 2001;) using SPSS version 17.0. The seedling height data (8^{th} – 14^{th} week) were transformed into ln (log

natural) and the outlayers were not used. The developmental stages of the seedlings were descriptively described without statistical analysis.

Botanical Terms of Seedling Parts

The botanical terms of *A. titanum* seedling parts used were based on those applied in Araceae according to Lobin *et al*. (2007) and Li (2013) (Figure 2).

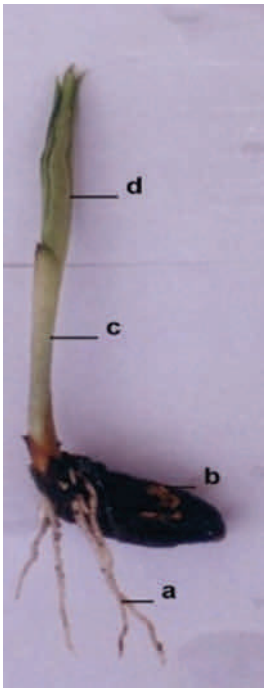

Figure 2. Seedling of *A. titanum*;80 mm height (a:Roots, b: Germinating seed, c: Cataphyll, d: Leaf/lamina before opening

RESULTS

Effect of the Fruit Pericarp and Pericarp on The Germination

The results are explained descriptively as follows because after 3.5 months the germination was absent and a little part of a few seeds had been consumed and party destructed by pests (a white ticks). They quite degraded some parts of exocarp, mesocarp and endocarp. These results suggested that the seed germination of *A. titanum* required a cultivation technique such as the peeling of the fruit epidermis (fruit peel) and the juicy pulp removal for extracting the seeds from the fruit (Experiment 2; the results as follow). Leaving the intact fruit (without extracting the seeds from the fruit/ removing all the fruit pericarp from theseeds) as they are in nature may delay the germination.

Effect of Testa on The Germination

The three treatments resulted in different responses on *A. titanum* seed germination rate and simultaneity and seedling height (Table 1-4). The treatments given did not affect the germination capacity of *A. titanum* seeds. However, the delay of seed germination caused by testa was evident (coefficient of germination rate was 1.7, the most delayed, $F(2,5) = 268$, $p < 0.01$).

Table 1. A. Germination capacity of *A. titanium* seedsafter 35 days; B. Analysis of variance (R Squared = 0.40).

A

Treatments	Germination capacity (%) Mean ± SE
With testa, without GA_3 (T0)	90 ± 10
Lack of testa, without GA_3 (T1)	100 ± 0
Lack of testa, GA_3 1000 ppm (T2)	100 ± 0

B

Source	DF	Sum of Squares	Mean Square	F Value	PR>F (Sig.)
Model	2	133.33	66.67	1.00	0.46
Treatment	2	133.33	66.67		
Error	3	200.00	66.67		
Corrected Total	5	333.33			

The germination of seeds with hard testa delayed for 7-35 days. This suggested that the dormancy period of the seeds was at least 7-35 days. However, after the dormancy was terminated and the seeds begun to germinate, the germination was simultaneous (coefficient of germination simultaneity was 0.8, the highest, $F(2,5) = 60$, $p < 0.01$).

Table 2. A. Germination rate of *A. titanum* seeds after 35 days; B. Analysis of variance (R Squared = 0.99); (1: Samples with the different lower case letters are significantly different; p = 0.05)

A

Treatments	Coefficient of germination rate (Mean ± SE)[1]
With testa, without GA_3 (T0)	1,7 ± 0,19a
Lack of testa, without GA_3 (T1)	1,8 ± 0.01b
Lack of testa, GA_3 1000 ppm (T2)	2.2 ± 0c

B

Source	DF	Sum of Squares	Mean Square	F Value	PR>F (Sig.)
Model	2	0.23	0.12	267.96	0.0004
Treatment	2	0.23	0.12		
Error	3	0.0013	0.0004		
Corrected Total	5	0.23			

Table 3. A. Germination rate simultaneity of *A. titanum* seeds after 35 days; B. Analysis of variance (R squared = 0.98).

A

Treatments	Coefficient of germination simultaneity (Mean ± SE)[1]
With testa, without GA_3 (T0)	0.8 ± 0.06a
Lack of testa, without GA_3 (T1)	0.5 ± 0.02b
Lack of testa, GA_3 1000 ppm (T2)	0.3 ± 0.02c

B

Source	DF	Sum of Squares	Mean Square	F Value	PR> F (Sig.)
Model	2	0.29	0.14	59.98	0.004
Treatment	2	0.29	0.14		
Error	3	0.0013	0.002		
Corrected Total	5	0.23			

The results (Figure 3) demonstrate the effect of the three treatments on the seedling growth during the experimental period. Theregression

Table 4. A. The seedling height of *A. titanum* seeds after 133 days; B. Analysis of variance (R squared = 0.50)

A

Treatments	Height of seedlings(cm) Mean ± SE[1]
With testa, without GA_3 (T0)	16.5 ± 2.2a
Lack of testa, without GA_3 (T1)	20.1 ± 1.5b
Lack of testa, GA_3 1000 ppm (T2)	23.6 ± 1.3c

B

Source	DF	Sum of Squares	Mean Square	F Value	PR>F (Sig.)
Model	2	20241.08	10120.54	10.50	0.0007
Treatment	2	20241.08	10120.54		
Error	21	20246.25	964.11		
Corrected Total	23	40487.33			

formulas for growth patternsshow that the seedlings from1000 ppm GA_3-treated seeds (T2) were the highest on 14th week (Table 1; Figure 3). However, the growth level of the seedlingsof T2-treated seeds was thelowest (beta 1=0.074) which means that > 14-week seedlings with testa-without GA_3 (T0)-treated seeds (beta 1= 0.184) as well as without testa-without GA_3 (T1)-treated seeds (beta 1=0.157) were potentially growing more rapidly. This result suggest that higher GA_3 (1000 ppm) enhanced the seedling growth but the growth level each week did not increase significantly.

Developmental Stages of *A. titanum* from Seed to Seedling

The seedling has at least three phases (Figure 4:1-9). The explanation as follows:

Phase 1: The Growth of the Seedling with Leaf Bud Protected by the Cataphylls (Figure 3: 1b-5)

The seed (Figure 4:1b) germinated on 2nd week after sowing (Figure 4:2). Between 2nd -3rd week (Figure 4:2) the whitish brown, the cone-like leaf bud covered by 3-4 cataphylls emerged and the height was variously 2-7 mm followed by the early

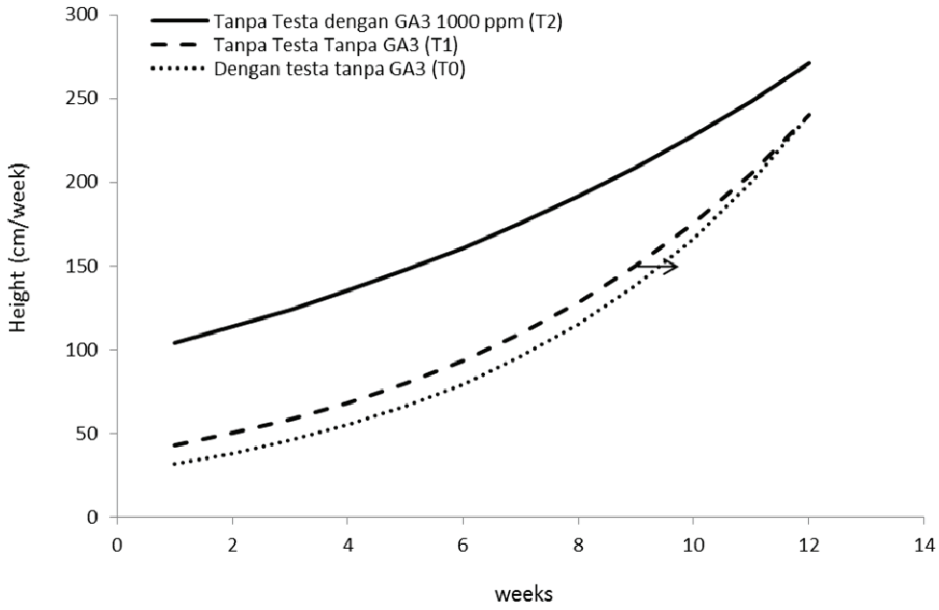

Figure 3. Comparison of the leaf/seedling growth level between treatments.

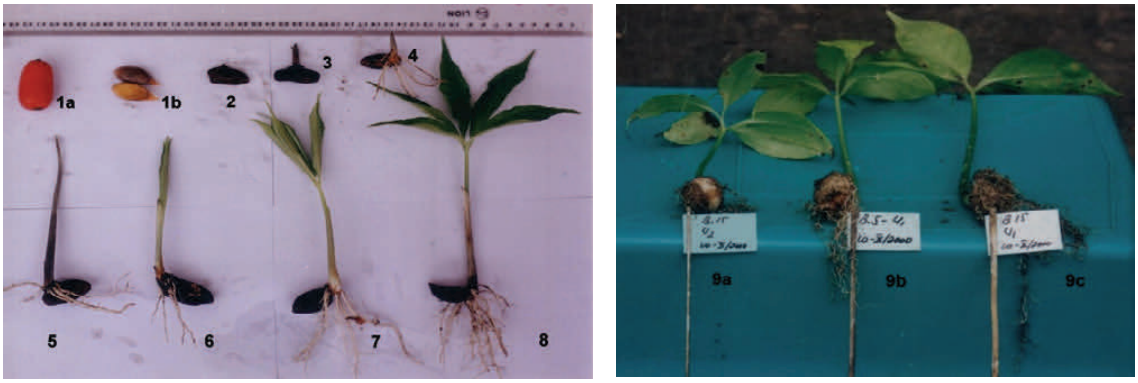

Figure 4. The developmental stages of *A. titanum*from seeds to seedling (stages 1-9) were shown from the seed germination (stage 2) to first-leaf emergence (stage 8) for 19 weeks and tuber development (stage 9) after 24 weeks); scale is in mm.

growth of a few roots. The leaf-bud height was varied between 20-80 mm after 2 weeks. On 5th - 8th week the number of the cataphylls were 5-7; the primary colour of the cataphyll was whitish-light green with dark green to black or reddish to brownish purple irregular blotches. On 5th week, the seedling (leaf-bud/the highest tip of the most outer cataphyll) was 80-150 mm high and started rooting (a few roots about 3-4 roots) then more rooted on 8th week. Between 5th – 8th week the number of cataphylls were 5-7, whitish light green with dark green to purplish brown blotches (Figure 3:3-3:4).Between 8th–11th week, the old cataphylls senesced and fell down, 3-4 cataphylls remained and began to be in a senescence state. On 11th week the seedling was 150-180 mm high and the tip of the seedling which was the most outer (the last) cataphylls appeared to swell indicating the lamina to come out (Figure 4:5).

Phase 2: The Early Growth of Leaf (Figure 4: 6-8)

On 11thweek the un-open leaf emerged from the tip of the enclosing last cataphyll (Figure 4:6).

After three weeks, all cataphylls had been senesced or at least one was left and being senesced. The petiole (leaf stalk) was already clearly seen with whitish irregular blotches. The whitish irregular blotches revealed on the petiole surface. Overall the petiole was between 50-80 mm high and a few seedlings reached 80-120 mm in height. The roots were quite massive with 7-10 roots as the tersier roots started growing.The leaf began to open on 14th week (Figure 4:7); the petiole was between 60-160 mm high.During 3-week observation, more whitish blotches of the petiole were evident.and the roots grew well (about 10-11 single roots) within 3 weeks. At this stage all cataphylls were already senesced and fell down. There was no significant seedling growth between 17th-18th week (Figure 4:8), the total height of the seedling was 125-270 mm and the leaf was not fully opened yet with 57-275 mm in diameter. The leaflets were varied from lanceolate to elliptical. The number of the leaflets were 5-7, only a few seedlings had 2-3 leaflets. The height of petioles was 65-238 mm. On 19th week, the lamina was fully opened which was 160-270 mm in diameter. The roots reached 11 roots and 85-210 mm long.

Phase 3 (Figure 4:9): Tuber Development

The 24-week seedlings had the tubers which were 50-100 mm in diameter without any significant increase of the seedling height.

DISCUSSION

The Germination of *A. titanum* Seed in Intact Fruit

The germination of *A. titanum* seeds in intact fruit is inhibited by the presence of thick fruit flesh (mesocarp) and hard testa. The fruit remained fresh -red suggesting that the fruit can maintain their freshness for at least 2 months after sowing. After 3.5 months, a little part of a few seeds had been consumed by a type of white ticks. They quite degraded some parts of exocarp, mesocarp and endocarp. In addition, the seeds inside the fruit remained ungerminated. This may also suggest that the seed conservation of *A. titanum* may be undertaken by storing the fruit with the seeds inside in moss for at least 3.5 months (this is called as an after-ripening treatment or matriconditioning treatment to stimulate the germination according to Nurmailah (1999). However, further studies on seed storage techniques for *A. titanum* seeds are required. In nature, these results may imply that although the exocarp, mesocarp and endocarp were not germination inhibitors as long as they degraded by soil organisms; moreover, they may protect the seed embryos before germination takes place. In addition, the degraded parts of the seeds stimulated the imbibitions that initiate the germination as oxalate crystals had been removed from these three parts of fruit (exocarp, mesocarp and endocarp). Oxalate crystals may inhibit the germination as many seed disperser agents such as some animals may be sensitive to this substance (causing allergy and fruit inedibility).

Germination Capacity of *A. titanum* SeedsWith and Without Testa

The germination of *A. titanum* seeds was inhibited by testa. This research resulted in the germination delay from 7-35 days. These results were in accordance to Hetterscheid (1996) who also reported that many of *Amorphophallus* spp. seeds were able to germinate after 7 – 21 days. The variation on the nature of the tough testa with its aril attached may cause this delay as it may inhibit the initial water imbibition (Koch and Dixon, 2000; Candiani *et al*., 2004). However, after the dormancy was ended and the seeds begun to germinate, the germination was simultaneous. GA_3 enhanced the germination rate (coefficient of germination rate was 1.8) because GA_3 mobilized carbohydrates to trigger germination (Abidin 1989, Atwell *et al*., 1999). However, the growth level of the seedlings each week did not increase significantly as GA_3 play only less important roles on the growth of the petioles (Atwell, 1999).

Bewley and Black (1994a) suggested that the germination rate can be enhanced when the imbibitions rate of the seeds were not inhibited by (1) testa or, (2) lack of soil moisture that can prevent the difference of water potency between the sowing media and the seeds.

A Phase of Life Cycle: Developmental Stages of *A. titanum* from Seed to Seedling

The adult *A. titanum* has vegetative (leaf) and generative (inflorescence or infructescence) phases; however, before the generative phase the tuber remains at dormancy phase for several months. Whereas at the early growth stage, the seedling of *A. titanum* has vegetative phase supporting photosyntheses to produce more photosynthat such as starch that contributes the tuber enlargement. The developmental stages of *A. titanum* from seeds to seedling were shown from the seed germination, first-leaf emergence and tuber development in series of photographs overtime during the experimental period (Figure 4) referred to Kikuta *et al*. (1938) in another species of Aroid. Three phases of seed-to-seedling life cycle in *A. titanum* were discovered and recorded consisting of (1) the growth of the seedling with leaf bud protected by the cataphylls, (2) early growth of leaf, and (3) tuber development.

CONCLUSION

The fruit pericarp and pericarp as well as seed coats/testa may inhibit the germination of *A. titanum* seeds; GA_3 hormone enhanced the germination rate. The fruit pericarp and the pericarp inhibited the germination for maximum 35 days (times to final germination). The delay of the germination for 7-35 days was caused by the testa/ seed coat. GA_3 hormone promoted the germination rate with 2.19 coefficient of germination rate; and higher GA_3 (1000 ppm) may enhance the seedling growth (reached the highest 23.6 ± 1.3c). We also recorded developmental stages from the seed germination, first-leaf emergence and tuber development in series of photographs overtime during the experimental period.

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