

OIL PALM (*Elaeis guineensis*) SEED CHARACTERISTICS AND GERMINATION POTENTIAL AS INFLUENCED BY MATURITY STAGE AND FRUITLET POSITION ON A BUNCH

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ABSTRACT

Oil palm is mainly propagated through dura × pisifera hybrid (DxP) seeds. In the natural environment, oil palm seeds require more than eight months of storage to achieve 25% germination due to a combination of morphological and physiological dormancy. Therefore, improvement in terms of the percentage of germination, uniformity and reduction in time to germination will benefit seed producers tremendously. In this study, the effect of harvesting at three maturity stages (18, 20 and 22 weeks after pollination or WAP) and six seed positions within a bunch (Proximal-Base, Proximal-Apex, Middle-Base, Middle-Apex, Distal-Apex and Distal-Base) on seed characteristics and germination potential were evaluated in a split-plot design. Seeds harvested at 20 WAP had 80% black-coloured seeds with the highest germination of 85.1%, compared with 70.5% and 80.9% at 18 and 22 WAP, respectively. Increasing seed maturity from 18 to 20 WAP showed more seeds shifting from semi-white and white to black (up to 40% more). Seeds located at the base region of the spikelet were smaller in size and were predominantly white with no differences in germination capacity.

Keywords: fruitlet position, maturity stage, oil palm, seed germination.

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INTRODUCTION

Seed maturity is an important element in determining the optimal harvesting time to produce high-quality seeds. Studies have shown the importance of seed maturity stages in the development of seed characteristics such as seed size, dry weight, the biochemical composition, including abscisic acid and gibberellin levels (Tetteh *et al.*, 2018; Vishal *et al.*, 2018), as well as the germination capacity (Lima *et al.*, 2012; Samarah and Mullen, 2004). In DxP oil palm seed production, the harvesting can generally

be done between 18 to 22 weeks after assisted pollination, according to the standard commercial practice (SDSAS, 2020). In practice, the 20 weeks after the pollination stage is perceived as the most practical for harvesting, with almost all the fruitlets still attached to the spikelets. However, germination is reported to range from 62.2% to 72.0%, with sporadic germination spread over 60 days (SDSAS, 2018). The oil palm seeds requirement in Malaysia stands at 50 million seeds per year in 2021, selling at an average of RM2.00 per germinated seed (MPOB, 2022). Hence, the relatively low (~68%) and non-uniform germination cause a tremendous loss to the industry. A detailed study on biochemical changes and histology of the oil palm seed development indicates that seed development is completed 18 weeks after anthesis (Kok *et al.*, 2015), raising the question of the advantage of early harvesting without affecting the oil palm seed quality. In oil palm, early harvesting has been carried out occasionally due to high demand from the nursery

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and seed buyer, however, the germination capacity has not been documented. Another aspect of seed development that contributes to a lower number of saleable seeds is the endocarp colour. Upon the depericarp process, around 20% of seeds are generally discarded due to their white colour, which was perceived as having low quality. A recent study by Norsazwan *et al.* (2022) indicated that seed endocarp colour was not associated with abnormality and lower quality, yet the issue of perception from potential customers remained.

Generally, an oil palm fruit bunch consist of 1000 to 3000 fruitlets, which develop from a single inflorescence (Corley and Tinker, 2015). Although assisted pollination is conducted thoroughly to ensure all the anthesizing female florets receive the intended *pisifera* pollen at the same time, variation may persist due to the differences from florets that are located at different sections within the bunch. Baydar and Erbaş (2005) reported that sunflower seed development was dependent on the seed position within the flower head. Seeds that were located at the outer side of the head were found to have higher dry weight with increased levels of palmitic, stearic and oleic acids. In addition, variation in seed characteristics was associated with spatial differences which influence the photosynthetic supply to the developing seeds (Bareke, 2018). In commercial seed production, the oil palm bunch is currently processed as a whole; without separating the seeds into different sections. While this is a practical system, it could also account for the variation in seed morphology, the asynchronous germination capacity and the incidence of white seeds. Therefore, this study aims to evaluate the effect of seeds harvested at different maturity stages on seed characteristics and germination. In addition, the characteristics of seeds located at different positions within a fruit bunch on germination and quality will be established.

MATERIALS AND METHODS

Seed Source

Oil palm D×P CALIX 600 seeds used in this study were obtained from Sime Darby Seeds and Agricultural Services (SDSAS). The *Dura* mother palm from a similar genetic lineage (code: BM 2515 to BM 2525) was selected from field PT 100 Ladang Dusun Durian, Sime Darby Plantations, Banting, Selangor (2°48'16.2"N 101°27'21.2"E). *Pisifera* pollen source (code: BM 2323/34) was used in controlled pollination. The procedure for controlled pollination of D×P seeds was conducted following the MS157: 2017 guidelines (Department of Standards, 2017). The harvested bunch was immediately transported to the seed processing facility of Sime Darby

Seeds and Agricultural Services (SDSAS), Banting, Selangor, Malaysia.

Experimental Design

The experimental design used was a split-plot, with the seed maturity stage (as the main plot) and the seed position (as the sub-plot). Three levels of seed maturity stages (18 WAP, 20 WAP and 22 WAP) and six positions on the bunch (Proximal-Base, Proximal-Apex, Middle-Base, Middle-Apex, Distal-Apex and Distal-Base) were evaluated. All the treatment combinations were replicated four times by using four different oil palm fruit bunches from similar CALIX 600 materials. The oil palm bunch was initially divided into three equidistance sections from the stalk and recorded as proximal, middle and distal. Each of the sections was further divided into the base and apex region of the spikelet (Figure 1).

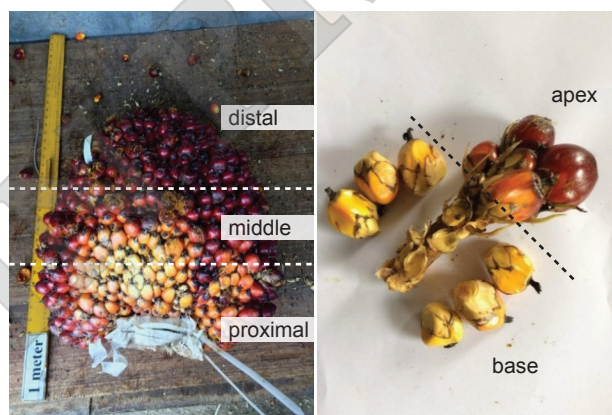


Figure 1. D×P bunch was divided and separated into three equal sections (distal, middle and proximal) (left) Spikelets from each of the sections were further divided into apex or base (right).

The detached fruitlets were then placed inside a mesocarp removal (also known as depericarper) machine. After the mesocarp removal, the seeds were immersed and continuously agitated with 0.05% Benomyl 50% WP (95% a.i) solution for 5 min to prevent fungal infection.

Seed Endocarp Colour and Physical Characteristics

Four replicates of 100 seeds from each treatment were randomly placed inside a plastic container for seed endocarp colour assessment. The percentage of black (endocarp surface is black or dark brown, with <10% white), semi-white (10%-90% endocarp surface is white coloured) and white (endocarp surface is white, with <10% black) seeds on each position was recorded as described by Norsazwan *et al.* (2022). Next, four replicates of 10 seeds were randomly selected for seed physical characteristics evaluation. The seed dry weight (g), shell thickness (mm), seed length/width (mm), kernel length

(mm), embryo length/width as well as operculum diameter (mm) were measured. For embryo measurement, a steel clamp was used to cut open the seed and expose the endosperm region, without damaging the embryo located directly underneath the fibre plug area. The embryo was then carefully excised from the seed using a scalpel blade. Embryo length and width (mm) were measured using a stereo microscope attached to a digital camera (Leica model EZ4D).

Seed Viability

Seed viability was measured using both tetrazolium (TZ) and germination tests to evaluate the germination potential and actual germination capacity of the dormant seed. Four replicates of 10 embryos were excised and soaked in distilled water for 3 hr. It was then transferred into a vial containing 5 mL of 1.0% (w/v) Tetrazolium chloride solution. The vials were kept in darkness for 17 hr prior to observation. The staining was categorised as red (viable), pink (non-viable) and white (non-viable). The mean percentage of the viable embryo was recorded. Prior to the germination test, seeds were subjected to heat treatment of $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 60 days. After that, 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 a germination room at $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ germination temperature. Germinated seeds were monitored daily by recording the number of seeds showing normal emergence of radicles from the fibre plug according to the Sime Darby Seed Production Unit (SPU) standard evaluation procedures. 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 percentage germination was determined as a percentage at time intervals over the course of the germination period, using the following Equation (1):

$$\text{Germination (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Number of seeds used}} \times 100 \quad (1)$$

Statistical Analysis

Analysis of variance (ANOVA) was performed by Microsoft Excel and Statistical Analysis Software, SAS 9.4 (SAS Institute, Cary, NC). A significance level of $P \leq 0.05$ was used for Duncan's Multiple Range Test (DMRT) throughout this study.

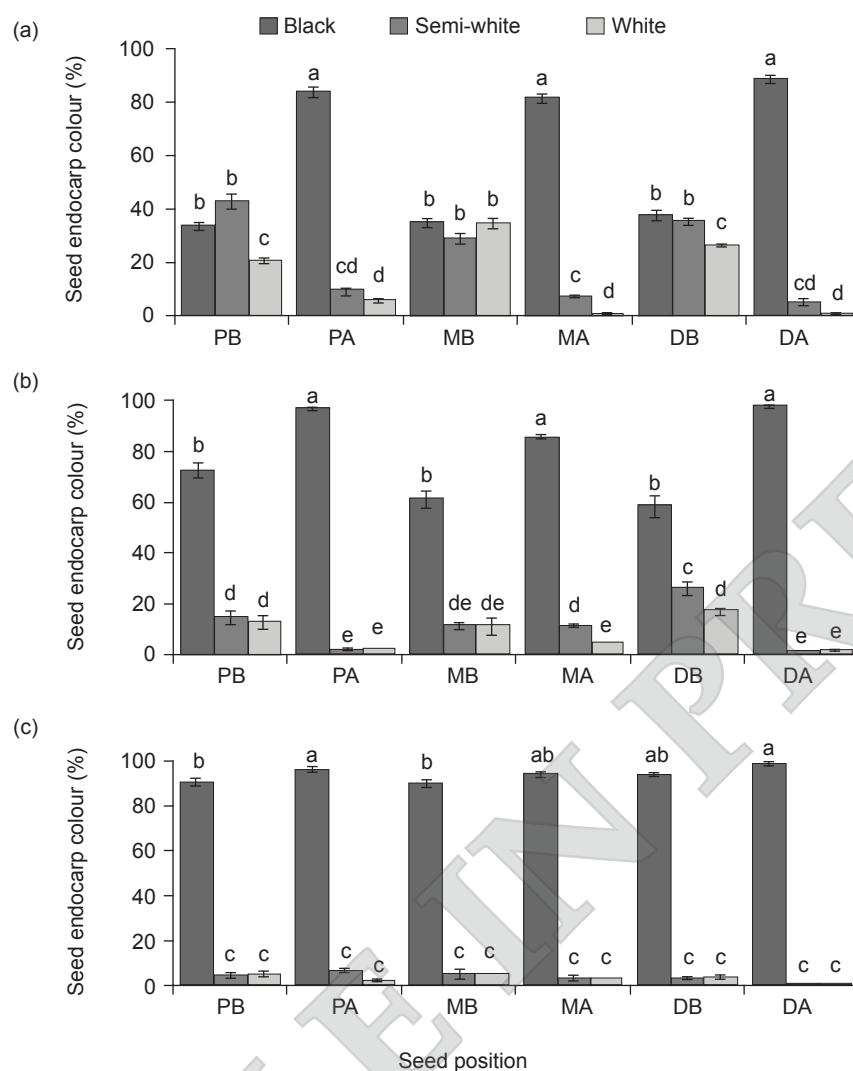
RESULTS AND DISCUSSION

The unique structure of oil palm inflorescence, which consisted of up to 3000 seeds, formed along

hundreds of spikelets tightly attached to the stalk (Corley and Tinker, 2015) was found to influence the fruitlets and seed morphological characteristics. In this study, it was recorded that as the maturity stages increased from 18 to 22 WAP, there was a clear shift in seed colour, from mostly white and semi-white to black. The percentage of black seeds increased significantly, while semi-white and white seeds decreased from 18 to 22 WAP, for every section of the oil palm fruit bunch. For instance, the proximal-base region had only 33.75% of black seeds at 18 WAP and this increased to 72.58% and 90.50% at 20 WAP and 22 WAP, respectively (Figure 2 and Figure 3).

Seeds that developed closer to the stalk (the base part of the spikelet), regardless of any position on the bunch (either proximal, middle or distal) had higher percentages of white seeds in comparison to the respective apex section of the spikelet. At 18 WAP, the proximal region of the bunch; proximal-base, recorded 33.75% of black, 46.00% semi-white and 20.25% of white seeds. On the other hand, the proximal apex was composed of 84.00% black, 10.00% semi-white and 6.00% white seeds. Similarly, the middle and distal sections had 20%-30% more semi-white and white seeds at the base, in comparison with the apex section of the spikelet.

However, as the harvesting was delayed, the differences between white and semi-white seeds between the proximal and apex of the spikelet decreased significantly, as can be seen at 22 WAP. Seeds located at the base region of the spikelet experience less exposure to sunlight, thus reducing pigmentation which resulted in lighter-coloured seeds (Norsazwan *et al.*, 2022). The pigmentation in seeds is known to be mainly caused by the accumulation of flavonoids, which is influenced by sunlight exposure (Marles *et al.*, 2003). Higher light intensities were reported to increase flavonoid content as a protective measure from harmful UV radiation (Su *et al.*, 2006; Xie and Wang, 2006). These include anthocyanins (red and purple), flavonols (pale yellow to colourless) and tannins (colourless, which later turn brown with oxidation) (Wan *et al.*, 2016; Yu, 2013). Earlier studies from Marles *et al.* (2003) had reported that dark brown coloured seed coat in *Brassica carinata* was mainly due to the presence of condensed tannins (proanthocyanidins) and the absence of anthocyanin. In terms of seed dry weight, several distinct differences were observed between the base and apex within the spikelet, for all three seed maturity stages. Seeds located at the apex region consistently showed higher seed weight. For example, at 18 WAP, proximal-apex, middle-apex and distal-apex recorded 3.79 g, 3.80 g and 3.88 g per seed, respectively (Table 1).



Note: PB - proximal-base; PA - proximal-apex; MB - middle-base; MA - middle-apex; DB - distal-base; DA - distal-apex. Different letters indicate significant differences within each column, at a 5% probability level.

Figure 2. Mean percentage of black, semi-white and white seeds at different sections of the oil palm bunch harvested at (a) 18, (b) 20 and (c) 22 weeks after pollination (WAP).

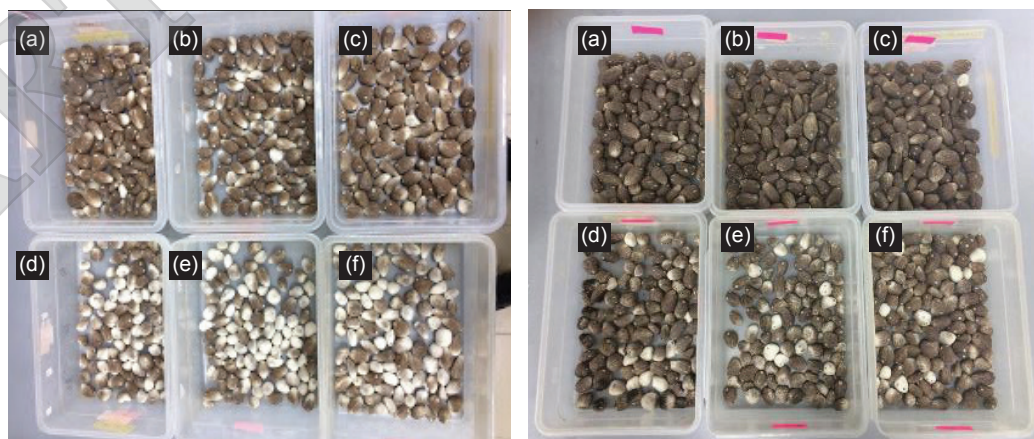


Figure 3. Seed endocarp colour changes from 18 WAP (left) to 22 WAP (right). (a) Proximal-apex, (b) middle-apex, (c) distal-apex, (d) proximal-base, (e) middle-base and (f) distal-base.

TABLE 1. SEED CHARACTERISTICS FOR DIFFERENT SEED MATURITY STAGES AND SEED SECTIONS

Seed stage	Seed position	Mean seed dry weight (g)	Mean seed length (mm)	Mean seed width (mm)	Mean operculum diameter (mm)	Mean shell thickness (mm)
18	PB	2.38g	17.08de	17.49a	3.62ab	3.17ab
	PA	3.79a-c	26.32a	17.83a	3.50a-c	3.18ab
	MB	2.97d-f	14.27e	17.95a	3.31c-e	3.01bc
	MA	3.80a-c	26.47a	16.98a	3.02f	2.45g
	DB	3.36b-d	16.49de	17.07a	3.39a-d	2.72d-f
	DA	3.88a-c	25.06ab	13.10b	3.07ef	2.96b-d
20	PB	2.79e-g	15.71de	16.84a	3.44a-d	3.08a-c
	PA	3.87a-c	24.82ab	17.31a	3.44a-d	3.03a-c
	MB	3.56bc	18.16d	16.71a	3.35b-d	2.99b-d
	MA	3.47b-d	25.76ab	16.84a	3.00f	2.50fg
	DB	2.70fg	14.51e	16.43a	3.31c-e	2.85c-e
	DA	3.67a-c	23.38bc	13.72b	3.24d-f	3.08a-c
22	PB	2.91d-g	16.81de	17.15a	3.35cd	3.00b-d
	PA	4.23a	23.32bc	17.97a	3.63a	3.30a
	MB	3.41b-d	21.75c	16.52a	3.06ef	2.51fg
	MA	4.23a	21.58c	17.92a	3.50a-c	3.10a-c
	DB	3.31c-e	16.33de	16.00a	3.25d-f	2.70e-g
	DA	3.95ab	18.28d	13.25b	3.25d-f	3.02bc

Note: PB - proximal-base; PA - proximal-apex; MB - middle-base; MA - middle-apex; DB - distal-base; DA - distal-apex. Different letters indicate significant differences within each column, at a 5% probability level.

In contrast, the proximal, middle and distal-base sections had 2.38 g (proximal), 2.97 g and 3.36 g, respectively. Similar trends were observed for the seeds harvested at 20 and 22 WAP. A similar seed length trend was observed at 18 and 20 WAP. The highest seed lengths were obtained at proximal-apex, middle-apex and distal-apex sections, with values ranging from 23.38 mm to 26.47 mm. The base region of the same spikelet, consistently showed lower seed length values, ranging from 14.20 mm to 18.16 mm. On the other hand, at 22 WAP, only the proximal and distal-apex regions had recorded significantly higher seed lengths, in contrast to the respective base regions. The middle-apex and middle-base showed no significant differences in length. The seed width showed less overall variation as compared to length. However, no specific trend was observed. The seed width ranged from 13.10 mm to 17.98 mm and varied throughout the different seed maturity stages. Interestingly, the operculum size was found to have a weak correlation with the seed weight (Pearson's Correlation Coefficients = 0.11073, p -value <0.05). The mean value ranged from 3.00 mm to 3.63 mm in diameter. In general, oil palm seeds did not show changes in shell thickness from 18 to 22 WAP and the values varied greatly among

different seed positions. The shell thickness was significantly correlated to the operculum diameter (Pearson's Correlation Coefficients = 0.3097). The value ranged from 2.45 mm to 3.30 mm. The proximal sections (for both base and apex) of the bunch consistently showed more than 3.00 mm shell thickness.

Generally, all seeds that were located at the base region of the spikelets showed a more rounded appearance due to the lower length-to-width ratio. In contrast, higher length-to-width ratio of seeds at the apex section resulted in a more elongated morphology. The effect of space competition towards the seed morphological characteristics had been reported in other oilseed species, including sunflower (Gupta *et al.*, 2009), mustard (Munshi and Kochar, 1994; Munshi and Kumari, 1994) and soybean seeds (Guleria *et al.*, 2008). The seeds that were in the central region of the whorl showed the significantly lower size and dry weight in comparison to the outer region (Gupta *et al.*, 2009). In the case of oil palm development, fruitlets located closer to the stalk will experience more spatial restriction due to the bunch position in between two fronds, thus reducing sunlight exposure (pigmentation), along with smaller, rounded fruitlets and seeds (Figure 4).

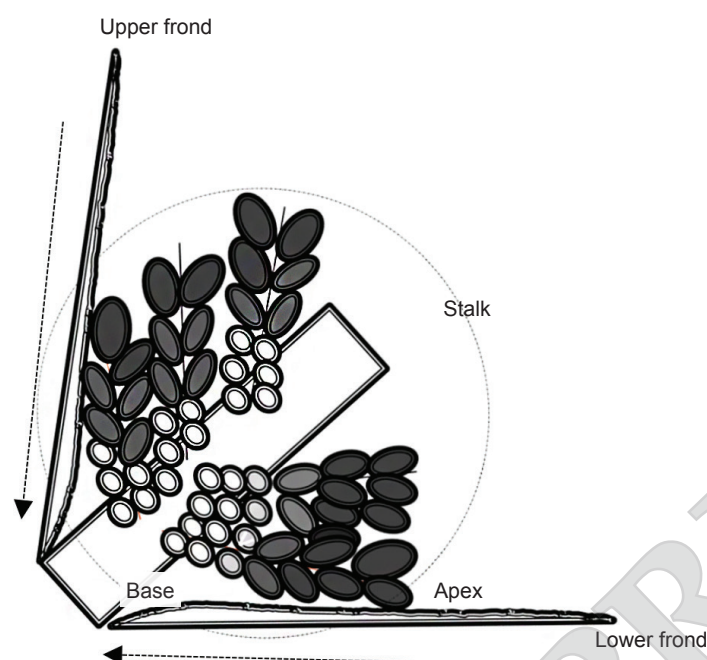
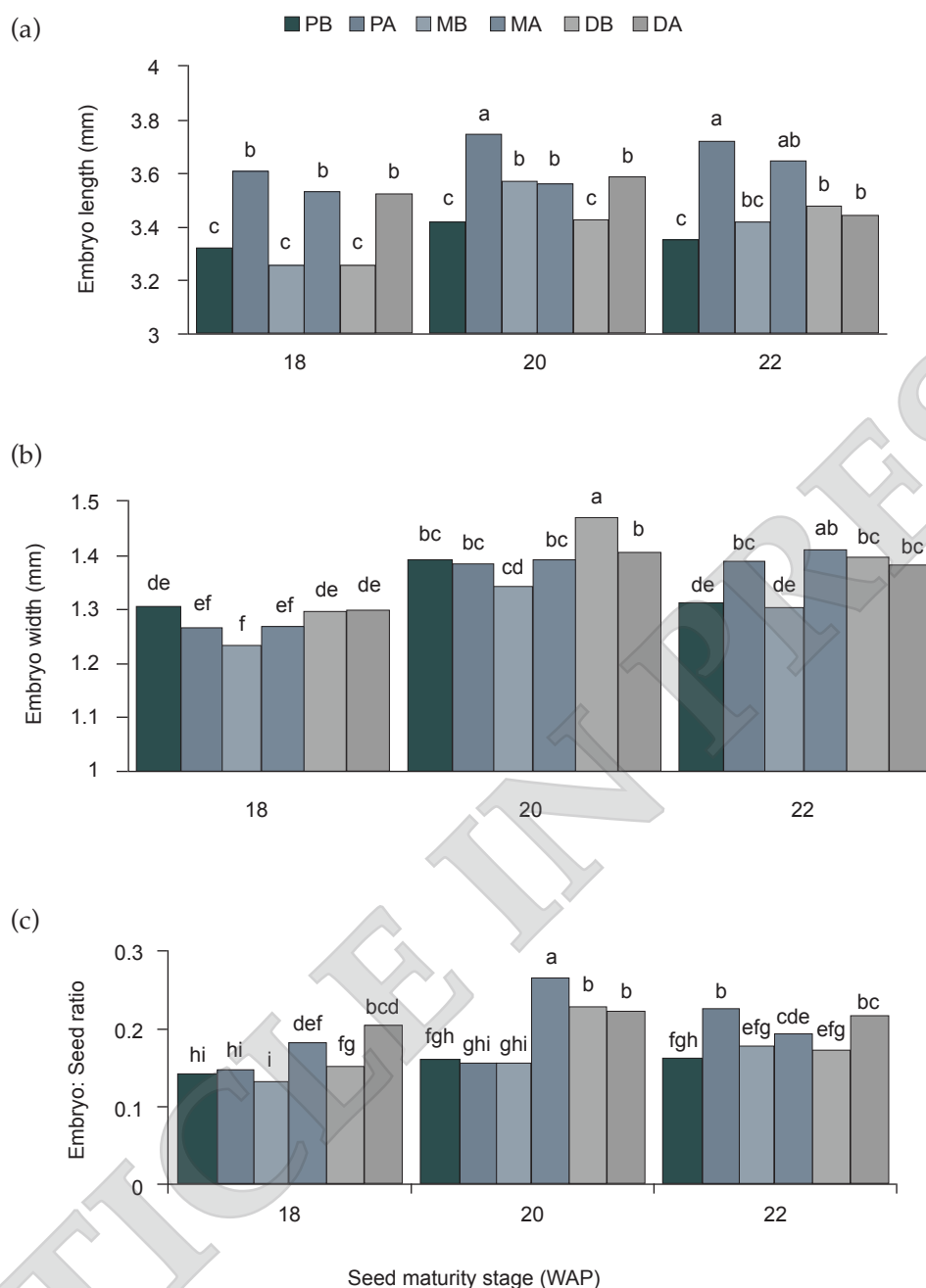


Figure 4. Diagram illustrating the seed developmental differences on seed endocarp colour and characteristics, based on its position in the spikelet and the bunch position in between two fronds. Arrow indicates the direction of increasing spatial restriction, thus resulting in smaller, rounded and less pigmented seeds at the base.

Seed producers generally harvest D×P bunches between 18 to 20 WAP, to ensure that fruitlets are still attached to the bunch and prevent loss from natural abscission (SDSAS 2020). Based on the seed endocarp colour assessment, it was evident that the white-coloured seeds gradually decreased with the maturity stages. This shows that natural pigmentation is still happening, even on the base section of the bunch. However, the process is slower due to the lower sunlight exposure as explained above. For this reason, the differences in seed endocarp colour could be due to natural developmental process and this only affected the external characteristics of the seed. Therefore, delaying the harvesting would greatly increase the percentage of black seeds, which translates to more saleable seeds since the white seeds are often perceived as being abnormal and mainly discarded during production (SDSAS, 2020). Thus, from the seed endocarp colour perspective, it would be advantageous for seed producers to harvest the seeds at 22 WAP. However, it is important to note that it also would significantly increase the incidence of loose fruits, which may cause potential losses of seeds during harvesting.

In this study, the evaluation for both morphological and germination parameters was compared at 18, 20 and 22 WAP; with a total of 6 weeks of evaluation. If we consider the whole oil palm seed developmental process from pollination to harvesting (approximately 20 weeks after pollination), the time of evaluation only

encompassed the final 2 weeks of the changes prior to and after the seed had reached the current standard harvesting stage. For this reason, most of the physical seed characteristics such as seed weight, seed length, seed width and operculum diameter were not significantly different, as the seed had already attained the maximum mass maturity at this point. It was reported that between 13 and 15 weeks after anthesis; the oil palm seed endosperm cavity had fully solidified, with the presence of an approximately 3 mm cylinder-shaped embryo underneath the operculum region (Kok *et al.*, 2013; 2015). In the case of sunflower seed, seed position was reported to influence the maturity as it happened progressively from the outer region towards the central part of the whorl (Gupta *et al.*, 2009). However, this is not the case for oil palm seeds. It was found that the embryo size increases with the seed maturity stage and is not influenced by the different seed positions. From the seed dormancy perspective, embryo development is often associated with both morphological and morpho-physiological dormancy attributes, having either rudimentary or underdeveloped embryos (Baskin and Baskin, 2014). Both embryo length and width showed up to 5% and 13% increment in size, from 18 to 20 WAP, respectively. If we look at the embryo-to-seed (E:S) length ratio, an average of 23% increment was recorded within 2 weeks of maturity, indicating an increase in embryo size in relation to the seed size (Figure 5).



Note: PB - proximal-base; PA - proximal-apex; MB - middle-base; MA - middle-apex; DB - distal-base; DA - distal-apex. Different letters indicate significant differences within each column, at a 5% probability level.

Figure 5. Interaction effect of seed maturity stages and seed positions on (a) mean embryo length, (b) mean embryo width and (c) mean Embryo: Seed (ES) Ratio.

An increasing E:S ratio is regarded as an indicator of morphological dormancy release, in seeds with underdeveloped embryos (Forbis *et al.*, 2002). For this reason, the germination was shown to be increasing, particularly from 18 to 20 WAP; with an average of 77.5% and 85.1%, respectively (Table 2). At 18 WAP, distal sections of the seeds had 5.0% higher germination as

compared to the proximal seeds, with 12% to 18% higher germination than the middle sections. Seeds that were harvested at 20 WAP showed a more uniform pattern, where no significant differences were observed at all seed sections (all germinated more than 83.0%) except for the proximal-base seeds; with 81.5 % normal germination.

TABLE 2. PERCENTAGES OF SEED GERMINATION AND VIABLE EMBRYO

Seed Stage	Seed Position	Mean seed germination (%)	Mean viable embryo (%)
18	PB	74.00bc	80.00d
	PA	79.50ab	87.50cd-e
	MB	67.50c	95.00a-c
	MA	73.50bc	100.00a
	DB	85.50a	95.00a-c
	DA	85.00a	97.50ab
20	PB	81.50ab	82.50d
	PA	86.50a	90.00b-d
	MB	84.00ab	97.50ab
	MA	87.00a	95.00abc
	DB	88.50a	100.00a
22	DA	83.00ab	100.00a
	PB	88.50a	95.00a-c
	PA	85.00ab	97.50ab
	MB	87.00a	95.00a-c
	MA	81.50ab	90.00b-d
	DB	72.00bc	85.00cd
	DA	71.50bc	95.00a-c

Note: PB - proximal-base; PA - proximal-apex; MB - middle-base; MA - middle-apex; DB - distal-base; DA - distal-apex. Different letters indicate significant differences within each column, at a 5% probability level.

In contrast, 22 WAP seeds that were located at the distal sections of the bunch, had recorded significantly lower germination (less than 72%), in comparison to the proximal and middle sections. The percentage of abnormal and diseased seeds was less than 5% throughout all seed maturity stages and seed positions. In addition, the TZ test conducted in this study clearly showed that the differences between the estimated germination potential with actual germination had decreased from 18 WAP to 22 WAP, suggesting a reduction in the depth of primary dormancy in the seed (Baskin and Baskin, 2014; Flores *et al.*, 2011; Soares *et al.*, 2016; Vankus, 1997). At 18 and 20 WAP, seeds located at the middle and distal sections of the bunch (for both the apex and base section) showed more than 95% viable embryos. In contrast, the proximal-base and proximal-apex regions had recorded less than 90% embryo viability. However, at 22 WAP, no differences in embryo viability were recorded as all the seed positions had resulted in higher than 90% embryo viability, except for the distal-base section. This scenario showed that earlier harvesting even for only two weeks, could significantly increase the percentage of dormant seeds within the bunch.

The germination was not significantly different for seeds located at different positions, despite the clear morphological differences observed, particularly between the base and apex sections of the spikelets. However, the difference in seed morphology was not reflected in the germination parameters. Referring to *Table 2*, while the germination was different among some of the seed positions at 18 WAP (for example the distal sections had 5% higher germination as compared to the proximal seeds and 12% to 18% higher than the middle sections), however as the seeds undergo further maturation into 20 WAP, germination was found to be more uniform across different positions. Indeed, variation was observed for germination percentage and mean germination time; however, there was no direct correlation between the morphological characteristics with germination. It is possible that at 20 WAP, the seeds exhibit similar physiological composition across different sections of the bunch. For instance, the oil palm has identical endogenous hormones or enzyme concentrations that are required for germination, such as GA, ABA and α -amylase (Baskin and Baskin, 2014). The intrapopulation variation observed in this study

can also be explained from a seed evolutionary point of view. As we know, the seed is formed as a means of reproduction and survival for all angiosperms. There is a concept in the evolutionary study referred to as 'bet hedging', where the plant increases its chance of survivability in an uncertain environment by having a range of phenotypic characteristics (Slatkin, 1974). From the ecological context, this strategy is indeed advantageous as it serves as an adaptive mechanism towards any changing environmental conditions by having staggered seed germination, or seedling establishment in a population (Gremer and Venable, 2014; Simons, 2009). However, in agricultural production, this strategy is termed disadvantageous as it reduces uniformity, which ultimately results in suboptimal crop establishment and makes crop management more challenging (Finch-Savage and Bassel, 2016).

CONCLUSION

Harvesting at 20 WAP resulted in the highest germination percentage, with more uniform germination across different sections within the bunch. In addition, there was also an increase in the percentage of black-coloured seeds, in comparison to semi-white and white seeds, rendering harvesting at 20 WAP should increase the number of saleable seeds for the producers. Seed positions had shown significant effects on morphological characteristics such as seed weight, length and width. The base region of the spikelets exhibits smaller, rounded and more white-coloured seeds in comparison to the apex region, regardless of its position within the bunch. The structure of oil palm inflorescences was found to influence the morphological differences observed, due to the spatial variation during development. Despite the prominent variations within the bunch, these morphological differences did not correlate with the seeds' germination. Therefore, harvesting and processing the whole bunch at 20 WAP is recommended to the seed producers, based on the germination and seed endocarp colour evaluation.

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