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EVALUATION OF DIFFERENT SEED DORMANCY BREAKING METHODS INCLUDING ENZYMATIC ASSAYS FOR GERMINATION IMPROVEMENT IN OIL PALM (*Elaeis guineensis* Jacq.)

MOHD NORSAZWAN GHAZALI[1]*; UMA RANI SINNIAH[1] and PARAMESWARI NAMASIVAYAM[2]

ABSTRACT

The oil palm (Elaeis guineensis) *is propagated by seeds for establishment in nurseries and commercial plantations. The seed is naturally dormant; thus, heat treatment is commercially used to alleviate this problem. This study evaluated eight seed dormancy breaking methods (operculum removal, 60 days storage, 60 days 40°C heat treatment, 120 days storage, 60 days storage + 40°C heat treatment, 180 days storage, 120 days storage + 40°C heat treatment and control) on seed germination, based on physical, morphological and physiological dormancy characteristics. Imbibition test indicated that less than 7% mass increment was recorded in all treatments. Germination of more than 82% was obtained for all heat-treated seeds with less than 13 days of mean germination time. The embryo was fully developed at 20 weeks after pollination, but applying heat treatment has accelerated its growth. The heat and storage treatments cause up to 36% reduction in peroxidase and 13% in catalase activities, with 9% (endosperm) and 26% (embryo) increment of α-amylase. It can be suggested that oil palm seeds exhibit a non-deep physiological dormancy, with heat treatment of 40°C as the most effective and practical dormancy-breaking method for commercial seed production.*

Keywords: enzymatic activity, germination improvement, heat treatment, oil palm.

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INTRODUCTION

Oil palm cultivations are mainly propagated through *dura* × *pisifera* hybrid (D×P) seeds (Corley & Tinker, 2015). The seeds are known to be highly dormant and recorded less than 25% germination after eight months of storage (Norsazwan et al., 2016). The use of heat treatment was first suggested by Hussey (1958) and Rees (1962). To date, the technique adopted by the seed producer to break dormancy and induce germination is by using heat treatment, at 40 ± 2°C for 60 days before the germination process (Corley & Tinker, 2015; Department of Standards Malaysia [DOSM], 2017). Since 1962, various alternative dormancy-breaking methods have been attempted, including the adoption of accelerated aging treatment (Murugesan et al., 2005) and extended heat treatment duration (Martine et al., 2009). However, the response does not surpass the standard heat treatment germination record of an average of 68% within 4 months. Recent studies, such as the use of neonicotinoid solution to trigger germination, gave 86% germination (Chanprasert et al., 2012), and stimulation with the electromagnetic field resulted

[1] Department of Crop Science,
Faculty of Agriculture, Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia.

[2] Department of Cell and Molecular Biology,
Faculty of Biotechnology and Biomolecular Science,
Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia.

* *Corresponding author e-mail: mohdnorsazwan@upm.edu.my*

in more than 90% germination (Sudsiri et al., 2017). However, these methods were not reproducible and were not practical for implementation at a commercial scale. Darkwah et al. (2021) suggested that there were variations in the germination percentage of oil palm seeds from different progenies, however, the role of heat treatment was found to be crucial in achieving more than 80% germination. The influence of heat on germination is intriguing as to what type of dormancy the oil palm has, and the mechanism that triggers germination. With an average of 68% germination upon heat treatment (Norsazwan et al., 2024), it appears that dormancy is not fully alleviated, or some seeds are not in the right stage to respond accordingly. In addition, germination after heat treatment takes up to 60 days with sporadic germination and spread across this duration. This raises the question of whether the heat treatment was effective in breaking dormancy or, whether a higher germination percentage and speed could be achieved. Seed dormancy is a complex process and can be influenced by various factors. The most systematic dormancy classification system used to date was developed by Baskin and Baskin (2004), where it can be divided into five main categories; physical dormancy (PD), morphological dormancy (MD), physiological dormancy (PYD), morpho-physiological dormancy (MPD) and combinational dormancy. According to Baskin and Baskin (2014), palms normally have MPD, however, dormancy mechanisms and the germination process have not been fully understood in oil palm seeds. To achieve the highest germination percentage, understanding these two processes is vital, since they are closely interrelated and regulated by various factors (Nautiyal et al., 2023).

Seed germination and dormancy are vital components of seed quality; hence, understanding these processes is essential for a sound seed production system. The two processes are closely interrelated and regulated, both by genetic as well as environmental factors. While dormancy provides an inherent mechanism aimed at the survival of the plant species to withstand adverse external conditions by restricting the mature seed from germinating, the ability of the dehydrated seed to remain viable and produce a vigorous seedling upon hydration under favourable conditions is the key to the survival and perpetuation of the plant species. In addition, quality seeds are expected to result in a timely and uniform germination, under favourable field conditions after sowing to establish a healthy crop stand. Therefore, in seed technology, dormancy is not considered a desirable trait to be monitored in the seed lots used for sowing. Thus, to achieve the highest germination percentage, understanding the factors controlling these two interlinked and contrasting processes are vital. In this study, several selected effective dormancy-breaking treatments were used to understand and elucidate the type of dormancy in oil palm seed with the heat treatment as the benchmark.

MATERIALS AND METHODS

Seed Source and Study Site

Freshly harvested *dura* × *pisifera* (D×P) seeds from four controlled pollinated bunches (replicates) of CALIX 600 *dura* mother palm sources were collected from Field PT 100, Sime Darby Research, Banting, Malaysia, at 20 weeks after pollination (WAP). The samples were subjected to standard seed processing methods as shown in *Figure 1*. All the laboratory experiments were conducted at the Seed Technology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia (2° 59′ 15.4″ N, 101° 44′ 03.7″ E).

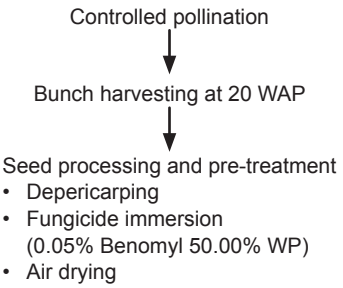


Figure 1. Flow of pollination and processing methods before seed dormancy evaluation.

Seed Treatments

Eight dormancy-breaking treatments, which include the fresh seed as follows: Treatment A – untreated control; Treatment B – operculum removal; Treatment C – ambient temperature (27°C) storage for 60 days; Treatment D – heat treatment (40 ± 2°C) for 60 days; Treatment E – storage at 20°C for 120 days; Treatment F – storage at 20°C for 60 days + heat treatment for 60 days; Treatment G – storage at 20°C for 180 days; and Treatment H – storage at 20°C for 120 days + heat treatment for 60 days.

The operculum removal was performed following Murugesan et al., (2008). The treatments were blocked according to their respective harvesting periods to analyse variations from the blocks.

Seed Imbibition

Four replicates of 20 seeds from each treatment were placed onto a sand medium inside a plastic container and completely submerged in water

for 10 days, at 27°C. The seed weight (g) was measured after 0, 6, 12, 24, 48, 96, 192 and 240 hr. The percentage of mass increase over the imbibition period was calculated to determine the water uptake by seeds. To further assess the effect of imbibition on different seed components, the change in moisture levels for both endosperm and embryo tissues were evaluated in two phases; at 0 hr (pre-imbibition) and 240 hr of imbibition. The moisture content was determined gravimetrically as a percentage of fresh weight basis, using the Low Constant Temperature Oven Method (103°C for 17 ± 1 hr) as described by the International Seed Testing Association (ISTA, 2024).

Embryo Growth

Four replicates of 10 seeds were dissected and the embryo was excised on respective days after imbibition. The embryo length (mm), width (mm), and embryo-to-seed length ratio (E:S) were recorded using a stereomicroscope attached to a digital camera (Leica Microsystems, Germany) EZ4D.

Peroxidase (POD) Assay

One hundred mg of excised embryo and endosperm from all the treatments were ground into powder and homogenised with 1 mL of 100 mM potassium phosphate buffer (pH 7.0). The samples were centrifuged at 12,000 g at 4°C for 20 min. The reaction mixture (3.00 mL) consisted of 0.05 mL of 20 mM guaiacol, 2.83 mL of 10 mM phosphate buffer (pH 7.0), 0.10 mL enzyme extract, and 0.02 mL of 40 mM H_2O_2. The enzyme activity was determined spectrophotometrically at 470 nm. The oxidation of guaicol was measured by the increase in absorbance at 1 min. Peroxidase (POD) enzyme activity was expressed per mg of extractable fresh tissue using Equation (1) and Equation (2).

Catalase (CAT) Assay

Excised embryos and endosperms from all treatments were homogenised and extracted by using a similar extraction protocol as mentioned in the POD (EC1.11.1.7) assay. The reaction mixture (3.00 mL) consisted of 1.50 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.50 mL of 75 mM H_2O_2, 0.05 mL of enzyme extract and 0.95 mL of distilled water. The unit of catalase (CAT) activity was recorded based on absorbance value at 240 nm after 2 min of reaction time. The calculation was done based on the following Equation (3), using an extinction coefficient of 43.6 M^{-1} cm^{-1} (Thant et al., 2017). CAT activity was expressed per mg of extracted fresh tissue (µmol min^{-1} mg fresh weight $[FW]^{-1}$) using Equation (4).

α-Amylase Assay

One hundred fifty mg of the ground powder (embryo and endosperm) from all treatments were homogenised with 1.5 mL of cold 20 mM sodium phosphate with 6.7 mM sodium chloride, pH 6.9 extraction buffer. The samples were centrifuged at 12,000 g at 4°C for 15 min. The α-amylase activity was determined based on the formation of reducing sugars using a colour reagent. A 100 µL of 10% starch solution was pipetted into a 2.0 mL microcentrifuge tube. Then 100 µL of the supernatant for analysis was added and incubated for 3 min at room temperature. Next, 100 µL of colour reagent that consisted of 5.3 M potassium sodium tartrate tetrahydrate, and 96 mM 3,5-dinitrosalicylic acid was added. The microcentrifuge tube was placed in a boiling water bath for exactly 15 min. The tube was then allowed to cool at room temperature. Distilled water (900 µL) was added and mixed by inversion. The absorbance value was measured at 540 nm using a Multiskan GO (Version 1.00.40) spectrophotometer (Thermo Fisher Scientific, USA).

$$\text{POD (nmol/min/mL)} = \frac{\left(\Delta \frac{470A}{\text{min}}\right) \times \text{Total volume} \times 1000}{26.6 \times \text{Sample volume}} \quad (1)$$

$$\text{POD (nmol/min/mg FW)} = \frac{\text{nmol/min/mL}}{\text{mg/mL enzyme}} \quad (2)$$

$$\text{CAT (µmol/min/mL)} = \frac{\left(\Delta \frac{240A}{\text{min}}\right) \times \text{Total volume} \times 100}{43.6 \times \text{Sample volume}} \quad (3)$$

$$\text{CAT (µmol/min/mg FW)} = \frac{\text{µmol/min/mL}}{\text{mg/mL enzyme}} \quad (4)$$

Seed Germination

The treated seeds were imbibed in water for 10 days, separated into 15 × 10 cm plastic bags containing four replicates of 100 seeds, and placed inside the germination room at 30°C. Seeds were monitored daily by recording the number of seeds showing the emergence of radicles from the fibre plug, according to Sime Darby Seed Production Unit (SPU) standard evaluation procedures (Norsazwan et al., 2024). Germinated seeds were removed from the bag and the remaining non-germinated seeds were kept until the end of the germination test period (day 60). The germination was determined as a percentage at time intervals throughout the germination period using the following Equation (5):

$$\text{Germination (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Number of seeds used}} \times 100 \quad (5)$$

Statistical Analysis

Data were analysed using Microsoft Excel and Statistical Analysis Software, SAS 9.4 (SAS Institute, Cary, USA). Significant levels of $p \leq 0.05$ were used for Duncan's Multiple Range Test (DMRT) for analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Seed Imbibition

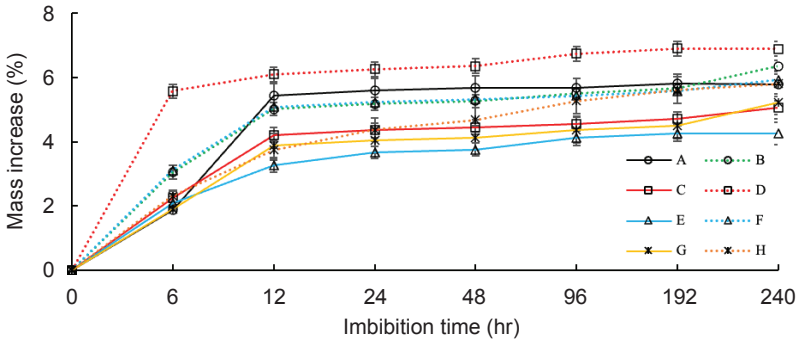
All treatments showed a gradual increase in seed mass from 0 to 240 hr after imbibition (*Figure 2*). Treatment D (heat treatment) showed the highest increment with 6.9%, whilst Treatment E (120 days storage) had the lowest overall mass increase (4.3%). Within the first 12 hr, a rapid mass increase was observed; ranging from 3.3% (Treatment E) to 6.0% (Treatment D). Thereafter, all the treatments showed no significant changes in the mass increase from 48 to 240 hr of imbibition, as the water uptake reached plateau.

Moisture Content in Endosperm and Embryo

In general, both endosperm and embryo tissues recorded a significant increase in moisture content due to imbibition, however, the magnitude of the increment differed markedly due to the treatments. Before imbibition, the endosperm tissue recorded moisture levels ranging from 15.4% (Treatment F) to 18.8% (Treatment A). After the 10 day imbibition period, all the treatments showed equal endosperm moisture content increment, with an average of 4.5% increase in comparison to the pre-imbibed stage (*Table 1*). In contrast, the embryo's initial (pre-imbibed) moisture content was significantly higher than in the endosperm, which ranged from 26.3% (Treatment C) to 30.7% (Treatment A). After 10 days of imbibition, the embryo showed an average of 11.4% increment in moisture. Interestingly, treatments that were heat treated or stored before heat treatment (Treatments D, F and H) recorded significantly higher embryo moisture content after imbibition (44.0%–48.8%), in comparison to other treatments. The lowest moisture values after imbibition were recorded by Treatments A, B and G, which showed no differences in moisture within the 35.0%–39.8% range.

Embryo Growth

Initially (day 0, immediately after imbibition), high variation was observed in embryo length with values ranging from 2.89–3.48 mm. The highest embryo length was recorded by Treatment H (3.48 mm), which differed significantly from most other treatments, except with Treatment F (3.35 mm). At day 20 after imbibition, a significant



Note: A - Treatment A; B - Treatment B; C - Treatment C; D - Treatment D; E - Treatment E; F - Treatment F; G - Treatment G; H - Treatment H.

Figure 2. The percentage of seed mass increases at 0, 6, 12, 24, 48, 96, 192, and 240 hr after imbibition from the initial seed mass.

TABLE 1. PERCENTAGE OF MOISTURE CONTENT FOR ENDOSPERM AND EMBRYO OF SEED TREATMENTS BEFORE AND AFTER IMBIBITION

Treatment	Moisture content (%)			
	Endosperm		Embryo	
	Pre-imbibition	Imbibed	Pre-imbibition	Imbibed
A	18.80b	21.20a	30.70d-f	39.80bc
B	18.60b	21.30a	30.20ef	36.60cd
C	17.80bc	21.50a	26.30f	38.10bc
D	16.18d	22.10a	29.03ef	48.80a
E	17.60b-d	23.20a	28.80f	37.20c
F	15.40e	22.10a	30.80d-f	46.30a
G	18.20bc	21.40a	28.80f	35.30c-e
H	16.60c-e	22.30a	30.50d-f	44.10ab

Note: Different letters (a-f) indicate significant differences within each column at a 5% probability level.

increase in embryo length was recorded only for Treatment D (heat treatment), and F (60 days storage + heat treatment), with 0.45 and 0.51 mm increments, respectively. From day 40 to 60 after imbibition, the pattern of embryo length remains the same, where Treatments D, F and H (120 days storage + heat treatment) recorded significantly higher embryo length, within the 3.23 to 3.82 mm range, in comparison to Treatment A, B, C, E and G (3.16–3.25 mm). The effect of each treatment on embryo length is summarised in *Figure 3a*. The embryo width showed less variation among the treatments, in comparison to the length. In general, Treatments A, C, E and G had no change in width throughout the 60-day evaluation period, with values ranging from 1.24–1.34 mm. On day 20 after imbibition, only Treatment D showed a significant width increment from 1.31–1.50 mm. From day 40 to 60 after imbibition, Treatments D, F and H showed higher embryo width in comparison to other treatments, with 1.62, 1.58 and 1.69 mm, respectively (*Figure 3b*). The embryo-to-seed length ratio (E:S) ranged between 0.10–0.16. Treatments A, B, C, E and G showed no significant change in the E:S (less than 0.12) within the 60-day evaluation period. Treatments D, F and H recorded a marked increase in ratio from day 0 to day 60 after imbibition, with 15%, 11% and 29% increments, respectively (*Figure 3c*).

POD Activity

All treatments recorded similar initial (pre-treated) activity levels, ranging from 184.7–205.9 nmol min^{-1} mg FW^{-1} of POD value. The application of heat treatment as well as storing the seeds before the heat treatment (Treatments D, F and H) resulted in more than 30% reductions in POD values. Treatments D, F and H recorded post-treatment POD activity of 130.4, 126.4 and 126.6 nmol min^{-1} mg FW^{-1} of POD value, respectively (*Figure 4*). Treatment A (204.0 nmol min^{-1} mg FW^{-1}), Treatment B (187.9 nmol min^{-1} mg FW^{-1}), Treatment C (193.3 nmol min^{-1} mg FW^{-1}), Treatment E (180.4 nmol min^{-1} mg FW^{-1}) and Treatment G (170.4 nmol min^{-1} mg FW^{-1}) showed no significant changes in POD activities in comparison to respective pre-treatment values.

CAT Activity

CAT showed a similar activity pattern as showed by the POD enzyme. The initial value for pre-treated seed ranged from 6.37–6.54 mmol min^{-1} mg FW^{-1} (*Figure 5*). Application of heat treatment and storage with heat treatments recorded a significant reduction in CAT activity, with 5.81 mmol min^{-1} mg FW^{-1} (Treatment D), 5.57 mmol min^{-1} mg FW^{-1} (Treatment F), 6.26 mmol min^{-1} mg FW^{-1} (Treatment G) and 5.84 mmol min^{-1} mg FW^{-1} (Treatment H) of post-treatment values. In contrast, Treatments A, B, C and E indicated no changes with respective pre-treatment CAT activity levels.

α-Amylase Activity

Endosperm. At the initial stage (0-hr of imbibition), Treatment D (heat treatment) indicated significantly higher α-amylase activity at 85.34 units uL^{-1}, in comparison to other treatments, which varied within the 56.20–59.00 units uL^{-1} range (*Figure 6a*). From 0 to 48 hr after imbibition, all treatments showed significant enzyme activity increment, however, some treatments (Treatments D, F and H) indicated a more rapid pattern than the others. Treatment D, Treatment F (60 days storage + heat treatment) and Treatment H (120 days storage + heat treatment) recorded high amylase activity; with 155.30, 132.10 and 135.90 units uL^{-1}, respectively. In contrast, Treatment A (untreated control), Treatment B (operculum removal), Treatment C (storage for 60 days), Treatment E (storage for 120 days) and Treatment G (storage for 180 days) indicated similar enzyme activity within 76.30–87.10 units uL^{-1} range. From 96 to 240 hr after imbibition, there were notable

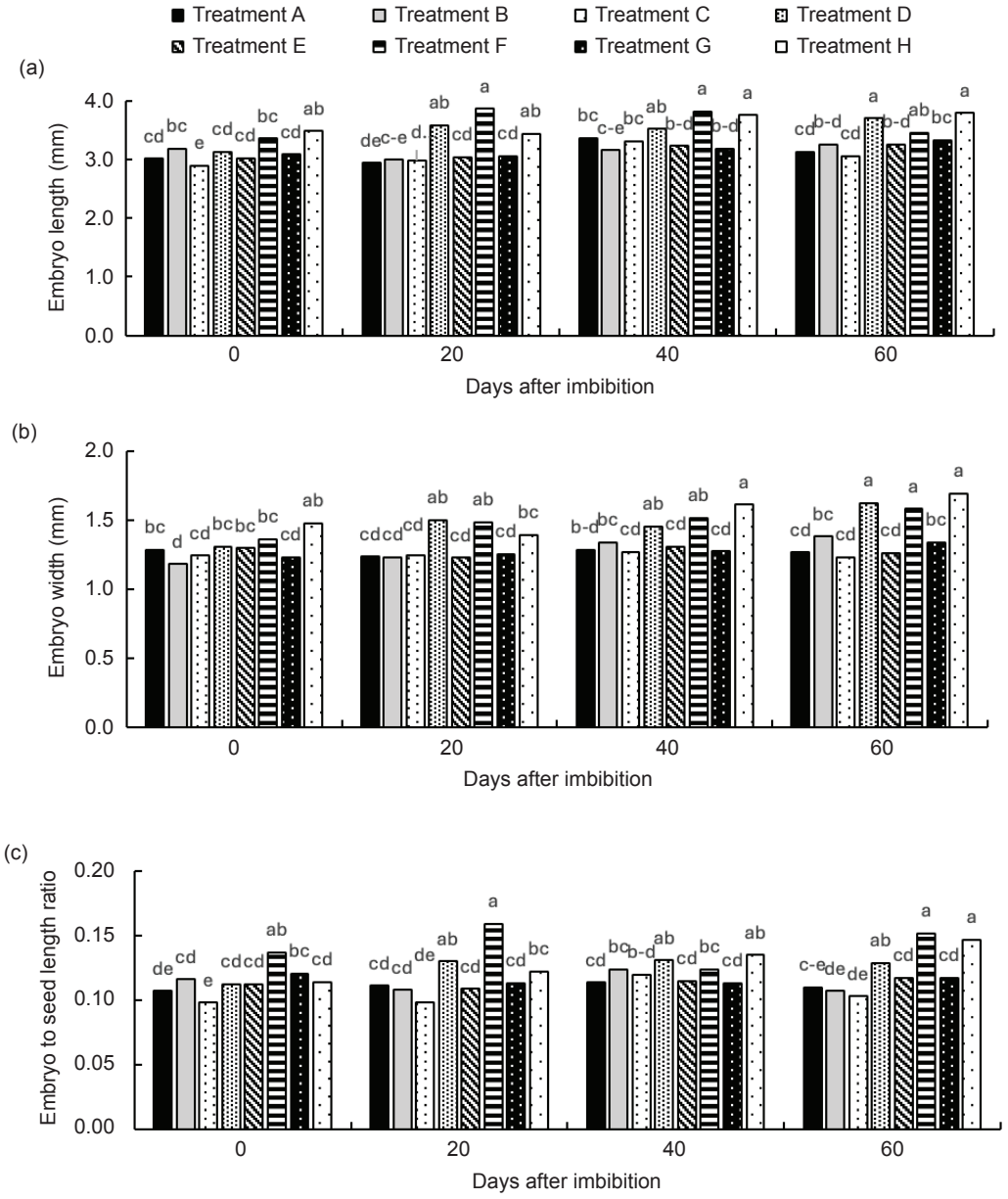


Figure 3. (a) Embryo length, (b) embryo width and (c) embryo-to-seed length ratio (E:S) at 0, 20, 40 and 60 days after imbibition.

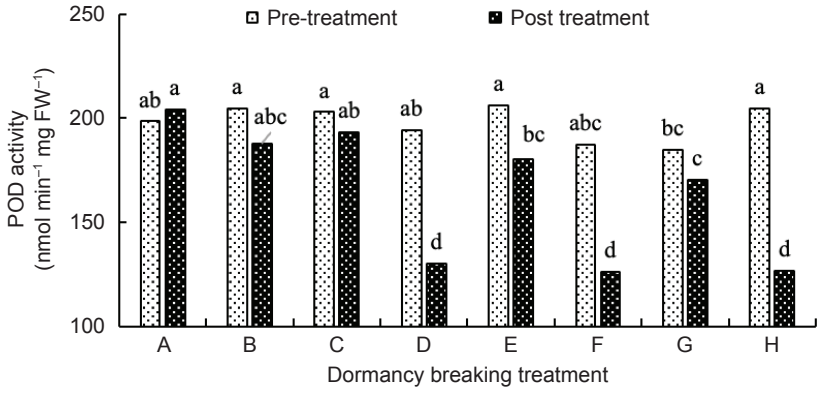


Figure 4. Peroxidase (POD) activities before and after dormancy-breaking treatments. Letters (a-d) indicate significant differences at 5% probability level.

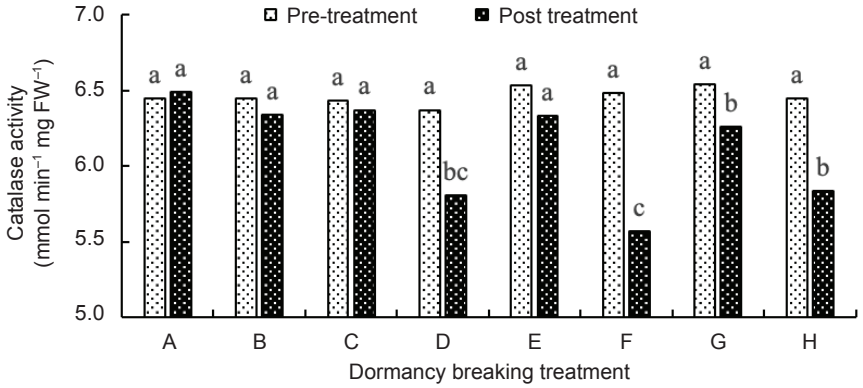


Figure 5. Catalase (CAT) enzyme activity before and after dormancy-breaking treatments. Different letters (a-c) indicate significant differences at a 5% probability level.

fluctuations in amylase activity; with a general gradual increment towards the end of the imbibition period. At 240 hr after imbibition, Treatment F (150.14 units uL^{-1}) recorded the highest α-amylase activity, followed by Treatments D, H, E, B and G (ranging within 125.00–135.70 units uL^{-1}), while the lowest activity was recorded by Treatment C (95.20 units uL^{-1}).

***Embryo*.** In general, the pattern of α-amylase activity in the embryo showed fewer fluctuations as compared to the endosperm tissue. At 0 hr after imbibition, no differences were recorded irrespective of treatments; within the 26.0–36.6 units uL^{-1} range (*Figure 6b*). From 0–48 hr after imbibition, a rapid increase in amylase activity was observed. The highest activity was recorded by Treatment F (115.0 units uL^{-1}), followed by Treatment D (108.9 units uL^{-1}) Treatment H (99.0 units uL^{-1}), Treatment E (89.5 units uL^{-1}), Treatment A (92.7 units uL^{-1}), Treatment B (86.3 units uL^{-1}), Treatment G (81.9 units uL^{-1}) and Treatment C (71.8 units uL^{-1}). From 48–96 hr after imbibition, all treatments indicated a more gradual amylase activity increment. Towards the end of the imbibition period (from 96–240 hr), only a slight activity increment was recorded by the treatments. Treatments D, F and H showed the highest final α-amylase activity (125.3, 129.3 and 114.0 units uL^{-1}, respectively), followed by Treatments G, E, A, B and C (within 90.2–100.3 units uL^{-1} range).

Seed Germination

All treatments that were heat-treated and stored before heat-treatments (Treatments D, F and H) recorded significantly higher final germination percentage (FGP) as well as normal seeds, in comparison to other treatments (*Table 2*). Treatment F showed the highest mean FGP at 89.8%, similar with Treatment H (87.3%) and Treatment D (82.8%), followed by Treatment G (12.5%), Treatment E (9.8%), Treatment A (7.5%), Treatment B (6.5%) and Treatment C (4.5%). A similar trend was observed for the percentage of normal seeds, since a majority of the germinated seeds indicated normal development, with less than 2.0% abnormalities and disease occurrence throughout all treatments. In terms of mean germination time (MGT), Treatments D, F and H recorded significantly faster germination (within 12–13 days), whilst other treatments showed more than 50 days of MGT.

Seed Water Uptake

According to the imbibition and moisture content analysis, it is evident that regardless of the treatment used water is still being absorbed into the embryos. Since the size of the embryo was significantly smaller in comparison to the whole seed, the imbibition test (which was conducted based on the whole seed weight) only showed less than 7% mass increment. Nevertheless, the application of heat treatment as well as storage before heat treatment was proven to increase the rate of water entry into the seeds. The role of heat treatment in increasing imbibition has been reported in several studies. In *Geranium carolinianum*, the application of dry-heat treatment could increase the seed mass by 95.0% within 10 hr of imbibition, and it is only 0.3% in the case of untreated seeds. Similarly, the application of hot-water treatment in *Adenanthera pavonina* seeds resulted in a 90.0% increase in seed mass with no change recorded for the control group. The increase in water imbibition upon heat treatment could be due to the heat causing dislodgement of the palisade layer on the seed surface, thus creating a 'water gap' in the embryo (Gama-Arachchige et al., 2010; Jaganathan et al., 2018). Therefore, based on the observation of imbibition, moisture content and germination data, it can be concluded that oil palm seed does not exhibit PD. This is because even in the untreated (control) sample, the seed still imbibed water like those treated, with heat-treated seeds having an accelerated rate.

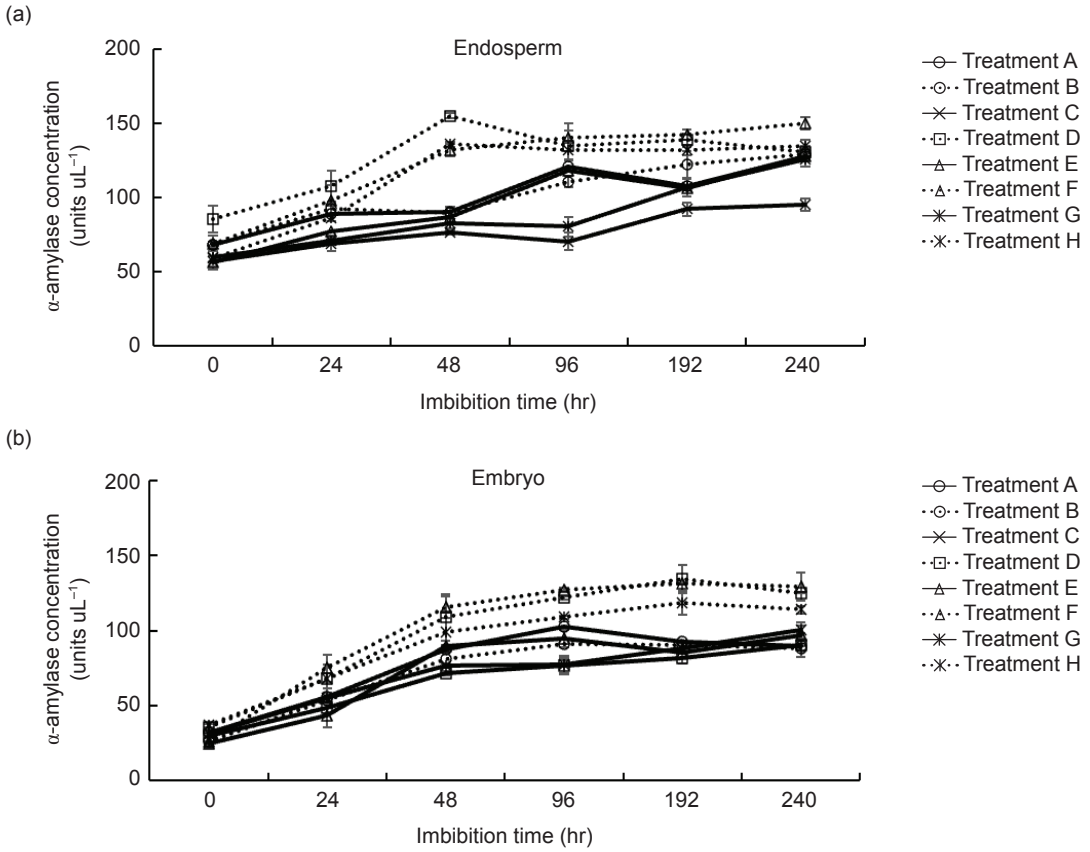


Figure 6. α-amylase activities for (a) endosperm and (b) embryo tissues from 0 to 240 hr after imbibition.

TABLE 2. MEAN VALUES OF FINAL GERMINATION PERCENTAGE (FGP), PERCENTAGE OF NORMAL, ABNORMAL, DISEASED SEEDS AND MEAN GERMINATION TIME (MGT)

Treatment	FGP (%)	Normal (%)	Abnormal (%)	Diseased (%)	MGT (days)
A	7.50bc	7.00bc	0.50ab	0.00b	50.08a
B	6.50c	6.50bc	0.00b	0.00b	50.50a
C	4.50c	3.00c	0.00b	0.50b	49.80a
D	82.80a	82.00a	0.25b	0.50b	12.30b
E	9.80bc	8.00c	0.25b	1.50a	50.00a
F	89.80a	87.80a	1.75a	0.25b	11.60b
G	12.50b	10.50b	0.50ab	1.50a	53.50a
H	87.30a	86.00a	1.25ab	0.00b	11.70b

Note: Letters (a-c) indicate significant differences within each column at a 5% probability level; FGP - final germination percentage; MGT - mean germination time.

Embryo Growth

Baskin and Baskin (2004) suggested seeds that exhibit MD simply require time for the embryo to develop and germinate. In this study, evaluation of embryo length, width and E:S showed that the application of heat treatment, and seeds that were stored before the heat treatment (Treatments D, F and H) indicated similar effects on embryo growth. On average, there was a 10% average increment in embryo length, 18% in width, and a 15% overall increase in E:S length ratio, shown by these treatments. Untreated (Treatment A), operculum removed (Treatment B) and seeds that were stored without any heat treatments (Treatments C, E and G) showed no significant changes within the 60 days evaluation period. Thus, this suggests that heat treating the seeds (with or without prior storage) accelerated the embryo growth. Earlier studies by Suranthran et al. (2011) and Kok et al. (2015) found that the embryo had completed the development and was able to resume growth into a normal seedling once excised from the seed. In contrast, morphologically dormant seeds would show a higher increment in embryo growth upon dormancy alleviation. For example, the underdeveloped *Podocarpus costalis* embryo showed a 54% increment in length before being

able to germinate (Chen et al., 2013). Therefore, this suggested that oil palm does not exhibit a standard MD.

Enzymatic Changes

The enzymatic assays of α-amylase, POD and CAT enzyme activities showed coherent results with the germination data. Three of the Treatments; Treatment D (heat treatment), Treatment F (60 days storage + heat treatment), and Treatment H (120 days storage + heat treatment) consistently showed significant increases in α-amylase, along with reductions in both POD and CAT activities in comparison to other treatments. The effect of heat treatments on α-amylase activities has been reported in *Hordeum vulgare*, *Cicer arietinum* L. and *Sorghum bicolour* seeds. In *H. vulgare* L. seed, high temperature (of more than 35°C) causes higher degradation of endosperm structure, particularly by reducing the crushed cell layer (CCL) thickness, thus increasing amylase production and embryo growth in comparison to non-heat treated seeds (Wallwork et al., 1998). Increasing heat treatment duration was also found to cause a decrease in alpha amylase inhibitor activity (AIA) in *C. arietinum* L., and *S. bicolour* seeds, thus directly increasing the amylase production and faster germination (Mulimani & Rudrappa, 1994). In terms of dormancy release, α-amylase was mainly associated with the balance of gibberellin (GA) and abscisic acid (ABA) productions in the endosperm and embryo tissues. Weiss and Ori (2007) suggested that seed germination can be characterised by transcriptional induction of hydrolytic enzymes including α-amylase, which are needed for the degradation of starch and the subsequent mobilisation into the embryo. Therefore, the changes in α-amylase activity were an indirect measure of the changes in the balance of seed ABA/GA levels, which is the known central regulatory mechanism underlying the maintenance and release of seed dormancy from the physiological perspective (Finch-Savage & Footitt, 2017; Shu et al., 2016). Zhang et al., (2022) reported that the genes related to ABA production negatively regulate the synthesis of GA and are strongly up-regulated in the mid-late stage of oil palm embryonic development. From *Figure 6*, it is clear that within the first 48 hr of imbibition, a marked increase in amylase activity was recorded, particularly for Treatment D, F and H. Seeds of *Melanoxylon brauna* recorded a significant increment of 60%–80% in α-amylase activity after four days of imbibition, which corresponds to more than 93% of germinated seeds (Ataíde et al., 2016). In *Oryza sativa* seed, α-amylase activity in the endosperm was shown to be positively correlated to GA synthesis in the embryo, which in turn hydrolysed the starch for energy supply during the germination process (Kaneko et al., 2002). Changes in endogenous signalling factors such as reactive oxygen species (ROS) were also reported to influence the balance between ABA and GA, thus affecting dormancy and germination in seeds (Ishibashi et al., 2017; Izydorczyk et al., 2017). The reduction of CAT and POD in this study suggested that the enzymes were utilised to protect the seeds from the accumulation of ROS resulting from heat treatment and storage. The resulting hydrogen peroxide (H_2O_2) from the initial reaction of superoxidase dismutase (SOD) was then converted to water and oxygen (Farooq et al., 2009). This is following findings from Kaushal et al. (2011), that the activities of antioxidants enzyme including CAT and SOD in chickpeas decreased under high-temperature stress between 40°C to 45°C. Similar findings were reported in soybean seeds, where both CAT and POD activities declined significantly when subjected to natural field weathering (Yadav, 2003). In this study, the concept of after-ripening was applied for Treatment E, F, G and H, where the seeds were stored at varying durations for up to 180 days to further alleviate the PYD in oil palm seed. Theoretically, if the seed does exhibit PYD; then an extended period of after-ripening could potentially assist in decreasing the ABA concentration along with higher GA sensitivity, thus decreasing the 'depth' of the dormancy (Finch-Savage & Footitt, 2017). However, it was found that the dormancy-breaking capacity remain after the storage period based on similar germination percentage and speed with seeds that were only heat-treated. Therefore, this suggested that oil palm seed may exhibit a non-deep PD since the dry storage period did not improve seed germination, which would be the case for intermediate PD (Baskin & Baskin, 2004). Nevertheless, the application of heat treatment was indeed proven to be crucial in alleviating the non-deep PD of oil palm seeds.

CONCLUSION

In this study, different dormancy-breaking treatments were employed in oil palm to understand the dormancy mechanism in this species, based on physical, morphological, and physiological perspectives and concluded that oil palm seeds exhibit non-deep PYD. It was observed that the seeds were able to imbibe water and have a fully developed embryo requires PYD alleviation. Application of heat treatment to fresh or stored seeds, resulted in improved seed germination of more than 82% and a low mean germination time of less than 10 days; indicating the necessity of heat treatment. The treated seeds had reduced CAT and POD enzyme activities but with increased α-amylase production in the embryo. Heat

treatment for 60 days at 40°C is confirmed as the most efficient and practical dormancy-breaking method in alleviating non-deep PD for commercial hybrid seed production.

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