Journal of Tropical Crop Science Vol. 12 No. 1, February 2025
www.j-tropical-crops.com

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

Protocol Development for Assessing Seed Moisture Content and Germination Testing in *Amorphophallus muelleri* Blume

Siti Fadhilah[A], Eny Widajati*[B], Satriyas Ilyas[B], Endah Retno Palupi[B], Abdul Qadir[B]

[A] Graduate Program in Agronomy and Horticulture, Bogor Agricultural University, Indonesia
[B] Department of Agronomy and Horticulture, Bogor Agricultural University, Indonesia

*Corresponding author; email: eny_widajati@apps.ipb.ac.id

Abstract

Seed quality testing involves the evaluation of germination capacity under a standardized moisture content. However, limited reports have been found for seed testing methodology, especially for *Amorphophallus muelleri*. Standardizing the seed testing method for *A. muelleri* is essential to ensure repeatability, reproducibility, and reliable seed germination results. This study aimed to develop procedures for assessing seed moisture content and germination tests. Three seed lots were used for seed moisture content determination. The necessity for seed cutting and efficacy of high-temperature oven methods (133°C for 4, 5, and 6 h) were compared to low-temperature oven methods (103°C for 17 h). Five lots of seeds of different ages were used for the seed germination test using the between-paper method in a constant temperature germinator of 25 and 30°C. There was no significant difference in the moisture content between the uncut/whole and the seeds cut. The high temperature of 133°C for four hours is an alternative to the low temperature. Germination rates at 25 and 30°C had no significant difference, i.e., 84 to 97% and 83 to 99%, respectively. However, at 30°C, it was shorter to 50% germination (T50) and germination period. Preheating the seeds for four weeks using the dry heat method shortened the seed germination time from 57 to 37 days. Seed lot exposed to dry heat had half the time required to reach T50, or 16.4 to 31.9 days after treatment, with a germination period of 37 to 52 days. The first count of germination at 25°C without preheating was 47 days after planting, and the final count was 73 days after planting, whereas at 30°C, it was 37 and 57 days after planting. The first and final count for germination with preheating treatment at both temperatures was 20 DAP and 37 DAP.

Keywords: between-paper method, normal seedling, preheating, first count, final count

Introduction

Amorphophallus muelleri Blume is one of the most popular cultivated food species; the tubers are usually marketed fresh and for processing, including chips and glucomannan flour. Glucomannan is a highly valuable raw material for food and non-food industries. *Amorphophallus muelleri* belongs to the Araceae family, widespread throughout tropical and subtropical areas, including Indonesia. *Amorphophallus muelleri* Blume has a synonym *A. oncophyllus* Prain. (Sharma and Wadhwa, 2022; Sumarwoto, 2005).

Amorphophallus muelleri can be propagated using bulbs and corms, however, both have low multiplication and inefficient bulking rates. The larger size and weight of bulbs and corms need more volume and space when they are to be used as planting material. Bulb and corm need more than 300 kg.ha^{-1} and 1500 kg.ha^{-1}, respectively. Propagation using botanical seeds may solve the problem of low propagation coefficient, which is only 30-40 kg per ha. The seed's physiological potential could be evaluated based on germination and moisture content testing. The International Seed Testing Association (ISTA) has published standard methods to assess seed quality, including germination and moisture content. Unfortunately, ISTA has not included standard methods for species like *A. muelleri* (ISTA 2021).

Seed moisture is an influential factor in seed longevity. Seeds with high moisture content deteriorate rapidly (Bakhtavar et al., 2023). Small changes in seed moisture content may affect the storage life of seeds. Various methods have been applied to determine seed moisture. However, there are three important factors in seed moisture content determination: grinding of seeds, drying temperature, and the drying period (Jaganathan et al., 2022). To evaluate the importance of these factors, whole and cut seeds were dried in a low-temperature oven, then the high-

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

temperature oven method was continued for various durations (ISTA, 2007).

Seed germination tests can provide an estimate of the crop's value in the field. Seed germination tests require optimum environmental conditions since testing under field conditions is usually unsatisfactory, as the results are inconsistent. Seed germination using straw paper takes 15 days (Sari et al., 2019) to three weeks (Harijati and Widoretno, 2019), whereas using a combination of soil, straw charcoal, and cocopeat takes up to 100 days (Wardani and Harijati, 2019).

There is limited information about the effects of temperatures and duration exposure to different temperatures on the seed moisture content, seed germination, and seedling quality. Therefore, it is important to develop a method with standardized conditions to reproduce the test result within a limited sample variation. Standardizing *A. muelleri* seed testing is important to ensure the testing method's repeatability, reproducibility, and reliability. This study was conducted to develop a protocol for determining seed moisture content and seed germination of *A. muelleri* seed.

Materials and Methods

Experiment 1. The Protocol Development for Seed Moisture Content Determination

Seed Lot Preparation

This method's development required three distinct levels of seed moisture content (MC). Three seed lots of *A. muelleri* var. "Madiun 2", used for seed moisture content determination, was harvested in May-July 2022 from Madiun (two lots, MDN1 MDN2), East Java, and Purwokerto (one lot, PWTA), Central Java, locations. Three different levels of seed moisture content (MC) were high (66.4 – 69.5%), medium (46-60%), and low (35-45%). Medium and low seed moisture content were obtained by reducing the initial seed moisture content.

The seed lots with different moisture content levels were prepared by drying seeds with high initial moisture content (67-68%). Seeds were placed in a tray and kept in an incubator at 70°C for 4-5 h per day until they reached 46-60% of their initial weight and 35-45% moisture content. The seeds were regularly weighed to estimate the level of moisture using the following equation:

Weight of seed lot at specific % moisture content = initial weight x (100-initial moisture content)/(100-desired moisture content)

When the seed lot reaches a specific moisture content level, it is kept in impermeable packaging for 24 hours and stored in a cold chamber at 7-10°C. Before testing, the seeds were moved to ambient room temperatures for 5-7 hours.

Seed Moisture Testing

The moisture of the whole and cut seeds was assessed using a completely randomized design consisting of two factors, i.e., different seed lots based on the location of the seed source (MDN1, MDN2, PWTA) and seed treatments before drying (whole and cut seeds). Seed moisture content was determined by the low-temperature oven method (LTOM) at 103$\pm$2°C for 24 h. Three replicates of seed lots were cut into pieces of < 7 mm. The seeds were cut due to the sticky nature and the high initial moisture content, which makes the grinding impractical. Each replicate used duplicate samples, which were independently drawn from working samples.

The efficacy of the high-temperature oven method was evaluated using a completely randomized design involving two factors: three seed lots (MDN2, MDN3, and MDN5) and drying temperatures of 103°C and 130°C. Each treatment was replicated three times. The oven temperatures were set at 103°C for 24 h, and the high temperature was set at 130°C for 4, 5, and 6 h.

The procedures for seed moisture testing used the ISTA method (2021). Each duplicate sample of 4.5 – 5 g of seeds was weighed. Porcelain cups and covers of 6 cm diameter were weighed before and after filling with the whole or cut seed. The containers were placed quickly next to their cover in an oven maintained at the required temperature. After heating, the containers were closed with the covers removed and cooled down in the desiccators for 30 minutes. The containers with their covers were weighed after cooling, and the following formula calculated the moisture content as a percentage by weight:

Seed MC (%) = (M2-M3)/(M2-M1) x 100%

Where:
MC : moisture content
M1 : weight of the container and cover (g)
M2 : weight of the container, cover, and contents before drying (g)
M3 : weight of the container, cover, and contents after drying (g)

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

Experiment 2. The Development of Seed Germination Test Procedures

Seed lot preparation

The seed germination testing was organized using a completely randomized design of two factors: seed lots and temperatures. Five seed lots of *A. muelleri* var. "Madiun 2" with different ages from different locations was used for this study: lot L1 from Panjaran village and lot L2 from Klangon village were 8 weeks after harvest (WAH), L3 from Klangon village and L4 from Tapos village were 3 WAH, L5 from Tapos village was 11 WAH. All seed lots were harvested from May to July 2023 and kept in a room with 20-24°C temperatures.

Three seed lots (L1, L2, L5) were tested for a dry heat preheating treatment to promote seed germination. Seeds were placed in a tray and kept in a germination incubator at 35°C for four weeks. The seed germination was then compared with the seed lot without the preheating treatment.

Germination testing

The seed germination test was conducted using the paper method at two different constant temperatures, 25°C and 30°C. The procedures of gemination testing were according to ISTA (2021). One hundred and fifty seeds were taken from each seed lot. With three replicates, each replicate consists of 50 seeds. The seeds were placed on a double-layer moist paper medium. Media could be rolled loosely and kept in a container to keep the humidity. The seed was incubated in a germinator with light at the required temperature. Seed growth was examined and documented during seed testing to determine normal and abnormal seedlings. Evaluation was carried out every three days until all seedlings grew normally, and seed germination reached 50% of final germination (T50).

The paper growing media used in germination testing should have sufficient strength to withstand the test, sufficient pore space for air and water, a pH value within the range of 6.0-7.5, salinity of $\leq$40 $mS.m^{-1}$, clean and free from toxic materials (ISTA, 2021).

The total period of seed germination was counted to indicate dormancy. The ungerminated seeds were checked by gently pressing with a thumb to determine their internal state. If a symptom of fungal infection or internal content oozed out or appeared rotten, it was considered dead. The germination test results are expressed as a percentage of the number of normal seedlings:

Germination (%) = (number of normal seedlings/ number of seeds) x 100

Calculations based on the nearest whole number, 0.5, were taken to the higher side (ISTA, 2018).

T50 was calculated according to Kumar et al. (2021):
T50 = Ti +[(N+1/2)-Ni] (Tj-Ti)/(Nj-Ni)

where T50 is the median germination time, N is the final number of germinated seeds, and Ni and Nj are the total numbers of seeds germinated in adjacent counts at time Ti and Tj, when Ni <((N+1)/2) < Nj.

Data Analysis

The necessity of cutting the seeds was assessed by comparing the whole versus cut seeds at various moisture levels using a t-test with a 5% significance level. The efficacy of oven temperature was assessed by comparing high and low-temperature ovens using a t-test with a 5% significance level.

The optimum temperature for the seed germination testing method was obtained by germinating five seed lots between constant temperatures of 25°C and 30°C using ANOVA at a 5% significance level. The effectiveness of preheating was determined by comparing the germination rate, T50, and period of germination testing using ANOVA at a 5% significance level.

Results and Discussion

Experiment 1 The Development of The Seed Moisture Content Determination Procedures

Seed Lot Preparation

Amorphophallus muelleri seeds had high moisture content and variable seed weights at harvest. Based on seed weight *A. muelleri* can be classified into large (0.2 to ≤ 0.3 g per seed), medium (0.1 to ≤ 0.2 g per seed), and small (0.01 to ≤ 0.1 g per seed) (Dewi et al., 2015). All seed lots used in this testing had > 65% moisture content at harvest. The 1000-seed weighed 185-242 g (Table 1). Seed lots MDN 2 and PWTA had a 1000-seed weight of 200.84 g and 242.66 g, so the seeds were categorized as large.

Amorphophallus muelleri seeds at harvest were sluggish and had high moisture content. Seed weight was reduced during preparation to obtain three moisture content levels. Seed moisture content decreased very slowly; it took up to three to five days to obtain 50-55% and 44-48% MC, respectively (Figure 1).

Journal of Tropical Crop Science Vol. 12 No. 1, February 2025
www.j-tropical-crops.com
Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

Seed moisture plays an important role in seed longevity; it is usually high at the physiological maturity stage and decreases until it reaches equilibrium with the environment's relative humidity. The seeds can retain moisture using different mechanisms (Hay et al., 2022).

Amorphophallus muelleri tubers and seeds have high amounts of glucomannan, consisting of β–glucose and β–mannose. It exhibits a high viscosity, solubility, and swelling property, good film–forming properties, and good gel properties in aqueous solutions. Hydrogen bonding and hydrophobic interactions establish the three–dimensional gel network in glucomannan, which closely relates to its hydrophilicity and gel properties (Harijati et al., 2018; Sharma and Wadhwa, 2022; Sun et al., 2023). Glucomannan has very high water absorbance and gelation properties (Sharma and Wadhwa, 2022) and prevents water from evaporating.

Whole Seed versus Cut Seed and the Low-Temperature Oven Method

Determining seed moisture content is one of the most critical and common assessments for a seed lot, as it estimates its value in commercial settings and the seed's physiological status for research and conservation. The most common method for determining seed moisture content is measuring the change in sample weight after drying at a constant temperature for a certain time (Hay et al., 2023).

The seed of *A. muelleri* had high moisture content and remained above 40% MC though it dried for days. The seed has mucilage that makes it sticky. Therefore, grinding the seed in a grinding mill is impossible. The seed is 0.5-1 cm long and cut into two pieces less than 0.7 mm (Figure 2).

The basic method for determining seed moisture content is to measure a sample's weight change after drying at a temperature that releases the water. The chemical composition and structure of the seeds will influence this process, and hence, the sample preparation, temperature, and drying duration vary among seeds of different species. The sample may need to be cut into pieces or ground for larger seeds before weighing and placing in the oven (Hay et al., 2023). The analysis of seed moisture testing using the low-temperature oven method showed different results, i.e., < 0.5% for K0 and 0.6% for K1. The difference was getting higher in K2 except for the PWTA lot. Thus, the seed cutting treatments were not statistically different for three levels of seed moisture lots (Table 2).

Water evaporation from the seed surface to the air and water movement from the inside part to the seed surface is the primary process during seed drying. Characteristics such as seed size and chemical composition will affect the suitable method for assessing the seed moisture content (Silva et al., 2020). Seeds cut in fragments had more expansive evaporation surfaces than the whole seeds. Therefore, the moisture content of the whole seeds tends to be lower than that of the cut seeds. Using the whole seed at the constant low-temperature oven method (17 h at 103°C) can be an efficient method to determine the seed moisture content and is more

Table 1. The 1000-seed weight and the initial seed moisture content of three seed lots

Seed lot	1000-seeds weight (g)	Initial seed moisture content (%)
MDN 1	185.65	68
MDN 2	200.84	67
PWTA	242.66	67

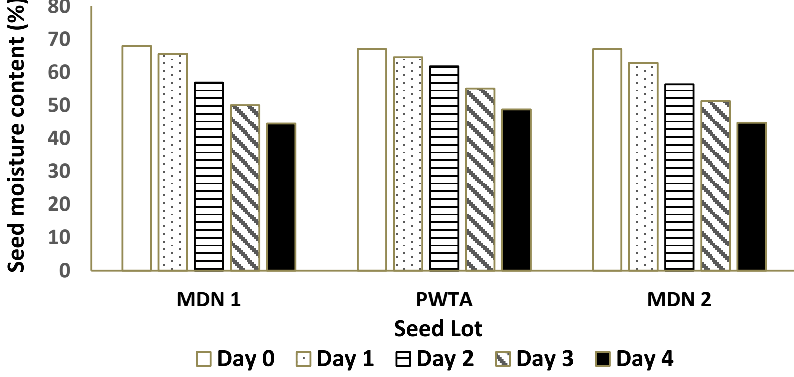

Figure 1. The moisture contents of *Amorphophallus muelleri* from different seed lots

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

accessible and practical to use in a laboratory. Since *A. muelleri* seeds were sticky and had mucilage, cutting them into fragments < 7.0 mm in a short time of environmental exposure was difficult.

Test the High-Temperature Oven Method

The average differences in seed moisture content using 103°C for 17 h and 130°C for four, five, and six hours were 0.69, 0.31, and 0.45, respectively (Table 3). Since there was no significant difference among high-temperature oven methods, the four-hour high temperature can be used to determine seed moisture content to substitute the low temperature for 17 h.

Seed moisture content determination at low temperatures takes 17 hours. Limited electricity supply, sample delivery on weekends, or the need for rapid testing become obstacles in the laboratory. Therefore, a high constant temperature method with a shorter time was needed. Since the comparison between the whole and cut seeds showed no significant difference, the testing method for seed moisture content determination should use the whole seed. There were no significant differences in germination between low and high temperatures (Table 3).

Testing moisture content immediately after seed harvesting is necessary to determine the proper seed handling to preserve its viability. *Amorphophallus muelleri* seeds might be recalcitrant since they had high moisture content at harvest. Research by Sari et al. (2019) showed that *A. muelleri* seeds have 63.7-66.5% moisture content, which can increase to 73.4% after six weeks of storage. Recalcitrant seeds usually germinate quickly and are sensitive to drying, so they cannot be stored for long periods. Furthermore, high

Table 2. The seed moisture content of the whole seed and the cut seed at the constant low-temperature oven (17 hours at 103°C)

Seed MC	Lot	Seed moisture content (%)		
		Whole seed	Cut seed	Difference
K0 (61-69%)	MDN 1	68.55	68.28	0.26
	MDN 2	67.27	67.64	0.37
	PWTA	67.10	7.48	0.38
t Test		ns		
K1 (46-60%)	MDN 1	50.17	50.67	0.50
	MDN 2	49.81	49.22	0.59
	PWTA	48.78	49.13	0.35
t Test		ns		NS
K2 (35-45%)	MDN 1	41.04	43.00	1.96
	MDN 2	41.19	41.89	0.70
	PWTA	43.29	43.61	0.32
t Test		ns		

Note: ns = values were not significantly different according to the t-test at α<0.05.

Figure 2. The whole seed with 1-cm length (A) and the half seeds (B)

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

water-content seeds are easily attacked by fungi.

Experiment 2 The Development of Seed Germination Test Procedures

Germination Test at 25°C and 30°C

The germination test provides information on seed viability in an optimum condition for the seed to germinate and develop normal seedlings. *Amorphophallus muelleri* grows in humid and wide-ranging temperature environments, either in lowland or upland tropical areas (Kurniawan et al., 2011; Sumarwoto, 2005). Therefore, similar environmental conditions of maternal plants must be tested to determine the optimum condition for seed germination in the laboratory (Rosa et al., 2021).

Seed germination at 25°C and 30°C gave similar results. Seed lots L1, L4, and L5 had higher germination at 30°C than at 25°C. Germination at 30°C was more homogenous among replicates, especially in L1, L4, and L5, indicated by the low standard deviations (Figure 3). However, there were no differences in the germination rate at 25°C and 30°C (Table 4).

The periods for seed germination to reach 50% (T50) varied among the seed lots. Seed lots harvested earlier have a shorter time to germination because the seed embryo has emerged (Figure 4).

The germination took longer at 25°C than 30°C for all seed lots tested. L3 and L4 with 3 WAH needed more than 70 days to reach T50 at 25°C, whereas L3 needed < 55 days at 30°C. Lot L1, L2, and L5 with 8, 7, and 11 WAH, respectively, needed < 50 days at 25°C and around 40 days at 30°C to reach T50.

The total L3 and L4 germination period was more extended than L1, L2, and L5 at 25°C (Table 4). Ten percent of the *A. muelleri* seeds germinated 24 weeks after anthesis, and the germination reached 100% in 36 weeks after anthesis (Sari et al., 2019). Furthermore, the seed needs 70 and 92 DAP to germinate when harvested from red and green fruits (Wardani and Harijati, 2018; Harijati et al., 2018). These differences in the germination period indicated that *A. muelleri* seed had dormancy. Even though the seed moisture content was high at harvest, it took 1-2 months before the seeds started to germinate (Sumarwoto, 2005), 4-6 months (Jansen et al., 1996), or during the winter (Zhao et al., 2010).

Determination of Normal and Abnormal Seedling

Amorphophallus muelleri is monocotyledon with hypogeal germination (Figure 5). The seed starts germinating when the plumula protrudes and emerges through the seed coat (Figure 4 B). The seedling was considered normal when it had all the essential structures, i.e., root and shoot (Figure 5 and Figure 6).

ISTA (2018) uses some criteria to assign seedling growth and divide into groups according to systematic class (monocotyledon, dicotyledon, and conifer), germination type (epigeal and hypogeal

Table 3. Seed moisture content at constant low and high temperatures

Moisture content (%)	Lot	Seed moisture content at different temperature treatments			
		103°C* 17 h	103°C 4 h	130°C 5 h	130°C 6 h
61-69	MDN 2	61.54	61.18	61.17	61.64
	MDN 3	63.39	63.53	63.38	63.75
	MDN 5	63.25	62.26	62.42	62.76
t-test			ns	ns	ns
46-60	MDN 2	52.44	52.11	51.77	52.96
	MDN 3	50.80	49.55	50.74	50.70
	MDN 5	49.02	47.98	49.13	49.96
t-test			ns	ns	ns
35-45	MDN 2	44.08	42.31	43.77	44.22
	MDN 3	40.27	40.22	40.47	40.17
	MDN 5	38.53	38.23	38.31	39.79
t-test			ns	ns	ns

Notes: *103°C 17 h is the standard method compared with 130°C for 4, 5, and 6 hours. ns= not.

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

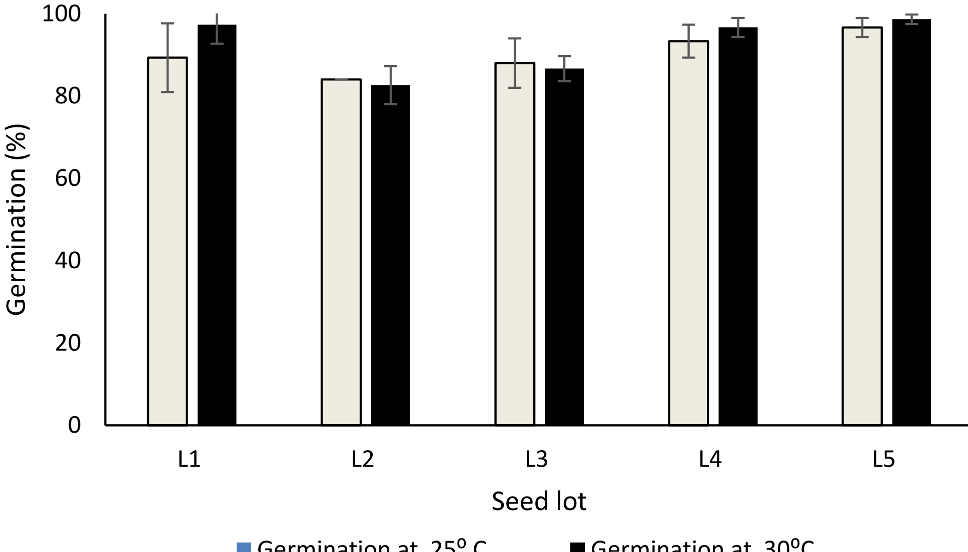

Figure 3. Germination of *Amorphophallus muelleri* seed lots at two different temperatures. Seed lot L1 is from Panjaran village; seed lot L2 is from Klangon village, 8 weeks after harvest (WAH); seed lot L3 is from Klangon village; seed lot L4 is from Tapos village, 3 WAH; seed lot L5 is from Tapos village, 11 WAH.

Table 4. *Amorphophallus muelleri* seed germination rate, T50, and total period of germination

Seed Lot	Germination (%)		T50 (DAP)		Germination period (DAP)	
	25°C	30°C	25°C	30°C	25°C	30°C
L1	89	97	45.4^{de}	38.9^{g}	74	54
L2	84	83	47.5^{d}	40.3f^{g}	74	54
L3	88	87	78.4^{a}	53.9^{c}	115	64
L4	93	97	70.5^{b}	42.2^{efg}	129	57
L5	97	99	43.4^{def}	42.7^{def}	79	69
Significance	ns		**			

Notes: Seed lots L1 and L2 are 8 weeks after harvest (WAH), seed lots L3 and L4 were 3 WAH, and seed lot L5 was 11 WAH. ** is significantly different, and ns= not significantly different according to the ANOVA at α<0.05.

Figure 4. *Amorphophallus muelleri* seeds after harvest (A) and after emergence (B).

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

germination), shoot development (with or without epicotyl elongation, no shoot elongation, with coleoptile, and tuberous hypocotyl), root system and its significance for evaluation (primary root essential, secondary roots may compensate for the primary root, several equal seminal roots). Unfortunately, a family of Araceae, especially *Amorphophallus* has not been listed in ISTA Rules. Tillich (2003) assigned Araceous seedlings and classified them into six types (Type A-F) based on the types of cotyledons (bifacial hypophyll and unifacial hyperphyll) and the existence of endosperm (with or without endosperm). Then, it was reduced to three basic types of seedlings, type A (with sub-type A1, A2), type B (with sub-type B1, B2), and type C (with sub-type C1, C2). *Amorphophallus* spp. belongs to the C2 type (Tillich, 2014).

Seedlings of type C have voluminous and green storage organs of cotyledonary hyperphyll, with no or only inconspicuous traces of endosperm. It has one or several cataphylls before the leaf emerges. Hypocotyl and primary roots were missing in the sub-type of C2 seedling, and then the first shoot-born roots broke through the body of the cotyledon at the base of the shoot (Tillich, 2014).

The normal seedlings of *A. muelleri* have developed complete and healthy essential structures such as several cataphylls and the first leaf, which grows inside the cataphyll or has emerged. Normal seedlings should be whole, have shoots and roots, and be free from minor defects and infection (de Medeiros et al., 2020; ISTA, 2021).

Abnormal seedlings did not show the potential for continued growth into satisfactory plants. It had significant defects such as deformed, fractured; the root is stunted or stubby, retarded or missing,

Figure 5. *Amorphophallus muelleri* germination and development into a normal seedling.

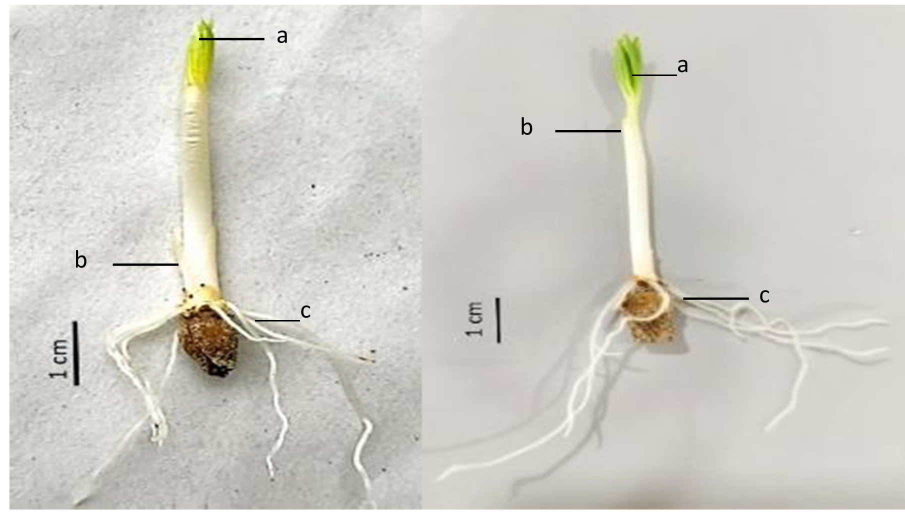

Figure 6. The structure of a normal *Amorphophallus muelleri* seedling; (a) leaf; (b) cataphyll; (c) roots.

Journal of Tropical Crop Science Vol. 12 No. 1, February 2025
www.j-tropical-crops.com

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

and decayed due to primary infection. Dead seeds at the end of the test period did not produce any part of a seedling but were not fresh or hard seeds. Dead seeds sometimes showed symptoms of fungal infection or rot (Figure 7).

Preheating to Speed Up Seed Germination

Preheating using the dry heat method for four weeks effectively speeded up the seed germination of *A. muelleri*. Control seed lots L1, L2, and L5 needed more than 74 days to reach maximum germination. Dry heat treatment accelerated germination by 52 days or less to reach a similar germination rate with control. Even though the germination rates were not significantly different between the control and dry heat treatments, all seed lots that were dry heat-tested were half shorter T50 and the total germination period (Table 5).

Amorphophallus muelleri seeds needed >100 days to germinate when they were sown three weeks after harvesting. The long germination period becomes an obstacle in seed trades because the quality of seed lot, especially seed germination, must be evaluated. Seed treatment, such as preheating, can promote germination or break dormancy (Lamont and Pausas, 2023). Furthermore, seed germination testing using 30°C gave a more satisfactory result than 25°C. Seed lots without preheating treatment had a shorter T50 and germination period but still needed four weeks for treatment before sowing.

Even though *A. muelleri* seed has a high moisture content at harvest, germination occurs slowly. The high level of glucomannan in the seeds inhibits germination. Increasing the temperatures at germination can reduce glucomannan elasticity and strength, which promotes germination (Herranz et al., 2013). Dry heat treatment enhances seed germination by increasing permeability, pore opening, and seed coat fracturing and tearing (Lamont and Pausas, 2023). In oil palm seeds, dry heat treatment at 40°C for 45 days promoted germination and delayed the increase of the seed ABA content during storage (Wongvarodom et al., 2024). Dry heat treatment could break physical dormancy in *Desmodium* and *Stylosanthes* species (Salazar et al., 2020).

Determination of The First and Final Germination Count

The first count can be determined when the normal seedlings reach a maximum number in daily evaluation

Figure 7. A dead seed (A) and abnormal seedlings (B and C) due to primary infection.

Table 5. Germination rate, T50, and germination period of *Amorphophallus muelleri* seeds

Seed lot	Germination (%)				T50 (DAP)				Germination period (DAP)			
	25°C		30°C		25°C		30°C		25°C		30°C	
	Preheating		Preheating		Preheating		Preheating		Preheating		Preheating	
	-	+	-	+	-	+	-	+	-	+	-	+
L1	89	93	89	99	45.4^{A}	16.4^{C}	45.4^{ab}	18.9c	74	48	74	37
L2	84	75	84	78	47.5^{A}	21.6^{B}	47.5^{a}	20.9c	74	52	74	40
L5	97	100	97	98	43.4^{A}	20.0^{BC}	43.4^{b}	20.2c	79	37	79	37
Significance	ns		ns			**		**				

Notes: Values followed by the same letters show no significant differences according to DMRT at α <0.05; ** = significant differences at α <0.01.

Journal of Tropical Crop Science Vol. 12 No. 1, February 2025
www.j-tropical-crops.com
Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

during seed germination testing or 50% of the total germination rate. The final count can be determined when normal seedlings accumulate steadily and there is no significant increase (Del Longo and Araoz, 2009; Gianinetti, 2020; Rosa et al., 2021).

Seed germination at 25°C and 30°C without preheating had various normal seedlings among seed lots. The maximum number of normal seedlings at 25°C occurred on 44-60 DAP, whereas at 30°C on 34-40 DAP. T50 at 25°C was on 43-37 DAP, whereas at 30°C was 38-42 DAP. Therefore, the first count at 25°C should be conducted at 47 DAP (Figure 8 A) and 30°C at 37 DAP (Figure 8 B). The maximum number of normal seedlings with preheating treatment at 25°C occurred at 17-20 DAP, whereas at 30°C at 20-26 DAP.

Amophophallus muelleri seed germination at 25°C without preheating treatment did not germinate uniformly. The final count for seed lots L1, L2, and L5 with 8 and 11 WAH occurred at 73 DAP; L3 and L4 with 3 WAH had a more extended period for the final count of 108 DAP. Meanwhile, the germination was faster at at 30°C without preheating (Figure 9A and 9B).

The final count can be determined at 57 DAP for all seed lots (Figure 9 A and B). The final count for germination testing with preheating treatment at 25°C or 30°C resulted in earlier and more uniform germination, 33 to 40 DAP. The final count can be determined at 37 DAP (Figure 9 C and D).

During seed germination testing, the first count is necessary to evaluate and remove the normal seedling and dead seed. This practice provides space for the other seedlings to grow and to avoid the spread of pathogen infection. The final count evaluates and determines the normal, abnormal, and dead seedlings. The seed germination testing period can be prolonged if many seedlings have not grown into normal seedlings or have not germinated. The seed lot should be re-tested if > 5% of the seed shows dormancy (ISTA 2018;2021).

Seed viability and seed germination are essential in determining the potential of seed germination and estimating the planting value (ISTA 2021). Non-uniform germination is affected by the time it

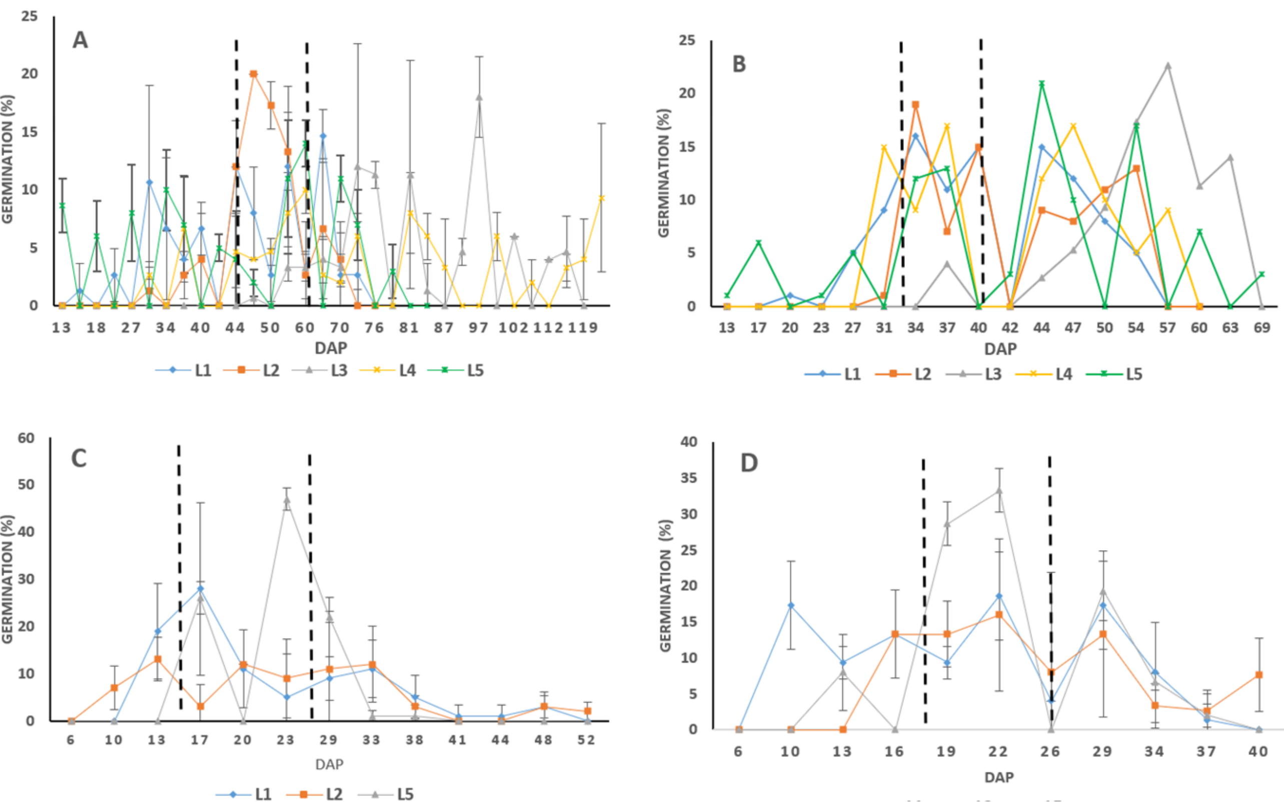

Figure 8. The determination of the first germination count based on daily evaluation without preheating at 25°C (A) and 30°C (B), with preheating treatment at 25°C (C) and 30°C (D). Vertical discontinued lines indicate the time range of the first germination count (days). Seed lots L1 and L2 are 8 weeks after harvest (WAH), seed lots L3 and L4 were 3 WAH, and seed lot L5 was 11 WAH.

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

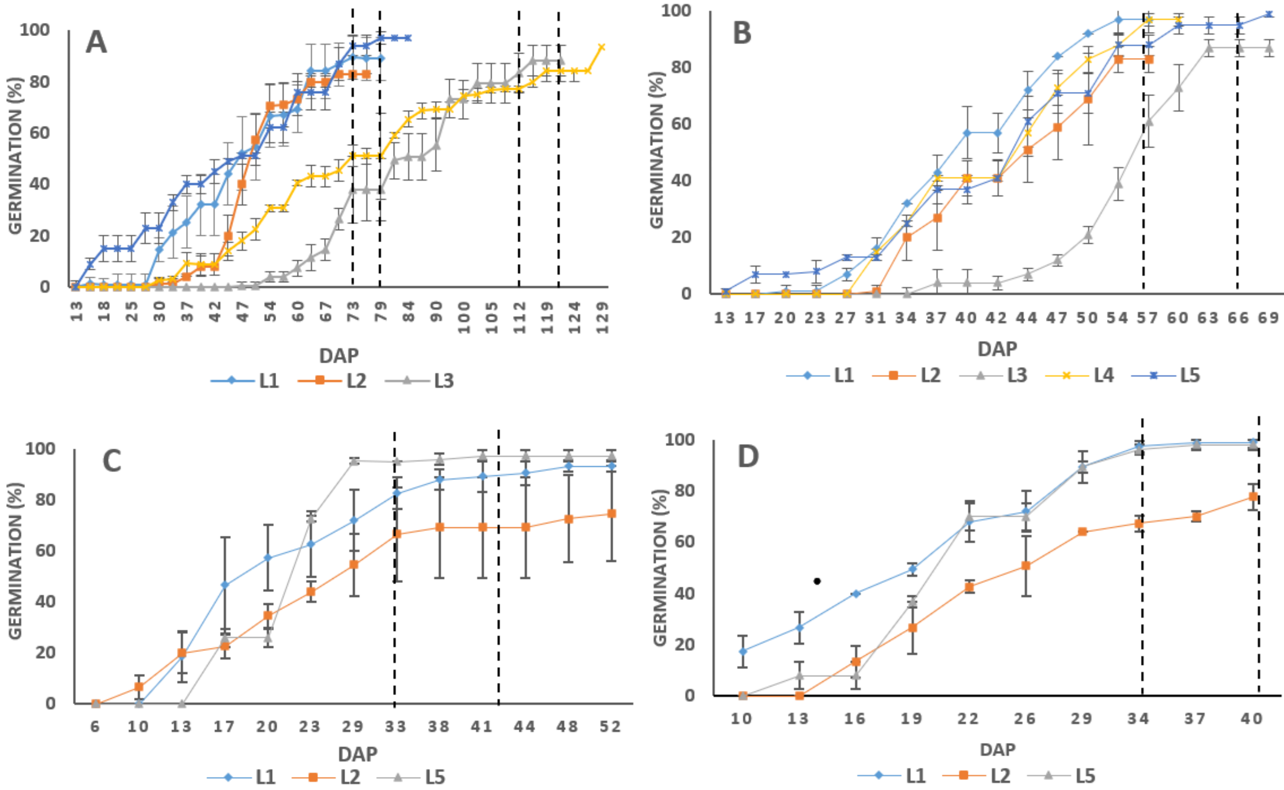

Figure 9. The determination of the final germination count based on the accumulation of normal seedlings without preheating treatment at 25°C (A) and 30°C (B), with preheating treatment at 25°C (C) and 30°C (D). Vertical discontinued lines indicate the time range of the final germination count (days). Seed lots L1 and L2 are 8 weeks after harvest (WAH), seed lots L3 and L4 were 3 WAH, and seed lot L5 was 11 WAH.

takes to harvest and seed dormancy. According to Wardani and Harijati (2019), fruit color can indicate seed maturation; seeds from the red fruit germinate earlier (70 DAP) than those from yellow (85 DAP) and green fruits (92 DAP). In Sari's study (2019), seed maturation influences the germination rates; at 24 weeks after anthesis, only 10% of the seeds germinated, whereas at 36 weeks after anthesis, germination reached 100%. Dry heat treatment can be applied soon after seeds are harvested to prevent the seeds from becoming dormant. The dry heat treatment can potentially process large batches of seeds to break dormancy.

Conclusions

The seed moisture testing of *Amorphophallus muelleri* may use either the low-temperature oven method of 103°C for 17 hours or the high-temperature oven method of 130°C for four hours using the whole/uncut seeds. The germination should use the between-paper method at 30°C, with the first count conducted at 37, and the final at 57 days after planting. A dry preheating at 35°C for four weeks can break seed dormancy and accelerate seed germination. High moisture content and seed dormancy after harvesting need proper handling and an optimum storage environment to maintain seed viability.

Acknowledgments

The authors thank the Indonesian Ministry of Agriculture and Balai Besar Pengembangan Pengujian Mutu Benih Tanaman Pangan dan Hortikultura (Balai Besar PPMBTPH) for funding the research and academic scholarship from 2021 to 2025.

References

Bakhtavarl, A.M., Afzal, I., Khalid, E., Jabeen, N., and Jabeen, R. (2023). Hermetic storage of okra seed maintains seed longevity in changing environments. *Plos One* **18**, e0287476. DOI: https://doi.org/10.1371/journal.pone.0287476.

Dewi, D.F.K., Azrianingsih, R., and Indriyani, S. (2015). Embryo structure of porang (*Amorphophallus muelleri* Blume) from various seed sizes. Biotropika 3, 146-150.

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

Gianinetti. A. (2020). Basic features of the analysis of germination data with generalized linear mixed models. *Data* **5**, 1-42. DOI: https://doi.org/10.3390/data5010006.

Harijati, N., and Widoretno, W. (2019). The effects of scarification on seed germination of porang (*Amorphophallus muelleri*) In "The 9th International Conference on Global Resource Conservation (IGRC) and Ritsumeikan University" (D. Siswanto, R. Mastuti, F.Z.B. Huyop, C. Treesubsuntorn, eds.), pp 1-6, Malang, Indonesia. AIP Conference Proceeding. pp 1-6. DOI: https://doi.org/10.1063/1.5061841.

Harijati, N., Mastuti, R., Chairiyah, N., Roosdiana, B., and Rohmawati, S.A. (2018). Effects of seeding material age, storage time, and tuber tissue zone on glucomannan content of *Amorphophallus muelleri* Blume. *International Journal of Plant Biology* **9**, 7626. DOI: https://doi.org/10.4081/pb.2018.7626.

Hay, F.R., Rezaei, S., and Buitink, J. (2022). Seed Moisture Isotherms, Sorption Models, and Longevity. *Frontiers in Plant Science* **13**, 891913. DOI: https://doi.org/10.3389/fpls.2022.891913.

Hay, F.R., Rezaei, S., Wolkis, D., and McGill, C. (2023). Determination and control of seed moisture. *Seed Science and Technology* **5**, 267-285. DOI: https://doi.org/10.15258/sst.2023.51.2.11.

Herranz, B., Tovar, C.A., de Zaldívar, B.S., and Borderias, A.J. (2013). Influence of alkali and temperature on glucomannan gels at high concentration, *LWT - Food Science and Technology* **51**, 500-506. DOI: https://doi.org/10.1016/j.lwt.2012.11.023.

ISTA. (2007). "ISTA Handbook on Moisture Determination". International Seed Testing Association.

ISTA. (2018). "ISTA Handbook on Seedling Evaluation". 4th edition. International Seed Testing Association.

ISTA. (2021). "International Rules for Seed Testing". International Seed Testing Association.

Jaganathan, G.K., Li, J., and Liu, B. (2022). Low constant temperature drying can be a satisfactory method to determine the moisture content of impermeable seed-coated species. *Southern Forests* **84**, 325–327. DOI: https://doi.org/10.2989/20702620.2022.2150586.

Jansen, P.C.M., van der Wilk, C., and Hetterschei, W.L.A. (1996). "Plants are yielding non-seed carbohydrates, *Amorphophallus Blume* ex Decaisne." Plant Resources of South-East Asia 9. Backhuys Publishers.

Kumar, S., Basu, S., Anand, A., Lal, S.K., and Tomar, B.S. (2021). Identification of the best germination indices represents seed quality status in unaged and aged onion seeds. *International Journal of Current Microbiology and Applied Sciences* **10**, 76-85. DOI: https://doi.org/10.20546/ijcmas.2021.1002.009.

Kurniawan, A., Wibawa, I.P.A.H., and Adjie, B. (2011). Species diversity of *Amorphophallus* (Araceae) in Bali and Lombok with attention to genetic study in *A. paeoniifolius* (Dennst.) Nicolson. *Biodiversitas* **12**, 7-11. DOI: https://doi.org/10.13057/biodiv/d120102.

Lamont, B.B., and Pausas, J.G. (2023). Seed dormancy revisited: Dormancy-release pathways and environmental interactions. *Functional Ecology* **37**, 1106–1125. DOI: https://doi.org/10.1111/1365-2435.14269.

Longo, O.T. D., and Aráoz, S.D. (2009). Criteria to determine the duration of the germination test for seeds of 'mistol' (*Ziziphus mistol* Grisebach). *Seed Science and Technology* **37**, 507-511. DOI: https://doi.org/10.15258/sst.2009.37.2.27.

Medeiros, J.A.D. de, Nunes, S.P.L., Félix, F.C., Ferrari, C.S., Pacheco, M.V., and Torres, S.B. (2020). Vigor test of normal intact *Amburana cearensis* (Allemão) A.C. Smith seedlings. *Journal of Seed Science* **42**, e202042011. DOI: 1 https://doi.org/10.1590/2317-1545v42221611.

Rosa, M.R., Dias, L.B.X., Nascimento, W.M., and Leão-Araújo, E.F. (2021). Standardized methodology for germination test on chickpea seeds. *Multi-Science Journal* **4**, 31-37. DOI: https://doi.org/10.33837/msj.v4i1.1371.

Salazar, D.E., Santos, L.G., and Hay, F.R. (2020). Effect of dry heat on seed germination of Desmodium and Stylosanthes species". *Seed Science and Technology* **48**, 419-437. DOI: https://doi.org/10.15258/sst.2020.48.3.11.

Received 28/08/2024; Revised 05/12/2024; Accepted 13/02/2025
https://doi.org/10.29244/jtcs.12.01.132-144

Sari, M., Santosa, E., Lontoh, A.P., and Kurniawati, A. (2019). Seed quality and seedling growth of iles-iles (*Amorphophallus muelleri* Blume) from different growing media. *Jurnal Ilmu Pertanian Indonesia* **24**, 144-150. DOI: https://doi.org/10.18343/jipi.24.2.144.

Sharma, S., and Wadhwa, N. (2022). Application of glucomannan. *Journal of Pharmacy Research*. **21**, 1–5. DOI: https://doi.org/10.18579/jopcr/v21i1.glucomannan.

Silva, R.C., Grzybowski, C.R.S., Belniaki, A.C., Vieira, E.S.N., and Panobianco, M. (2020). Determination of moisture content and storage potential of guanandi seeds. *Floresta* **50**, 953–960. DOI: https://doi.org/10.5380/rf.v50i1.59373.

Sivritepe, N., Sivritepe, H.Ö., and Türkben, C. (2018). Determination of moisture content in grape seeds. *Seed Science and Technology* **36**, 198-200. DOI: https://doi.org/10.15258/sst.2008.36.1.21.

Sumarwoto. (2005). Iles-iles (Amorphophallus muelleri Blume): deskripsi dan sifat-sifat lainnya. *Biodiversitas* **6**,185 – 190. DOI: https://doi.org/10.13057/biodiv/d060310.

Sun, Y., Xu, X., Zhang, Q., Zhang, D., Xie, X., Zhou, H., Wu, Z., Liu, R., and Pang, J. (2023). Review of konjac glucomannan structure, properties, gelation mechanism, and application in medical biology. *Polymers* **15**,1852. DOI: https://doi.org/10.3390/polym15081852.

Tillich, H.J. (2003). Seedling diversity in Araceae and its systematic implications. Feddes *Repertorium* **114**, 454 – 487. DOI: https://doi.org/10.1002/fedr.200311010.

Tillich, H.J. (2014). A new look at seedlings of araceae. *Aroideana* **37**, 1-14.

Wardani, I.B., and Harijati, N. (2019). The study of growth and its polyembryonic properties of porang seeds (*Amorphophallus muelleri* Blume) from various fruit colors in different planting media. *Journal of Experimental Life Science* **9**, 122-127. DOI: https://doi.org/10.21776/ub.jels.2019.009.02.10.

Wongvarodom, V., Sookkarn, R., Muangnapho, N., Eksomtramage, T., Thippan, S., and Lerslerwong, L. (2024). Dry-heat treatment for releasing the dormancy of stored oil palm (*Elaeis Guineensis* Jacq.) seeds. *Journal of Oil Palm Research* **36**, 234-245. DOI: https://doi.org/10.21894/jopr.2023.0015.

Yuzammi. (2009). The genus Amorphophallus Blume ex Decaisne (Araceae – Thomsonieae) in Java. *Reinwardtia* **13**, 1–12. DOI: 10.55981/reinwardtia.2009.8.

Zhao, J., Zhang, D., Jianping, Z., Srzednicki, G., Borompichaichartkul, C., and Kanlayanarat, S. (2010). Morphological and growth characteristics of *Amorphophallus muelleri* Blume - a commercially important konjac species. ISHS Proceedings of Southeast Asia Symposium on Quality and Safety of Fresh and Fresh Cut Produce pp 501–508. DOI: https://doi.org/10.17660/ActaHortic.2010.875.65.