

Breaking Seed Dormancy: Revisiting Heat-Treatment Duration on Germination and Subsequent Seedling Growth of Oil Palm (*Elaeis guineensis* Jacq.) Progenies

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Abstract: The role of varying heat-treatment durations on the germination of oil palm seeds is well documented. However, to the best of our, the influence of prolong heat-treatment upon oil palm seedling growth and survival has not been studied. We investigated the effects of different heat-treatment durations on germination and seedling growth of 10 oil palm progenies. Batches of 250 oil palm seeds from each progeny were heat-treated at a temperature of $39^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 60, 80, 100 and 120 days, to break dormancy. Progenies with germination percentage of 50 % or more for all heat-treatment durations were planted out in the nursery using a Randomized Complete Block design, with heat-treatment durations as main plots and progenies as subplots. Seedling growth parameters measured included rate of leaf production, leaf area and relative leaf area growth rate, rate of leaf elongation and diameter of base of seedlings stem. Progenies A5240, A5291, A5234 and A5221 were found to be the most outstanding progenies in terms of germination. Prolonged heat-treatment resulted in reduced germination and less vigorous seedling growth. Heat-treatment of 60 days gave the best seedling growth performance and seedling growth was adversely affected when heat-treatment exceeded 100 days. The study indicated that heat-treatment duration of 60 days and not 80 days was effective for breaking dormancy of the oil palm seeds and successful seedling growth. This study shows that oil palm seeds germination can be obtained successfully within a short period (60 days) of heat-treatment compared to the current 80 days application by PAMOL Plantation Ltd. Lobe estate. These results could provide significant solutions to the numerous demands of oil palm seeds by local farmers.

Key words: Oil palm (*Elaeis guineensis* Jacq.) % Progeny % Dormancy % Germination % Leaf area % Relative leaf area growth rate

INTRODUCTION

The oil palm, *Elaeis guineensis* Jacq., is an important source of vegetable oil cultivated in plantations and small farms in Southeast Asia and also Western Africa [1, 2]. The oil palm produces oil from the fruit mesocarp (crude palm oil) and palm kernel oil from the seed [1]. However, fossil and historical evidence, including personal communications suggest that the oil palm originated in the tropical rainforest of West Africa [1, 3, 4]. Morphologically, the oil palm is an erect, un-branched tree up to 35 m tall with a stout trunk of persistent leaf bases on which epiphytes often grow. The stem diameter varies from 20 cm to 75 cm, with progressive thickening towards the base [1, 2]. At the top, the plant terminates in a crown of leaves, produced in spiral succession from the leaf meristem [4]. The fruit (drupe) varies in shape from ovoid

to near spherical, bulging at the tip. The fruit is about 1 cm to 2.5 cm in length and weigh from 3 g to over 30g [1]. Like most fruits, the oil palm fruit consist of a pericarp, which consist of exocarp, mesocarp and endocarp.

In commercial seed production, parental parameters such as slow stem elongation, high fruit weight to fruit bunch ratio, high oil to bunch ratio, reasonable kernel size to bunch ratio, early fruiting, thin endocarp and a thick mesocarp and tolerance to *Fusarium* wilt are studied for a period of not less than eight years before selection for a parent plant is made [5]. Thus, the selection for high-yielding genotypes and the production of tenera hybrid seeds require careful controlled pollination [4]. The commercial oil palm tenera hybrids are a cross between the dura type (thin mesocarp, thick endocarp, with generally large kernel) and the pisifera type (thick mesocarp, no endocarp with small or no kernel). The

hybrid tenera type has a thin endocarp (0.5 to 3 mm thick), a medium to high mesocarp and reasonable kernel size. The improved oil palm progenies ensuing from the hybrid tenera type go into production 3 to 4 years after field plantings. However, the timing and productivity of the hybrid tenera type depends greatly on how well the plantation is managed [6].

Like most seeds with very thick seed-coat (testa), the oil palm seeds pose difficulties in seed germination because of a long "rest" after it is harvested [7, 8]. This long rest or dormancy was attributed to some mechanical constraints to embryo growth and the oxygen uptake by the compact "stony" endospermous tissue of the oil palm seeds [7, 9]. As such, oil palm seeds have to undergo a period of increased temperature under sufficient seed moisture for rapid and maximal germination [7, 10]. Naturally, oil palm seeds are pre-treated with heat resulting from the exothermic decomposition of the oily mesocarp by fungi and other microorganisms [11]. However, this natural process is very slow and few seeds germinate. On the other hand, in controlled heat-treatment, the seeds for germination are stored at a temperature range of 38 to 40°C for 90 days at 18 % moisture content [10]. Germination of the oil palm seed by heat-treatment has been described in some detail by Mok [7], Hussey [9], Rees [10], Corley [12] and Periasammy *et al.* [13]. Though these studies investigated the effects of different pre-treatment temperatures with varying durations on oil palm seed germination, none of the study investigated the physiological effects of varying durations on subsequent seedling growth. Currently, the research laboratory at PAMOL Plantation Ltd, Cameroon, which commercially produced germinated oil palm seeds, incubates seeds at $39 \pm 1^\circ\text{C}$ for 80 days and this generally gives a germination rate of less than 50 %. As a result, large quantity of seeds is wasted making it difficult for the plantation to meet up with the demand for germinated seeds. It is therefore necessary to investigate how different durations of heat-treatment could change this deficit. The objectives of this study were: (1) To investigate the effects of different heat-treatment durations on the germination of some PAMOL commercial tenera oil palm progenies. (2) To investigate the effects of different heat-treatment durations upon seedling growth. Our experiment was designed to investigate these objectives. The physiological effects related to prolong heat-treatment on oil palm seed germination and subsequent seedling growth are discussed.

MATERIALS AND METHODS

Location of Study and Plant Species: This study was conducted in the seed production unit and demonstration nursery at the research laboratory of PAMOL Plantation Ltd in Ekondo Titi Sub-Division (04°34 to 04°40 N and 09°04 to 09°10 E), the South West of Cameroon. The location benefits from the equatorial climate, which is characterized with an annual rainfall of 3,559 mm, relative humidity of 90% and an annual temperature of 27°C. The year is divided into two seasons: dry season that runs from December to February, but interrupted with light rain and a rainy season, which runs from March to November each year. The soil is sandy clay and chemically poor, but generally appreciable for oil palm cultivation, [14]. Oil palm seeds used for this study were obtained from controlled pollinations intended for commercial tenera seed production by the PAMOL research unit (Table 1). The fresh fruit bunches for seed production, were harvested at 1 to 10 loose fruits stage of ripeness [15]. To extract the seeds, the fruits were retted and de-pericarped mechanically. The seeds of each bunch were air-dried for four hours and batches of 400 seeds were stored at 22 to 25°C.

Pre-treatment Measurements (Seed Size and Viability): Samples of 20 oil palm seeds from each of the 10 progenies (Table 1) were used for seed size determination. The length and breadth of each seed were measured with a digital caliper (Mitutoyo, Aurora, IL) and the seed size (cm) was the mean of length and breadth. Seed viability was tested on 50 sampled seeds from each progeny of seeds by physical visualization and chemically by tetrazolium chloride viability test of the seed embryos. The endocarp of each seed was removed to obtain the endosperm. The embryo of each endosperm was carefully removed and observed with a hand lens. Yellow embryo indicates viable seed while brown embryo, dead seed. Chemically, the embryo of each endosperm was placed in 1% (10 gm/l) aqueous solution of tetrazolium chloride and kept under dark condition for 6 to 8 hours. A change in embryo coloration to partially or completely red or pink indicated viable seeds. Progenies with viability greater than or equal to 86 % were selected for germination.

Application of Heat-treatment Duration: Random sample of 250 seeds of each progeny selected from three months stored seeds were sprayed with a mist sprayer of distilled-water to raise the moisture content required

Table 1: The parentage and origin of the oil palm (*Elaeis guineensis* Jacq.) progenies used for heat-treatment germination

Progenies	Parentage Dura x Pisifera	Grand Dura Progeny Code	Grand Dura Origin	Grand Pisifera Progeny Code	Grand Pisifera Origin
A5207	12/1918 x 12/2709	Lb 130	Ekona x Ekona	Lb 135	Angola x Angola
A5240	13/1736 x 12/2709	Lb 240	Ekona x INEAC	Lb 135	Angola x Angola
A5291	13/1640 x 12/12433	Lb 235	Deli (NDN) x INEAC	Lb 158	Ekona x Ekona
A5234	13/1066 x 12/2709	Lb 240	Ekona x INEAC	Lb 135	Angola x Angola
A5200	13/0251 x 12/12428	Lb 235	Deli (NDN) x INEAC	Lb 158	Ekona x Ekona
A5228	13/3249 x 12/12433	Lb 275	Ekona x Ekona	Lb 158	Ekona x Ekona
A5281	13/2937 x 12/12433	Lb 258	Ekona x Ekona	Lb 158	Ekona x Ekona
A5221	13/1154 x 12/12433	Lb 230	Deli x (Deli x Ekona)	Lb 158	Ekona x Ekona
A5268	13/3249 x 12/12433	Lb 257	Ekona x Ekona	Lb 158	Ekona x Ekona
A5224	12/7978 x 12/12433	Lb 8	Aba x (Angola x Deli)	Lb 158	Ekona x Ekona

for germination (approx. 18 %). The moist seeds were kept in two 40 cm by 40 cm polythene bags for heat-treatment at 60, 80, 100, 120 days durations at temperature of $39\pm1^{\circ}\text{C}$ in a germinator. After heat-treatment, the 250 seeds of each progeny were removed from the germinator and brought to the required moisture content for germination by soaking in distilled-water for 7 days. The water was changed daily to avoid microbial contamination. The seeds were then stored in a 40 cm by 40 cm polythene bag and assessed for germination after 14 days of storage for first sorting and 7 days later for the second sorting. Percentage germination was estimated as follows:

$$\% \text{ Germination} = \frac{\text{Number of germinated seeds}}{\text{Number of seed incubated (250 seeds)}} \times 100$$

Nursery Establishment and Management: Polythene bags of size 40 cm by 35 cm were filled with free-draining sandy clay soil. Progenies A5240, A5291, A5234 and A5221, with optimal % germination rate (Table 3) were combined factorially with heat-treatment durations and tested on a Randomized Complete Block design, in three replicates. Heat-treatment durations of 60, 80, 100 and 120 days were main plots and progenies as sub plots (36 polythene bags per sub-plots). Each sub-plot was separated from each other by a path of 100 cm wide. The germinated seeds from the heat-treatment were planted 1 cm deep ensuring that the plumule (shoot) was placed upward and the radicle (root) downwards in an open top nursery. For each sub-plot, 20 border seedlings acted as guard plants to take care of border effects while the remaining 16 internal seedlings were used for data collection.

Drip-irrigation technique was applied when needed and weeds were carefully removed by hand ensuring that the tender roots of the seedlings were neither disturbed nor damaged. A small quantity (0.0025 % per

seedling) of Carboforan (FMC Corporation, Philadelphia, PA) and 4g/L Mancozeb (DuPont Agricultural Products, Wilmington, DE) were used for insects and fungi control. Liquid fertilizer mixture was applied weekly at a rate of 40 ml per seedling. The liquid fertilizer consist of 14.00 g sulphate of ammonia (2.94 g nitrogen), 14.00 g kieserite (2.27 g magnesium) and 28.00 g muriate of potash (13.94 g potassium) in 10, 000 ml of water.

Vegetative Growth Measurements: Measurements and calculations on vegetative traits on the nursery oil palm seedlings were according to the non-destructive methods suggested by Corley and Breure [16] with minor modifications. These measurements includes: rate of leaf production, diameter of base of stem, plant growth increment, leaf area and relative leaf area growth rate (R_A). For rate of leaf production per week, all leaves whose tips were visible above the older leaf bases, between 12 and 16 weeks after planting were counted. The rate of leaf production per week was estimated as: Number of leaves produced between 12 and 16 weeks of growth divided 4 (duration of growth). Diameter of base of stem at widest point was measured with a digital caliper (Mitutoyo, Aurora, IL) at right angles to the seedlings at 16 weeks of growth after planting. For plant growth increment, the height from soil surface to the tip of the youngest spear leaf of each seedling was measured at 12 and 16 weeks of growth after planting. Plant growth increment was estimated as: The height difference at 12 and 16 weeks of growth after planting.

Leaf area for both lanceolate and bifurcate leaves were measured with a portable LI-3300 area meter (LICOR, Inc., Lincoln, Nebraska). Total leaf area of a seedling was calculated as the sum of all leaf areas (lanceolate and bifurcate leaves) produced at the time of measurement.

The first measurement for total leaf area (A_1) was estimated at 12 weeks (t_1) of growth after planting. The second measurement (A_2) was repeated at 16 weeks (t_2) of growth after planting. Relative leaf area (% R_A ($\text{cm}^2/\text{cm}^2 \text{ day}^{-1}$)) were estimated as:

$$\% R_A (\text{cm}^2/\text{cm}^2 \text{ day}^{-1}) = \frac{\log_e A_2 - \log_e A_1}{t_2 - t_1} * 100$$

Data Analysis: Where appropriate, data from parameters measured were analyzed using two-way or one-way Analysis of Variance (ANOVA) using the statistical analysis software Sigmasat 3.1 (SYSTAT, San Jose, CA). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Pre-treatment Measurements (Seed Size and Viability):

Our results showed that seed size range from 1.45 cm to 2.38 cm. Progeny A5224 had the smallest seed size while progeny A5234 had the highest seed size (Table 2). For both physical visualization and tetrazolium chloride viability test of seed embryos, test showed that all ten progenies were physically and chemically viable (viability $> 86\%$). Though all ten progenies were potentially viable, six of the ten progenies had percentage viability greater than 90 whereas four progenies (A5200, A5228, A5281 and A5268) had less than 90 % viable seeds. However, all ten progenies were selected for germination by heat-treatment.

Germination Rate: The germination percentages of the ten oil palm progenies after heat-treatment for 60, 80, 100 and 120 days in the germinator at $39 \pm 1^\circ\text{C}$ are shown in Table 2. Our results indicated that for all ten oil palm progenies under the same heat-treatment condition ($39 \pm 1^\circ\text{C}$ and approx. 18 % moisture content), 60 days duration in the germinator produced the highest mean percentage germination (66.22 ± 9.58) and this was followed by 80 days duration (44.36 ± 10.82). However, germination rates were lethal for 100 days and 120 day durations with mean germination rate of less than 30 % (Table 2). Within the ten progenies, germination rates for 60, 80, 100 and 120 days combined were highest for progenies A5240 and A5221 ($> 80\%$) but very low ($< 5\%$) for progenies A5228 and A5268. However, all four progenies (A5200, A5228, A5281 and A5268) with seed viability less than 90 % had a mean germination rate of less than 25 % (Table 2).

Based on the percentage germination rate, four progenies (A5240, A5291, A5234 and A5221) in Table 3 were selected for further assessments of varying heat-treatment durations on seedling growth. Within these four progenies, the mean percentage germination rate was significantly higher for 60 days compared to 80, 100 and 120 days. However there was no significant difference in the mean percentage germination rate for both 100 and 120 days durations (Table 3). Within progenies, germination rates for 60, 80, 100 and 120 days combined were significantly higher for progenies A5240 and A5221 compared to progenies A5291 and A5234 (Table 3).

Table 2: Percentage germination rate of ten oil palm progenies exposed to different durations of heat-treatment to break dormancy. Values are cumulative number of seeds germinated after second sorting as a percentage of seeds incubated.

Progenies	Heat-Treatment Duration						SE \pm
	Seed Size	60 days	80 days	100 days	120 days	Means	
A5207	1.49	59.61	40.41	00.00	00.00	25.01	14.96
A5240*	1.70	92.43	86.89	81.61	82.84	85.94	2.44
A5291*	2.38	84.00	68.85	41.29	30.81	56.24	12.25
A5234*	1.81	85.21	75.20	10.01	30.03	50.11	17.96
A5200	1.54	64.47	08.87	14.17	00.80	22.08	14.40
A5228	1.46	15.63	02.04	00.00	02.02	04.92	3.60
A5281	1.52	52.00	21.23	06.09	04.09	20.85	11.07
A5221*	1.81	94.81	85.21	90.00	68.12	84.54	5.81
A5268	1.49	17.64	01.63	00.00	00.83	05.03	4.22
A5224	1.45	96.42	53.24	32.82	05.63	47.03	19.14
Means		66.22	44.36	27.60	22.52		
SE \pm		9.58	10.82	10.68	9.62		

The symbol * indicates progenies with optimal mean germination = 50.00 %. These progenies were selected for nursery establishment.

Table 3: Percentage germination rates of selected progenies for nursery establishment with mean germination rate of 50 % or more. Values are cumulative number of seeds germinated after second sorting as a percentage of seeds incubated

Progenies	Seed Size	Heat-Treatment Duration				Means	SE \pm
		60 days	80 days	100 days	120 days		
A5240	1.70	92.43	86.89	81.61	82.84	85.94 ^a	2.44
A5291	2.38	84.00	68.85	41.29	30.81	56.24 ^b	12.25
A5234	1.81	85.21	75.20	10.01	30.03	50.11 ^b	17.96
A5221	1.81	94.81	85.21	90.00	68.12	84.54 ^a	5.81
Means		89.11 ^a	79.04 ^b	55.73 ^c	52.95 ^c		
SE \pm		2.66	4.26	18.58	13.35		

Progeny means (column) and heat-treatment means (row) with the same letters are not significantly different ($P < 0.05$)

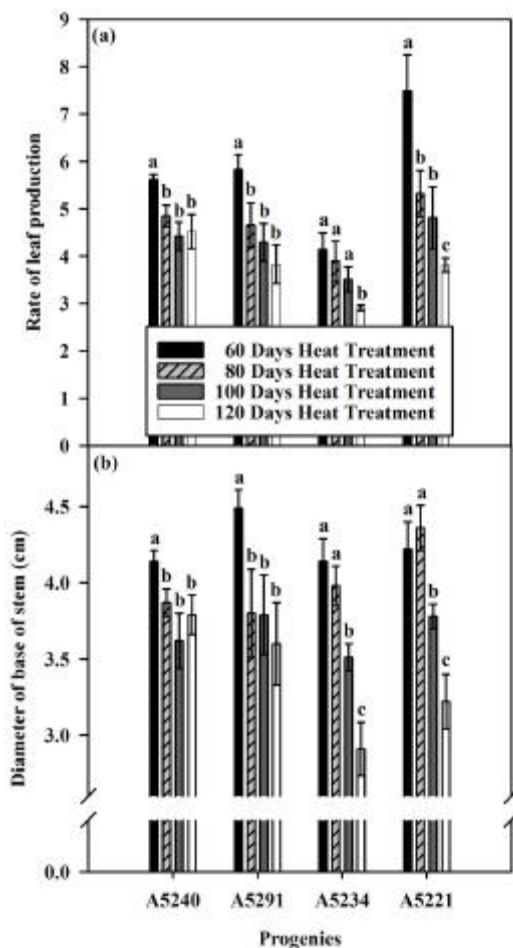


Fig. 1: Rate of leaf production (a) and diameter of base of stem (b) of four oil palm progenies exposed to different durations of heat-treatment to break dormancy. The bars are means \pm S.E of 16 oil palm seedling for each progeny in three replicates. Bars with the same letter within a progeny are not significantly different ($P < 0.05$).

Vegetative Growth Measurements: Seedling rate of leaf production for progenies A5240 and A5291 was not significantly different for 80, 100 and 120 days but was significantly higher for 60 days heat-treatment duration. For progeny A5234, no significant difference in the rate of leaf production was observed for 60, 80 and 100 days heat-treatment durations, however, 120 days heat-treatment duration significantly reduced the rate of leaf production. Finally, progeny A5221 also showed a significant reduction in the rate of leaf production with heat-treatment durations, however, there was no significant difference between 80 and 100 days heat-treatment durations. Overall, the rate of leaf production for progenies A5240, A5291 and A5221 was significantly higher for 60 days compared to 80, 100 and 120 days durations of heat-treatment (Fig. 1a).

Like rate of leaf production, the diameter of base of stem for progenies A5240 and A5291 was not significantly different for 80, 100 and 120 days heat-treatment durations but was significantly higher for 60 days heat-treatment duration. For progenies A5234 and A5221, our result showed no significant difference in the diameter of base of stem between 60 and 80 days heat-treatment durations, but was significantly different for 100 and 120 days heat-treatment durations. Overall, the seedling diameter of base of stem for all four progenies showed decreasing trend with heat-treatment durations (Fig. 1b).

Plant growth increment showed decreasing trend with heat-treatment durations (Fig. 3). For progeny A5240, plant growth increment was significantly higher for 60 days heat-treatment durations and also significantly different for all four heat-treatment durations. For progeny A5291, plant growth increment was significantly higher for 60 days compared to 80, 100 and 120 days heat treatment durations. However, for this progeny (A5291) there was no significant difference in plant growth increment for 80, 100, 120 days heat-treatment durations.

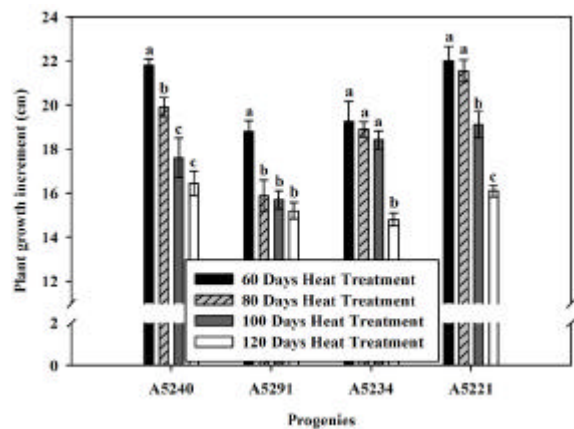


Fig. 2: Seedling growth increment of four oil palm progenies exposed to different durations of heat-treatment to break dormancy. The bars are means \pm S.E of 16 oil palm seedling for each progeny in three replicates. Bars with the same letter within a progeny are not significantly different ($P < 0.05$).

Whereas for progeny A5234, plant growth increment was significantly lower for 120 days compared to 60, 80 and 100 days heat-treatment durations. However, this progeny (A5234) did not show any significant difference in plant growth increment for 60, 80 and 100 days heat-treatment durations. Finally, for progeny A5221, plant growth increment was significantly higher for 60 and 80 days compared to 100 and 120 days (Fig. 2).

Seedling leaf area for progenies A5240 and A5221 was significantly higher for 60 and 80 days compared to 100 and 120 days heat-treatment durations. For progenies A5291 and A5234, leaf area significantly higher for 60 days and significantly lower for 120 days but was not significantly different for 80 and 100 days heat-treatment durations. Overall, seedling leaf area also showed a decreasing trend with heat-treatment durations (Fig. 3a).

Seedling relative leaf area growth rate like leaf area for progeny A5240 was significantly higher for 60 and 80 days compared to 100 and 120 days heat-treatment durations. For progenies A5291, A5234 and A5221, relative leaf area growth rate was significantly higher for 60 days compared to 80, 100 and 120 days heat-treatment durations. However, for these progenies, there was no significant difference in seedling relative leaf area growth rate between 80 and 120 days for progeny A5291, between 80 and 100 days for progeny A5234 and between 100 and 120 days for progeny A5221. Overall, seedling relative leaf area growth rate also showed a decreasing trend with heat-treatment durations (Fig. 3b).

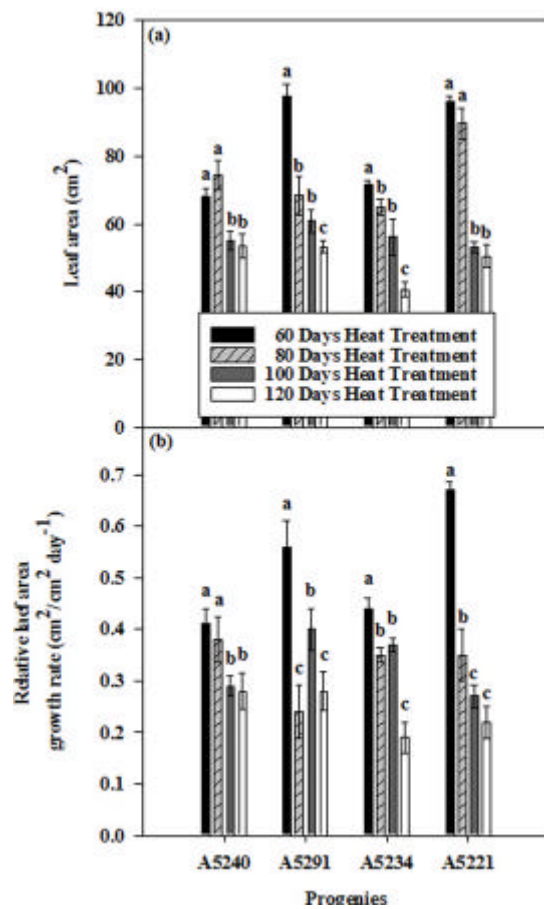


Fig. 3: Leaf area (a) and relative leaf are growth rate (b) of four oil palm progenies exposed to different durations of heat-treatment to break dormancy. The bars are means \pm S.E of 16 oil palm seedling for each progeny in three replicates. Bars with the same letter within a progeny are not significantly different ($P > 0.05$).

DISCUSSION

The present study has elucidated the potential of 60 days heat-treatment durations as optimal for germination of the oil palm seeds. In a recent study, Beugré *et al.* [17] also reported significant success in oil palm seeds germination for 60 days compared to 40 and 80 days heat-treatment durations. On the other hand, Soh *et al.* [18] suggested a 50 days heat-treatment at a temperature range of 37 to 39°C will provide an optimum germination for oil palm seeds. Our results and that of Beugré *et al.* [17] contradict earlier reports by Hussey [9] and Rees [10] suggesting an optimum heat-treatment duration of 80 days at a temperature range of 38 to 40°C.

According to Hussey [9] the optimum temperature range of 38 to 40°C accelerated the germination process of oil palm seeds in pure oxygen. Hussey [9] postulated that the germination of oil palm seeds is dependent upon a minimal oxygen concentration within the embryo of the oil palm seed. Like in most seed plants, the complete absence of oxygen inhibits the process of seed germination.

Within each treatment, there were differences in percentage seed germination with the ten oil palm progenies tested (Table 2). These differences may be attributed to the genetic constitution of each oil palm progeny [19]. The decrease in germination with increasing duration was consistent with the findings of Mok [7] and Gashaw and Michelsen [20]. They [7, 20] attributed the adverse effects of germination to prolonged heating of the oil palm seeds in the germinator. It should be noted that the endocarp of the oil palm seeds functions as a poor insulator, conducting small amount of heat to the endosperm during the heat treatment. Thus, prolonged heating will result in temperature increase in the endocarp, which may be transferred to the endosperms, causing reduction in enzyme activities, thus adverse effects to germination and seedling growth [21].

The seedling growth responses reported here following varying durations of heat-treatment to break dormancy in the oil palm progenies may provide some clues as to how prolong heat-treatment durations would influence the patterns of development in post nursery establishment. Our results showed that all seedling growth parameters measure decreased with increasing heat-treatment durations. These results suggest that the successful growth of these oil palm seedlings were impaired by the prolong heat-treatment in the germinator. The heat-treatment germination of oil palm seeds for more than 80 days are especially not worthy given that under natural conditions, oil palm seed germination require successive short periods of heat-treatment. These successive short periods of heat-treatment usually occur during the dry season and in most cases, the germination rate is very slow (1 to 3 years) with very small percentage germination compared to commercial germination. However, field/natural germination is influenced by other factors, which include seed age, moisture content, the presence of pathogens and seed predators.

CONCLUSIONS

In summary, increasing duration of heat-treatment to break dormancy in the commercial oil palm progenies negatively affected seed germination and also subsequent

seedling growth. This study has shown that heat-treatment duration of 60 days and not 80 days was effective for breaking oil palm seed dormancy and successful seedling growth. With respect to seed germination, progenies A5240, A5291, A5234 and A5221 were the most outstanding progenies, out of the ten progenies tested. These four progenies can be used for the genetic selection program for commercial oil palm seed production. The study described here shows that oil palm seeds germination can be obtained within a short period (60 days) of heat-treatment compared to the current 80 days application by PAMOL Plantation Ltd. Lobe estate. This shorter duration of heat-treatment, which results in significant percentage germination could provide considerable solutions to the numerous demands of oil palm seeds by local farmers and also more profit to the plantation estate.

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