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Fruit Maturity and Intra-Spadix Position Affected Seed Quality of *Amorphophallus muelleri* Blume

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Abstract

The seed harvesting process for *Amorphophallus muelleri* Blume is complicated by asynchronous flowering and fruiting. These factors affect the quality of the fruit and seeds, including fruit color, seed viability, and vigor. Pericarp color, as a simple indicator of fruit maturity, can be used to determine the best harvest time. At the M30% fruit maturation stage (30%–49% red pericarp), pericarp colors were more diverse across positions, including dark red, orange, yellow, and greenish yellow. The M50% (50%–74% red pericarp) included red, orange, yellow, and greenish yellow. The pericarp colors at M75% (>75% red pericarp) were more uniform, consisting of red, orange, and yellow. There were no significant differences in seed moisture content among spadix maturity stages, 62.49% (M30%), 63.42% (M50%), and 61.07% (M75%). Maturity stage and position affected the physical and physiological quality of the fruit and seeds. The well-filled and rotten fruits in the spadix increased as fruit maturity advanced. The highest number of empty fruits was found at M30%, while the highest seed dry weight was found at M50%. The proximal position had a higher percentage of empty fruits and a greater dry weight than the distal and middle positions. M75% had the highest average rate of well-filled fruit (90.83%) and the lowest average dry weight (8.1 mg per seed). The M50% maturity stage had the highest average dry weight (9.3 mg per seed) and the highest average viability of 93.1%, indicating that the seeds had reached physiological maturity.

Keywords: dry weight, moisture content, pericarp color, physiological maturity, viability

Introduction

Amorphophallus muelleri Blume has several propagative parts, including tubers, bulbils, and seeds. The plant has vegetative, generative, and dormant growth cycles during growing period. Planted tubers produce leaves during the rainy season and then become dormant during the dry season (Lontoh et al., 2019). Generative growth of *A. muelleri* occurs after at least two vegetative growth cycles (Santosa et al., 2019) the objective of this study was to evaluate the effect of gibberellic acid (GA_3). Using botanical seeds as propagation material is more profitable than using other materials, as only 56 plants are required per hectare. Each plant can produce 675 seeds or 450 fruits (Harijati et al., 2017). Furthermore, botanical seeds are relatively small and are well-suited for meeting the demand for large quantities of propagation material, long-distance distribution, and breeding activities. However, the asynchronous flowering and fruiting of *A. muelleri*, which cause various pericarp color changes on the spadix, are obstacle to seed harvest. Harvesting at an inappropriate time can affect the quality of the seeds produced. Pericarp color is a reliable indicator of maturity. Moreover, it is an easy way to choose the best harvest time (Dranski et al., 2010). Seed quality, including physical and physiological qualities, is an essential factor in successful field planting. High-quality seeds can be produced when the

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fruit has reached physiological maturity on the parent plant, whereas pericarp color is used as a parameter in evaluating seed quality. The pericarp color of the spadix turns orange at 28 weeks after anthesis (WAA) and red at 32 WAA. The color composition of the spadix at 28 WAA includes orange, light green, and green, while at 32 WAA it is red, yellow, and light green (Sari et al., 2019). Since fruit ripens asynchronously in the spadix, it is necessary to study the proportions of various colors at different positions in the spadix and their effect on fruit quality and seed viability. Therefore, seeds can be harvested at the appropriate time to meet seed certification standards. This experiment aimed to determine the effects of maturity stage and intra-spadix position on the physical and physiological quality of the seeds, and to determine the optimal harvest time based on fruit quality and seed viability, vigor, and dry weight.

Materials and Methods

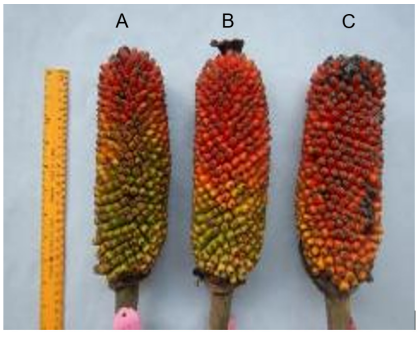
The field trials were conducted from May 2022 to October 2023 at Balai Besar PPMBTPH, Tapos Village, Depok, West Java. The experiment was conducted at coordinates 6°24′4″S, 106° 29′44″E, with an altitude of 115 m above sea level. This experiment involved observing the physical and physiological quality of fruit and harvesting at various stages of spadix maturity based on pericarp color composition. Tubers were obtained from farmers in the Klangon area, Madiun, East Java, Indonesia.

The average rainfall during the experiment was above 100 mm per month. The minimum temperature ranged from 19 to 20 °C, and the maximum temperature ranged from 24 to 26 °C in 2022. The minimum temperature ranged from 18–20 °C, and the maximum temperature ranged 28–30°C in 2023. The average relative humidity was 85%–89%, and the intensity of solar radiation was 6–8.6 MJ/m^2 per day. The rainfall was sufficient to supply the water needs of plants.

The maturity stage of *A. muelleri* spadix was determined by pericarp color changes from green to yellow or red. The experiment consisted of two factors: three maturity stages based on pericarp color composition in the spadix — M30% (30%–49% red pericarp), M50% (50%–74% red pericarp), M75% (>75% red pericarp) — and three intra-spadix positions: distal (P1), middle (P2), and proximal (P3). The treatments were arranged in a randomized complete block design (Figure 1). The various spadix color compositions were determined by comparing pericarp color with a color scale. The color was grouped and compared with the Pantone color chart at each position, and the number of pericarps for each color was then calculated. The harvested fruits were removed from the spadix sown and grouped by position. The fruits were tested for physical quality and then extracted to assess seed germination and T50. Seeds were extracted by soaking the fruits overnight, then manually removing them.

Figure 1

Various Spadix Maturity Stages: *(A) M30% (30%–49% Red Pericarp), (B) M50% (50%–74% Red Pericarp), (C) M75% (>75% Red Pericarp)*



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Color Composition, Well-filled, Empty, Rot and Dry Weight of *A. muelleri* Fruit and Seed

Three spadices at each maturity stage were physically observed to determine color. The fruit in each position were counted and classified as well-filled, empty, or rotten. The seeds were tested for moisture content and dry weight.

Seed Moisture Content Testing

Seed moisture was tested in duplicate according to International Seed Testing Association Rules (2021). Each sample weighed 4.5–5 g. The crucible and covers (6 cm diameter) were weighed before and after placing the whole seed into the crucible. The crucibles were then placed in an oven maintained at 103 °C for 17 hr. The crucibles and their covers were weighed after cooling, and the moisture content was calculated using the following formula.

$$\text{Seed MC (\%)} = \frac{(\text{M2}-\text{M3})}{(\text{M2}-\text{M1})} \times 100\%$$

Where:
M1: the weight of the crucible and cover (g)
M2: the weight of the crucible, cover, and seeds before drying (g)
M3: the weight of the container, cover, and seeds after drying (g)

Seed Dry Weight

About 3 g of seeds were weighed, placed in the crucible, and heated in an oven at 80 °C for 24 hr. The seeds were then cooled in a desiccator and weighed. Dry weight was calculated as the weight of the sample after drying divided by the number of seeds dried. Each test was repeated three times (Kumar et al., 2018).

Seed Germination and T50

Seed germination was tested using the between-paper method. About 150 seeds (50 seeds per replication) were randomly selected from the seed lot, sown between double-layer sheets, and incubated in the germinator at a constant temperature of 25 °C with light. Germination was evaluated every 3 days until no further germination was observed (285 days after sowing (DAS)), and the results were documented. Seed criteria for evaluation included normal seedlings, abnormal seedlings, ungerminated fresh seeds, and dead seeds. The media were replaced due to the long test period. The germination test results are expressed as the percentage of normal seedlings. Germination is calculated based on the following formula:

$$\text{Germination (\%)} = \frac{(\text{number of normal seedlings})}{(\text{number of seeds})} \times 100\%$$

T50 was calculated using the formula of Kumar et al. (2021):

$$T50 = \frac{[Ti + \left(\frac{N+1}{2} - Ni\right)(Tj - Ti)]}{Nj - Ni}$$

Where T50 is the median germination time, N is the final number of germinated seeds, and Ni and Nj are the cumulative numbers of seeds germinated in adjacent counts at time Ti and Tj, respectively, when Ni < $\left(\frac{N+1}{2}\right)$ < Nj.

There were more than 5% fresh seeds at the end of the germination test (285 DAS). Therefore, a tetrazolium test was conducted to determine whether the seeds were viable or non-viable. The tetrazolium test was carried out by cutting the seeds vertically, then soaking them in a 1% tetrazolium solution and incubating them at 35 °C for 48 hr. The staining results were examined under a stereo microscope.

Data Analysis

The pericarp color observations were analyzed descriptively by comparing colors with the Pantone color chart and are presented in a pericarp color identification table for each treatment. The experimental results on fruit and seed quality at various maturity stages were analyzed using analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) test at α = 5% in SAS 9.4.

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Results and Discussion

Color Composition, Well-filled, Empty, Rot and Dry Weight of *A. muelleri* Fruit and Seed

A prompt harvest is necessary to obtain maximum seed viability and vigor. Delaying harvest results in a decline in seed quality due to stresses such as high temperature, humidity, and rainfall, as well as pests and diseases attacks (Shah et al., 2021). High-quality seeds should be obtained by harvesting fruits after reaching maximum physiological maturity. Fruit maturity was neither simultaneous among plants nor within the spadix. Spadix maturity started at the distal end and progressed to the proximal end, so pericarp color varied: red at the distal end, yellow in the middle, and green at the proximal end of the spadix.

The spadix harvested during the research ranged from 16 to 22 cm in length, with a diameter of 5.5–9.0 cm. The spadix harvested at the M30% maturity stage exhibited different pericarp color compositions across positions (Figure 2). Greenish yellow comprised 47.3% in the spadix. Most of these fruits were found in the proximal position. There were two pericarp color compositions (dark red and orange) at the distal end, with orange being dominant (29.1%). There were three pericarp colors (orange, yellow, and greenish yellow), with greenish yellow dominating at the middle position (Table 1).

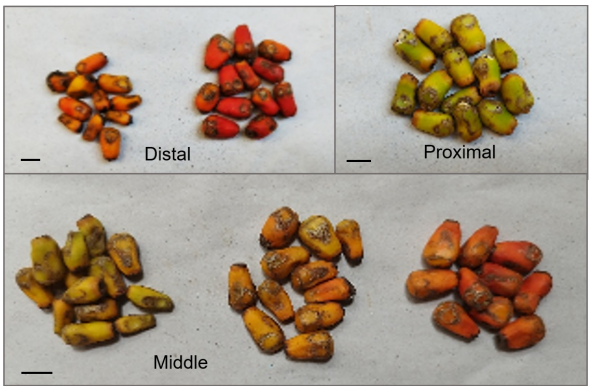
There were four pericarp colors in the spadix when harvested at the M50% maturity stage. Red pericarp color was dominant in the distal position, while orange pericarp color prevailed in the middle position. There was no red color at the proximal position, and many of the fruits were greenish yellow (21.5%) (Table 1) (Figure 3).

The M75% maturity stage showed that red pericarp color was dominant in the spadix (55.4%), especially in the distal position (34.5%). There were orange and yellow pericarp colors in the middle and proximal positions with different compositions (Figure 4).

The maturity stage greatly influences the pericarp color. The intensity of the red color increased in line with fruit maturity. The pericarp color composition was also more diverse when the spadix was harvested early. Fruit maturity, both within and among spadices, was asynchronous, resulting in differences in pericarp color: red at the distal end, yellow in the middle, and green at the proximal end. The maturity process affected the composition of the fruit's pigments. There is a dynamic color change during maturity, making the fruit attractive and serving as a visual indicator of maturity. Unripe fruits usually contain chlorophyll, which plays a role in photosynthesis. Color changes in the fruit from green to yellow to reddish yellow

Figure 2

The Pericarp Color Composition of M30% (30%–49% Red Pericarp) Maturity Stage at Intra-spadix Position



Note. The line scale (-) in the image is 10 mm.

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Table 1

The Pericarp Color Composition at Three Spadix Maturity Stages (M30%, M50%, M75%) and Three Intra-spadix Positions

Maturity stage	Fruits/ spadix	Fruit position	Pericarp color composition		
			Color	Number (%)	Pantone color chart number
M30%	428	Distal	Red orange	1.5	P 158U, P 172U, Orange U21
		Middle	Orange	29.1	P 1375U, Orange U21
			Orange	15.4	P 1375U
			Yellow	8.7	P 109U, P 110U
			Greenish yellow	4.6	P 395U, P 396U
		Proximal	Greenish yellow	40.7	P 388U, P 389U
M50%	502	Distal	Red	23.3	P 186C, P 179C, P 1665U
			Orange	9.7	P Orange U21, P 137U
		Middle	Red	9.5	P 176C, P 172U
			Orange	22.8	P Orange 21, P 1375U
			Yellow	0.5	P 116U
		Proximal	Orange	5.0	P 1375U, P Orange21
			Yellow	7.6	P 1235U, P 109U, P 110U
			Greenish yellow	21.5	P 605U, P 387U, P 389U
M75%	287	Distal	Dark red	34.5	P 485U, P 179C, P180C
		Middle	Red	16.8	P 485U, P 172U
			Orange	11.2	P Orange21
			Yellow	1.1	P 116U
		Proximal	Red	4.1	P 485U
			Orange	20.2	P Orange21, P 1505U, P 137U
			Yellow	12.1	P 129U, P1235U, P116U

Note. The maturity stage of M30% (30–49% red pericarp), M50% (50–74% red pericarp), M75% (>75% red pericarp).

Figure 3

The Pericarp Color Composition of M50% (50%-74% Red Pericarp) Maturity Stage at Three Different Positions of Spadix



Note. The line scale (-) in the image is 10 mm.

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were caused by chlorophyll degradation and the synthesis of anthocyanins and carotenoids. Chlorophyll content increases during early fruit development, then declines through maturation (Kapoor et al., 2022; Rahmadini et al., 2020).

The effect of spadix maturity stage and intra-spadix position on the physical quality of *A. muelleri* seeds can be assessed by the number of ripe fruits, seed moisture content, and seed dry weight. The results showed that the average seed moisture content among maturity stages was similar, 62.49% (M30%), 63.42% (M50%), and 61.07% (M75%). Seed moisture content across positions showed that the proximal position tended to be higher than the distal and middle positions. The seed moisture content began to decrease at harvest, as part of the natural drying process. Although the seed moisture content remained high, it decreased slightly at the M75% maturity stage compared to the M30% maturity stage (Figure 5). Seed moisture content decreases at the final ripening phase of the parent plant. At the same time, metabolic activity decreases, and the plant transitions to a resting or dormant period. However, there is no significant decrease in moisture content in recalcitrant seeds, so metabolic activity continues (Lah et al., 2023; Sripathy & Groot, 2023). The maturity phase, followed by the seed drying process characterized by a decrease in recalcitrant seed moisture content in *Reutealis trisperma*, showed that the seed moisture content

Figure 4

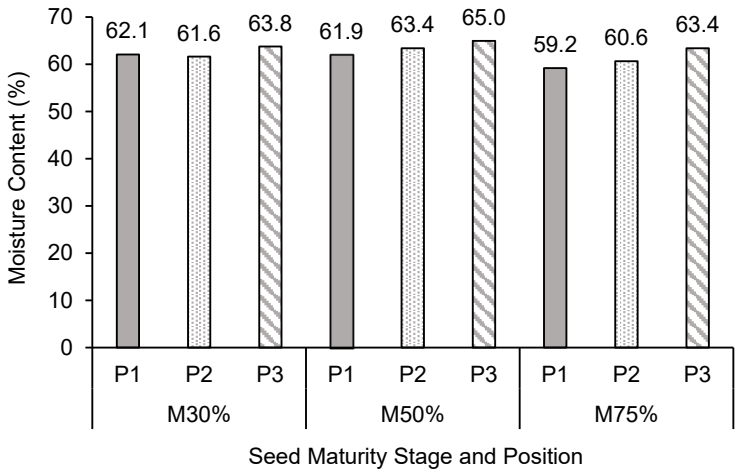
The Pericarp Color Composition of M75% (>75% Red Pericarp) Maturity Stage at Three Different Positions in Spadix



Note. The line scale (-) in the image is 10 mm.

Figure 5

Seed Moisture Content at Various Spadix Maturity Stage: M30% (30%–49% Red Pericarp), M50% (50%–74% Red Pericarp), M75% (>75% Red Pericarp), and Intra-positions at Spadix: Distal (P1), Middle (P2), and Proximal (P3)



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was higher at 24 WAF (weeks after flowering) (43.97%) compared to 26 WAF (34.27%) and 28 WAF (19.36%) (Tresniawati et al., 2014).

The number of well-filled fruits increased with spadix maturity stage (Table 2). The average number of well-filled fruits at M30% differed significantly from that at M75% but not significantly from that at M50%. The average number of well-filled fruits at was 83.78% (M30%), 89.52% (M50%), and 90.83% (M75%). The number of well-filled fruits did not differ significantly between the distal and middle positions. The number of well-filled fruits tended to be lower depending on fruit position, with the lowest number at the proximal position. The empty fruit percentage decreased as seed maturity increased. The M30% maturity stage had the highest percentage of empty fruits (16.03%) and was significantly different from M50% (7.51%) and M75% (6.15%). However, the highest number of empty fruits was at the proximal position in M30% (26.97%). Conversely, the percentage of rotten fruits increased with fruit maturity stage. M75% had the highest percentage of rotten fruit (3.88%) and differed significantly from the M50% (0.90%) and M30% (0.20%). The number of rotten fruits at each fruit position was not significantly different at all maturity stages. Seed dry weight increased at the beginning of the maturation phase and decreased after reaching physiological maturity. The seed dry weight increased at M50% (9.3 mg per seed) and then slightly decreased at M75% (8.1 mg per seed). Seed position also had a significant effect on dry seed weight; the distal position had the lowest dry weight, whereas the proximal position had the highest. Research on *Eremopyrum distans* seeds indicated that seed position significantly influenced seed number, size, mass, and germination. Seed number was higher at the proximal position than at the middle and distal positions. Seed size and mass decrease with more proximal seed position, but germination increases with more proximal seed position (Wang et al., 2010).

The seed position significantly affected seed quality, since fruits at the distal and middle positions were more exposed to sunlight and temperature than the fruit at the proximal position. Sunlight exposure plays a role in photosynthesis and in the formation of energy required for fruit development. Fruits located at the base of the spadix and protected by the spathe at the beginning of their development receive less sunlight, which affects their development (Kviklys et al., 2022). The pericarp's red color gradually

Table 2

Well-filled Fruit, Empty Fruit, Rotten Fruit, and Dry Weight of A. muelleri Seeds at Various Maturity Stages (M30%, M50%, M75%) and Intra-positions in the Spadix

Maturity stage	Position	Number of fruits	Well-filled fruits (%)	Empty fruits (%)	Rotten fruits (%)	Seed dry weight (g)
M30%	Distal	131	92.44 a	7.23 b	0.32 bc	8.09 bc
	Middle	131	88.31 a	11.42 b	0.27 bc	8.53 b
	Proximal	166	73.03 b	26.97 a	0.00 c	9.21 ab
M50%	Distal	161	89.58 a	9.35 b	1.08 bc	8.33 b
	Middle	171	91.88 a	7.47 b	0.65 bc	9.27 ab
	Proximal	170	87.11 a	11.90 b	0.98 bc	10.19 a
M75%	Distal	126	92.36 a	1.87 b	5.47 a	7.24 c
	Middle	105	92.79 a	3.65 b	3.56 ab	8.16 bc
	Proximal	135	87.32 a	9.20 b	2.61 abc	8.94 b

Notes. The maturity stage of M30% (30–49% red pericarp), M50% (50–74% red pericarp), M75% (>75% red pericarp). The numbers in the table followed by the same letters in each column show no significant difference at α = 5% level (Duncan's multiple range test).

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changed depending on the fruit's position on the spadix. The process of seed development and maturation began at the distal end of the spadix and then spread to the middle and proximal ends. This indicated that seeds in the distal position had reached physiological maturity before those in the proximal position. Therefore, seeds in the distal and middle positions tend to have better quality than seeds in the proximal position. The effect of fruit position on seed dry weight is thought to be influenced by several factors, including assimilate translocation, maturity stage, and seed moisture content. *A. muelleri* does not produce leaves during the generative or flowering phase, so the primary source of assimilates is the tubers. The M75% seed maturity stage had the lowest dry weight because the seeds had passed physiological maturity. The fruit is fleshy, so the seeds remain moist. The metabolic process in seeds with high moisture content during the maturity phase remained active, so the seeds continued to respire. The use of food reserves during respiration decreased seed dry matter (Alan & Eser, 2008).

Seed Germination and T50

The asynchronous seed maturity of *A. muelleri* can be indicated by various pericarp colors. In that regard, seeds need to be tested to determine seed viability and vigor. The seeds were sown directly after the extraction process. Even though the germination period was up to 285 DAS, more than 5% of the seeds were fresh at the end of testing. The tetrazolium test was performed to assess the viability of ungerminated fresh seeds. A viable seed is one that can germinate normally and produce a normal seedling under favorable conditions. It has a bright red color that is evenly distributed on the surface of the seed tissue (Figure 7). *A. muelleri* fruit maturity affects seed germination and germination period. The seeds from red, yellow, and green fruit had different germination periods, such as 70, 85, and 92 DAS, respectively (Wardani et al., 2019). Although *A. muelleri* seeds are recalcitrant, the study showed that the seeds had a dormancy period for 1–2 months (Nasir et al., 2015). Seed treatment using dry preheating at 35 °C for four weeks breaks seed dormancy and accelerates *A. muelleri* seed germination (Fadhilah et al., 2025)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 hr).

Seed germination was significantly influenced by the maturity stage and intra-spadix position. Normal seedlings of *A. muelleri* consist of a leaf, a cataphyll, and a shoot-borne root. Some leaves may not pass through the cataphyll, but growth was already visible in the cataphyll during seed germination testing (Figure 6). The seed germination process is characterized by the emergence of a plumule enclosed by cataphylls. *Amorphophallus muelleri* seedlings are monocotyledonous with a hypogeal germination type (Nurshanti et al., 2022).

Seed germination increases with maturity. Spadices harvested at the late-maturity stage (M75%) yielded the highest average germination (56.4%). Seed position within the spadix also affected viability. Seeds of the distal and middle positions had better germination than those at the proximal position, while seeds at the proximal position had higher percentage of fresh seeds than those at the middle and distal positions. Seeds at the distal position in M75% had the highest germination (65%) (Table 3).

The higher the seed maturity stage, the faster the seeds grow and develop into normal seedlings (T50). Intra-spadix position in the spadix significantly affected T50, with the distal position having a shorter T50 than the middle and proximal positions. M75% had the lowest T50 (103.8 to 151.9 DAS), while M30% in the distal had a similar T50 with M50% in the middle and proximal position (182.1 DAS) (Table 3).

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Figure 6

Normal Seedling of A. muelleri: Leaf (a), Cataphyll (b), Shoot-born Root (c)

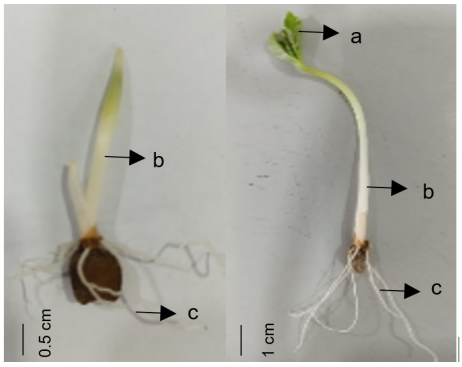


Figure 7

The Viable Seed in Tetrazolium Testing: (A) M30% (30–49% Red Pericarp), (B) M50% (50–74% Red Pericarp), (C) M75% (>75% Red Pericarp)

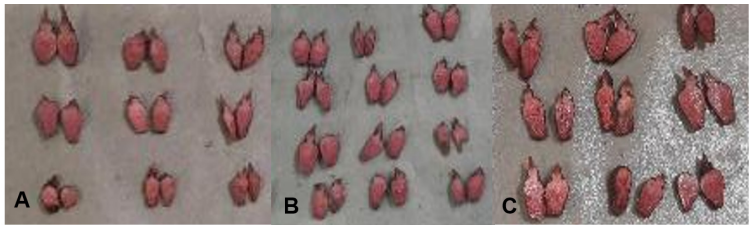


Table 3

Germination Percentage, Viability, and T50 of Seeds at Three Maturity Stages and Three Spadix Positions

Maturity stage	Position	Germination (%)	Ungerminated fresh seeds (%)	Viable seeds (%)	T50 (DAP)
M30%	Distal	50.0	26.0	76.0 c	182.1 bc
	Middle	58.7	29.3	88.0 ab	205.4 b
	Proximal	43.3	36.0	79.3 bc	237.8 a
Average		50.7	30.4	81.1	208.4 a
M50%	Distal	48.0	44.7	92.7 a	165.8 c
	Middle	45.3	50.0	95.3 a	182.9 bc
	Proximal	55.3	36.0	91.3 a	183.5 bc
Average		49.5	43.6	93.1	177.4 b
M75%	Distal	64.7	5.3	70.0 c	103.8 e
	Middle	61.3	16.0	77.3 c	123.9 de
	Proximal	43.3	28.7	72.0 c	151.9 cd
Average		56.4	16.6	73.1	126.5 c

Notes. The maturity stage of M30% (30–49% red pericarp), M50% (50–74% red pericarp), M75% (>75% red pericarp). The numbers in the table followed by the same letters show no significant difference at α = 5% level (Duncan's multiple range test). T50= the median germination time, DAP = days after planting.

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The tetrazolium test showed that seeds harvested at M50% had the highest average viability (93%), indicating that they had reached physiological maturity across all intra-spadix positions. The low viability at M30% showed that some seeds, especially at the proximal end, had not reached physiological maturity. There were many rotten seeds at M75%, indicating that the seeds were harvested too late, thereby increasing the risk of disease. The higher germination and viability at the distal and middle positions indicated that these positions reached physiological maturity faster than the proximal position (Table 3).

Seed maturity significantly affected seed viability and vigor. Seeds at optimal maturity had higher germination and lower T50 because they were fully developed and had sufficient food reserves to support germination, including root and shoot formation. Therefore, the seeds germinated faster because they were ready to begin the process as soon as water, oxygen, and suitable temperature were available. Physiological maturity is achieved when the seed has maximized its food reserves to produce the maximum seed dry weight (Nugroho et al., 2022). *Michelia champaca* seeds are recalcitrant and reach physiological maturity when the seed color turns black. At this phase, the seeds have the lowest moisture content and maximum fat and protein content (Pramono & Rustam, 2017).

Early harvesting reduces seed food reserves, thereby suppressing seed viability and vigor. Meanwhile, harvesting too late results in seeds losing viability due to extreme environmental conditions such as heat and rain, as well as disease. Food reserves accumulate during seed maturation. Seed moisture content decrease, followed by a decrease in metabolic activity at the end of physiological maturation, and seeds enter the resting or dormant period. However, there is no significant decrease in seed moisture content, indicating that metabolic activity remains active in the seeds (Sripathy & Groot, 2023).

Conclusions

The optimum quality of *A. muelleri* seeds at all fruit positions on the spadix was reached when more than 50% of the pericarp turned red. At this stage, the average well-filled fruit percentage was 89.25%, average seed moisture was 63.4%, average dry weight of 9.3 mg per seed, average germination percentage was 49.5%, ungerminated fresh seed percentage was 43.6%, and a total viable seeds percentage was 93.1%.

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