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A MODEL FOR PREDICTING FLOWER DEVELOPMENT IN *Elaeis guineensis* Jacq.

NORASHIKIN SARPAN*; SAU-YEE KOK*; SOOK-KEAT CHAI; ANWAR FITRIANTO‡; AZIMI NURAZIYAN*; ISHAK ZAMZURI*; MEILINA ONG-ABDULLAH* and SIEW-ENG OOI***

ABSTRACT

The proper development of oil palm fruit is important as the source of oil is the fruit mesocarp and kernel. Prior to fruit formation, the development of flowers is therefore also important. Determination of the flower development stages in oil palm generally involves tedious histological analyses of each sampled inflorescence, making it a costly and inefficient way of gauging the developmental state. In this study, a statistical model was established from the association of physical or macroscopic measurement data to flower development, which was determined via histological analyses. The final reduced ordinal logistic regression model is a partial proportional odds model that uses inflorescence length and palm age as predictors to predict the flower development stage. The likelihood-ratio χ^2 test suggested the model adequately fits the data ($p < 0.01$). The model, with a prediction accuracy of 78.5%, can be used for selecting inflorescences of specific development stages from palms aged three to 10 years of field-planting. These stages can be further verified by histological analyses. This lowers the overall costs and time by reducing the number of samples requiring histological analysis prior to downstream studies.

Keywords: flower development, histology, ordinal logistic regression.

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INTRODUCTION

Oil palm is one of the most efficient oil producing crops in the world (Murphy, 2014). The most important part of oil palm is undoubtedly the fruit, from which the oil is extracted. This leads to the importance of studying flower development in oil palm as proper flower development would be crucial to fruit development.

* Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43400 Kajang, Selangor, Malaysia.
E-mail: oseng@mpob.gov.my

** Department of Cell and Molecular Biology,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia.

‡ Department of Mathematics, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

Being a monoecious plant, oil palm produces the male and female inflorescences on the same palm. Inflorescences are formed in the axils of their subtending fronds. Each inflorescence is enclosed within a prophyll and peduncular bract (Corley and Tinker, 2003; Adam *et al*., 2005; Montoya *et al*., 2014; Mgbeze and Iserhienrhien, 2014). A female inflorescence can bear several hundreds to thousands of florets. The inflorescence can range from less than 1 cm to more than 30 cm in length and the flower development stage of most inflorescences is generally indistinguishable at the macroscopic view. In-depth microscopic anatomical analysis of oil palm flower development indicated five key developmental stages (Adam *et al*., 2007; 2005). These stages encompass the formation of the floral meristem up to the development and maturity of the floral reproductive organs.

An attractive alternative for the propagation of elite oil palm planting materials is by clonal

propagation. However, one of the primary risks of clonal propagation is the production of a homeotic variant called mantled (Corley *et al*., 1986; Konan *et al*., 2010; Jaligot *et al*., 2011). Fruit development is usually aborted in severe mantling. A mantled fruit contains several supernumerary carpels that surround the main carpel due to the transformation of the stamens and staminodes into these carpel-like structures (Adam *et al*., 2005). The floral triad initiation in a mantled flower is similar to that in normal flowers and no difference is seen up till the reproductive organ initiation stage.

Generally, the current practice for selecting target flower development stages for downstream experiments would be to conduct anatomical via histological analysis of inflorescences sampled from each palm (Beulé *et al*., 2011). As the palm ages, the fruit bunch size also changes. Planters generally use a forecasting system that takes into account the palm age to predict total production (Loh and Sharma, 1999; Yong and Wong, 2012). Fruit production from young palms (four to nine years from planting) depends more on the bunch numbers, while in older palms (more than eight years) yield comes more from increased bunch weight (von Uexkull and Fairhurst, 1991). As the age of the palm has an influence on the size of the bunch and therefore the inflorescence sizes as well, histological staging of inflorescences sampled from palms of different ages would also be necessary to determine their stage of flower development.

The objective of this study was to generate a model to estimate the flower developmental stage of inflorescences sampled from normal and mantled clonal palms based on macroscopic evaluations. Histological analysis of these inflorescences enabled the categorisation of the inflorescences into the five key flower developmental stages based on Adam *et al*. (2005). Together with the macroscopic measurements of these inflorescences, statistical analysis was conducted to generate a model to predict the developmental stage of a sampled inflorescence. This would allow an estimation of the flower developmental stage of inflorescence samples based on their macroscopic data.

MATERIALS AND METHODS

Plant Materials

Normal and mantled clonal palms were selected for sampling from MPOB and the Rubber Industry Smallholders Development Authority (RISDA) research stations in Perak, Malaysia. Inflorescence samples were obtained from a total of 31 clonal palms that were of the *tenera* variety with ages ranging from 3 to 10 years of field-planting. These inflorescence samples were processed for histological analysis. Inflorescences had also been previously sampled from 85 clonal palms (normal and mantled) from various MPOB research stations across Malaysia. The lengths, subtending frond numbers and sexes of all sampled inflorescences were recorded.

Histology

Histological analysis was conducted on 172 female inflorescences of lengths more than 1 cm. Histological staining was conducted according to Fisher (1968) with minor modifications. Briefly, inflorescence samples were fixed for two days at room temperature in fixation buffer [10% paraformaldehyde, 25% glutaraldehyde, 0.5 g (w/v) caffeine, 0.2 M phosphate buffer pH 7.2]. For inflorescences with lengths of more than 2 cm, individual spikelets were dissected from the inflorescence rachis, cut into lengths of 0.5 cm or smaller and fixed. Fixed tissues were then dehydrated through a graded ethanol series - 30% ethanol for 30 min, 50% ethanol for 45 min, 70% ethanol for 45 min, 80% ethanol for 1 hr, 90% ethanol for 1 hr, 95% ethanol for 1 hr and twice in 100% ethanol for 1 hr each. Softer tissues were obtained by incubating samples in 100% butanol for a minimum of 24 hr which was repeated twice, and subsequently in a mixture of 1:1 ratio of Technovit®7100 impregnation solution (Heraeus Kulzer, Germany): 100% butanol for a minimum of two days. Following that, samples were infiltrated with Technovit impregnation solution for at least one week before embedding in Technovit®3040 resin (Heraeus Kulzer, Germany). The Technovit impregnation and embedding solutions were prepared according to the manufacturer's instructions (Heraeus Kulzer, Germany). Blocks were then sectioned at 5 μm thickness using a Leica microtome (Leica, Germany). Slides were stained in Periodic acid-Schiff reagent for 20 min and Naphthol blue black at 60°C for 5 min. Distilled water (pH 4.5) was applied in between staining steps to wash off excess dye. Periodic acid-Schiff specifically stains polysaccharide (starch reserves and walls) while Naphthol blue-black specifically stains soluble or reserve proteins blue-black in colour (Fisher, 1968). Images of the stained sections were then viewed and photographed with a camera attached to a LEICA DM6000 B light microscope (Leica, Germany) and processed with the Progress Research Pro software (Leica, Germany).

Statistical Analysis

Statistics was conducted using Genstat 17th Edition (VSN International, 2014) and Stata 14.0 (StataCorp, 2015). Kendall's tau correlation analysis was done to check the relationships among the variables frond number, inflorescence length and age of sampled palm. For statistical modelling, ordinal

logistic regression was used as the dependent variable (flower development stage) could be ranked. The gologit2 program was used in Stata 14.0 to estimate generalised ordered logit models for ordinal dependent variables (Williams, 2006).

RESULTS

Inflorescence Sampling and Macroscopic Data Measurements

A total of 760 inflorescences were sampled from 15 normal clonal palms and 16 mantled clonal palms, aged 3, 5 and 10 years. The sampled mantled palms produced fruits with six to eight supernumerary carpels (*Figure 1*). Numbering attributed to the inflorescences corresponded to that of their subtending fronds (Adam *et al.*, 2005). Frond numbering was done as explained by Adam *et al*. (2005), however with the first fully opened leaf numbered as +1 (Breure, 2003). Inflorescence lengths, their subtending frond numbers and sexes were recorded (*Table 1*). Lengths were only measured for inflorescences longer than 0.1 cm, with prior removal of the prophyll and peduncular bract before measurement was done. Histological analyses were conducted on 172 female inflorescences of more than 1 cm in length. Generally, sex of inflorescences with lengths less than 1 cm could not be visually determined.

For each sampled palm, the subtending fronds were removed prior to the removal of the inflorescences at their bases. The outer prophyll of the inflorescence was removed, followed by the bracts. The female inflorescence is a compound rachis composed of approximately 150 rachillae (Adam *et al*., 2005). Each rachilla bears 5-30 floral triads for a female inflorescence. The length of the inflorescence was then measured from the base where its lowest rachilla was located till the tip of the inflorescence (*Figure 1G*). For inflorescences longer than 2 cm, several rachillae were excised and immersed into fixative solution for histology. For inflorescences longer than 25 cm, individual florets were removed and fixed. Only the female inflorescences were analysed in this study. Depending on the age of the palms, the inflorescence length for each stage also varied. The differences in inflorescence length from one stage to the next appeared more distinct in older palms (*Figure 2*).

Histological Analysis and Flower Developmental Stage Categorisation

Histological analyses were conducted for the inflorescences sampled from the 31 palms, amounting to a total of 172 female inflorescences. Based on their histological observations, the inflorescences could be categorised into the key developmental stages as defined by Adam *et al.* (2005). Each category or stage comprised of defined anatomical characteristics (*Figure 3*). Based on histology, some of the inflorescences were at a development state between the defined flower stages and as such, were excluded from the generation of the predictive regression model. However, these inflorescences were used instead for testing of the predictive model. The first key flower development stage involved the presence of the floral meristem, which was observed as a cluster of actively dividing and cytoplasmic-rich cells that were stained dark-blue by Naphtol blue-black. The second stage showed the development of the floral triad, comprising of the floral meristem flanked by two perianth organs which gave rise to the accompanying staminate flowers. Several sections may have to be searched as sometimes only the floral meristem flanked by one perianth organ

Figure 1. Inflorescences and fruits from normal and mantled palms. A: Inflorescence (indicated by arrow) located between chopped subtending frond (sf) and trunk of palm. B: Normal fruit bunch. C: Mantled fruit bunch. D: Normal fruit (N) and mantled fruit (M). E: Cross-sectioned mantled fruits (M) and normal fruit (N). F: Inflorescence still enclosed within the prophyll and bracts. G: Female inflorescences after removal of prophyll and bracts. Red arrow indicates length of the inflorescence. H: Mantled florets. sc - Supernumerary carpels; asf - accompanying staminate flower.

TABLE 1. DATASET ON INFLORESCENCE SAMPLES FROM 31 PALMS USED FOR STATISTICAL MODELLING

Floral stage	Palm age (years)	Frond number	Inflorescence length (cm)	Normal (N) / mantled (M)	Floral stage	Palm age (years)	Frond number	Inflorescence length (cm)	Normal (N) / mantled (M)
1	5	5	2	N	3	5	11	7.8	N
1	5	6	2	N	3	5	12	8	N
2	10	11	4.8	N	3	5	13	8.5	N
2	10	15	6.6	N	3	5	10	4	N
2	10	14	6.2	N	3	5	8	5	N
2	10	13	5.7	N	3	5	12	5	N
2	10	14	5	N	3	5	10	4.5	N
2	5	5	2	N	3	5	11	4.5	N
2	5	5	2.1	N	3	3	15	4.2	N
2	5	10	4	N	3	3	16	3.1	N
2	5	11	4	N	3	3	20	6.5	N
2	5	4	2.5	N	3	3	11	4.3	N
2	5	5	3	N	3	3	12	2.3	N
2	5	6	2.5	N	3	3	14	3	N
2	5	7	2.5	N	3	3	17	5	N
2	5	11	4.5	N	3	3	15	5	N
2	5	8	2.5	N	3	3	21	3.5	N
2	5	9	2.5	N	4	10	14	12	N
2	5	10	4	N	4	10	20	15.5	N
2	5	7	2.6	N	4	10	17	14.7	N
2	5	8	2.8	N	4	10	17	20	N
2	5	9	3.5	N	4	5	14	12	N
2	3	17	1.2	N	4	5	15	14	N
2	3	19	1.5	N	4	5	14	16	N
2	3	4	1.3	N	4	3	18	7.5	N
2	3	9	0.7	N	4	3	16	8.5	N
2	3	11	0.7	N	4	3	22	5.5	N
2	3	19	1.8	N	4	3	23	3	N
2	3	20	1.5	N	5	10	15	23	N
3	10	13	9	N	5	10	21	27	N
3	10	15	8	N	5	10	18	25.5	N
3	10	12	9.5	N	5	5	14	17.5	N
3	5	11	4.5	N	5	5	15	24	N
3	5	12	6.5	N	5	5	16	29	N
3	5	13	9	N	5	5	13	13	N
3	5	12	4.5	N	5	5	14	15	N
3	5	13	9	N	5	5	15	29.5	N
5	5	16	26	N	2	3	16	3	M
5	3	19	11.3	N	2	3	8	1.1	M
5	3	17	21.5	N	2	3	13	1.6	M
5	3	19	26	N	3	10	14	14	M
5	3	22	24	N	3	10	15	14	M
1	5	4	1.8	M	3	10	14	12	M

TABLE 1. DATASET ON INFLORESCENCE SAMPLES FROM 31 PALMS USED FOR STATISTICAL MODELLING (continued)

Floral stage	Palm age (years)	Frond number	Inflorescence length (cm)	Normal (N) / mantled (M)	Floral stage	Palm age (years)	Frond number	Inflorescence length (cm)	Normal (N) / mantled (M)
1	5	3	2.2	M	3	10	14	9.2	M
1	3	5	1	M	3	10	16	9.5	M
1	3	4	1.2	M	3	5	10	5	M
1	3	12	1.5	M	3	5	11	6.5	M
2	10	11	5	M	3	5	10	6	M
2	10	13	9.8	M	3	5	9	3	M
2	10	13	6.8	M	3	5	10	4	M
2	10	12	5.5	M	3	5	11	5.2	M
2	10	13	7.4	M	3	5	11	5.5	M
2	10	12	5	M	3	3	17	4.5	M
2	10	11	6.5	M	3	3	18	5	M
2	10	10	5	M	3	3	14	4	M
2	10	14	9.3	M	3	3	15	5.5	M
2	10	12	6.2	M	3	3	18	7	M
2	5	4	2	M	3	3	19	4.3	M
2	5	12	4.3	M	3	3	15	3.5	M
2	5	8	4.1	M	3	3	16	4.5	M
2	5	5	2.1	M	3	3	17	5.5	M
2	5	6	2.4	M	4	10	15	20	M
2	5	7	2.5	M	4	10	16	29	M
2	5	6	2.5	M	4	10	15	16.6	M
2	5	7	2.1	M	4	10	15	14.5	M
2	5	8	3	M	4	10	13	17.5	M
2	5	9	3	M	4	10	16	26.5	M
2	5	8	3.4	M	4	5	13	13	M
2	5	9	3	M	4	5	11	10.5	M
2	5	5	2.4	M	4	5	12	12	M
2	5	6	2.4	M	4	5	12	14.5	M
2	5	8	2.4	M	4	5	13	16	M
2	5	9	2.4	M	4	3	19	9	M
2	5	10	5	M	4	3	20	12	M
2	3	16	3	M	4	3	21	14.5	M
2	3	12	2.5	M	4	3	16	8	M
2	3	13	3	M	4	3	17	12	M
2	3	15	2.5	M	4	3	18	19.5	M
4	3	19	14	M	5	5	14	23	M
4	3	20	11	M	5	5	15	34	M
4	3	21	15	M	5	5	15	26.5	M
4	3	22	10	M	5	5	13	25.5	M
4	3	20	13.5	M	5	5	14	17	M
4	3	18	9.5	M	5	3	21	18	M
4	3	19	12	M	5	3	22	26	M
5	10	17	25.5	M	5	3	21	24.5	M

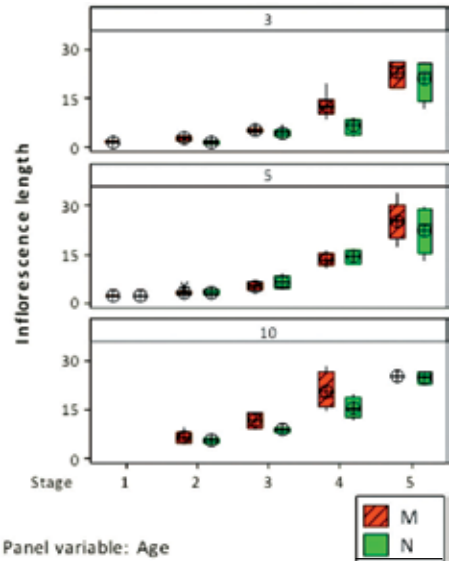

Figure 2. Inflorescence length ranges for each flower stage based on palm age and phenotype. Numbers on top of each panel represent palm ages (3, 5 and 10 years of field planting). Symbols ⊕ in the middle of the box represent mean values. Phenotype: normal (N); mantled (M). Boxplot generated by Minitab® 16.2.4.

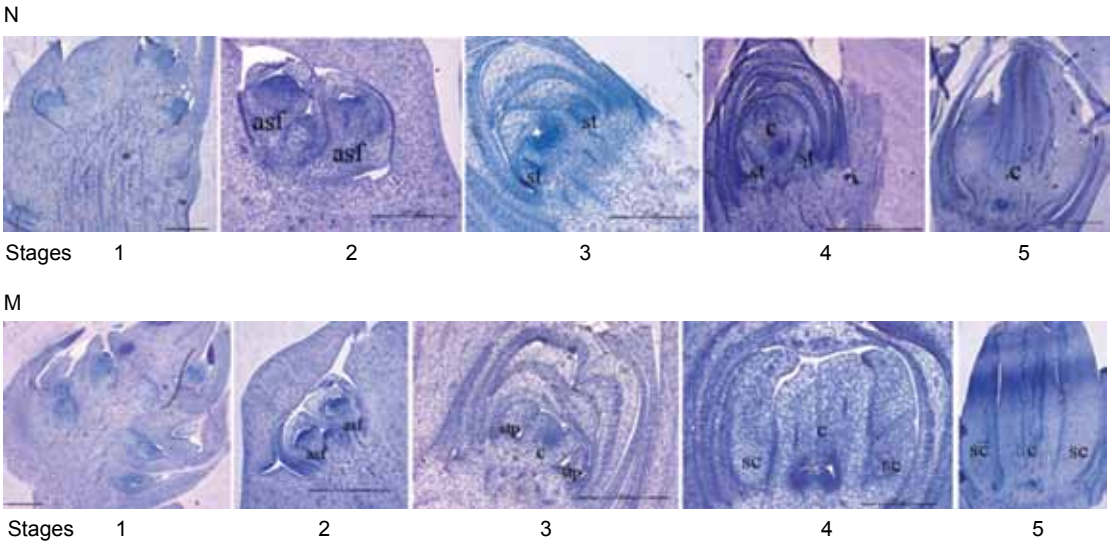

Figure 3. The five key stages of flower development in oil palm. Flower developmental stages from normal (N) and mantled palms (M). Bar = 400 µm. asf - Accompanying staminate flower, st - staminodia, c - carpel, stp - staminodia primordia, sc - supernumerary carpel.

could be seen. This is due to the angle of the tissue in the mould when sectioned. Stage 3 involved the development of the perianth organs and the initiation of the reproductive organs. At this stage, several layers of tepals, staminodium and gynoecium were seen. The staminodium was observed developing next to the gynoecium. Generally, the accompanying staminate flowers were no longer seen flanking the pistillate flower as their peduncles have elongated and were therefore located above the pistillate flower (Adam *et al*., 2005). Floral anatomy at stages 1 to 3 was quite indistinguishable between the normal and mantled inflorescences. Only at stage 4 onwards, the elongation of the carpel in normal inflorescences was flanked by developmentally halted staminodes (rudimentary stamens), but in mantled inflorescences, the formation of carpel-like structures or supernumerary carpels in place of the staminodes was observed. At stage 5, the pistillate flower contained a fully formed ovule.

The inflorescences were thus categorised into stages 1 to 5 according to their histological observations. The staging, together with their inflorescence lengths, ages of the palms they were

sampled from and fruit phenotype of the palm (*Table 1*) were used as the training data set for the generation of the statistical prediction model.

Statistical Analysis and Predictive Model

For the inflorescence dataset of the 31 palms of which histological staging was conducted, inflorescence length and flower developmental stage were highly correlated with a Kendall's tau correlation coefficient (τ) of 0.7314 (p value = 0.0000). Although frond numbers were moderately correlated to developmental stage (τ = 0.5432; p value = 0.0000), it was also moderately correlated to inflorescence length as well (τ = 0.4763; p value = 0.0000). Moreover, the correlation between the frond numbers and inflorescence length was higher on the entire dataset that included another 85 sampled palms, with an overall τ correlation coefficient of 0.6851 (*Table 2*). Three-year old palms portrayed higher frond numbers for the same inflorescence lengths compared to the older age groups (*Figure 4*).

As the flower developmental stages or categories could be ranked, ordinal logistic regression analysis was conducted with the age, inflorescence length and phenotype data as predictors, from the dataset of 31 palms. Once we obtained the final reduced model, the necessary assumptions for the model were checked. However, the proportional odds assumption for ordinal logistic regression was violated. Hence, a generalised ordered logit model was fitted as an alternative to model the dependent and predictor variables. This analysis was conducted using the gologit2 routine in Stata14.0.

In this model, frond number was not included as a predictor in the model as it was moderately correlated to inflorescence length. In addition, inflorescence length exhibited a higher Kendall's tau correlation coefficient to flower development stage. Results of the full model indicated that the fruit phenotype and interaction between inflorescence length and age did not have a significant contribution to the flower stage. The test of proportional odds assumption under gologit2 indicated that the assumption was violated by the variables age (p = 0.01969) and inflorescence length (p = 0.00001). However, gamma parameterisation indicated that the gammas for the age predictor were statistically insignificant and it may thus be safe to impose the proportionality constraint on this predictor variable. Therefore, a partial proportional model and a non-proportional odds model were generated, with

TABLE 2. CORRELATION OF INFLORESCENCE LENGTH WITH FROND NUMBERS FOR DIFFERENT PALM AGE GROUPS

Age (years)	Total No. of palms	Total No. inflorescences	Kendall's tau, τ (p-value)
23	19	237	0.8242 (0.000)
17.5	34	265	0.7691 (0.000)
10	12	220	0.8892 (0.000)
7.5	26	275	0.7617 (0.000)
5	14	264	0.8767 (0.000)
3	11	252	0.7570 (0.000)
Overall	116	1 513	0.6851 (0.000)

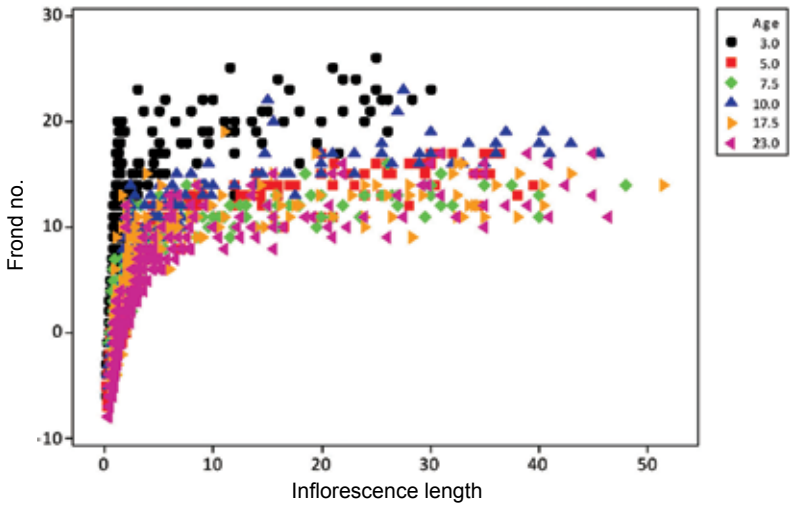

Figure 4. Association between frond numbers and inflorescence length with increasing age. n= 116 palms.

different coefficients for the predictors at each flower stage. The partial proportional odds model was more parsimonious in that proportionality was not imposed on the inflorescence length predictor, but imposed on the age predictor. Comparison of these two models suggested that the models did not differ much from each other (*Table 3*). The BIC statistic favoured the partial proportional odds model while the AIC statistic leaned towards the non-proportional odds model. However, the non-proportional odds model generated 21 cases of negative predicted probabilities out of the 172 cases, which suggested some flaw in the model. The partial proportional odds model did not generate any cases of negative predicted probabilities. Thus, regression analysis showed that the final reduced partial proportional odds model with two predictors (inflorescence length and palm age) was statistically reliable in distinguishing the flower stage categories (p value < 0.05) (*Table 4*). The estimated ordinal logistic regression equation of the final reduced model is:

$$P(Y_i > j) = \frac{\exp(\hat{\beta}_{0i} - 1.058Age + \hat{\beta}_{1j} Length)}{1+\exp(\hat{\beta}_{0j} - 1.058Age + \hat{\beta}_{1j} Length)}, \text{ j=1,2,3,4:}$$

where j is the flower stage category, $\hat{\beta}_{0j}$ and $\hat{\beta}_{1j}$ are the intercepts (constant) and logit coefficients for length respectively, in the 'Coefficient' column of *Table 4*. The generalised ordered logit model generates j-1 sets of parameter estimates for a series of binary logistic regressions where categories of the dependent variable are combined (Williams, 2006).

The likelihood-ratio χ^2 analysis suggested the model adequately fits the data (p value < 0.01). The regression coefficients are presented in *Table 4*. Both predictors were found to contribute significantly to the flower stage. Testing of the model back on the training dataset of 31 palms returned a prediction accuracy of 78.5%. The model equation indicated that the inflorescence length has a positive relationship with a higher ordered flower stage while age has a negative relationship, as indicated by the signs of their estimated regression coefficients.

We tested the model on predicting the flower developmental stage of four inflorescence samples, using their corresponding sampled palm ages and their inflorescence lengths as predictors (*Table 5*). The flower developmental stage of samples U1, U3 and U4 were predicted to be most likely at stage 2 while sample U2 was predicted to be most likely at stage 3. Histological analyses of these samples indicated that the flower stages of all four inflorescences to be between stages 2 and 3 (*Figure 5*).

DISCUSSION

Studies on oil palm flowering require sampling of inflorescences that are located at the base of the fronds, usually embedded within the trunk. Inflorescences larger than 25 cm are usually at the later stage 5 of flower development. Generally, for inflorescences of lengths smaller than 25 cm, it is difficult to morphologically determine their flower stage. Anatomical analysis would be required to determine the flower stage of these inflorescences (Beulé *et al*., 2011). To reduce the tediousness of anatomical analyses, this study was conducted to generate a predictive model that would help to narrow down the number of samples for anatomical analysis when targeting a specific flower stage.

Staging of the inflorescences based on their histological observations was conducted by the identification of several characteristics (Adam *et al*., 2005), as detailed in the Results section. The most difficult flower development stage to determine is usually stage 3, as the transition between stages 2 to 4 occurs quite rapidly in a palm.

In clonal palms, mantling sometimes occurs which results in the formation of supernumerary carpels on the fruits. In a mantled fruit bunch, the extra carpels may probably influence the overall size of the fruit bunch and perhaps in relations to that, the inflorescence length prior to fruit development. However, the fruit phenotype (normal/mantled) of the sampled palm did not contribute significantly to the flower development stage dependent variable in the full regression model.

TABLE 3. COMPARISON OF THE MODELS GENERATED BY GOLOGIT2

Model	Observations	df	AIC	BIC	Likelihood-ratio χ^{2*}	Pseudo-R2
Proportional odds	172	6	223.21	242.09	285.42	0.5747
Non-proportional odds	172	12	188.64	226.41	331.99	0.6685
Partial proportional odds	172	9	192.99	221.32	321.63	0.6476

Note: * p<0.01.

TABLE 4. GENERALISED ORDERED LOGIT MODEL FOR FLOWER DEVELOPMENT STAGE BASED ON AGE AND INFLORESCENCE LENGTH

Flower stage	Predictor	Coefficient	SE coefficient	Z-score	*p*-value
1	Age	-1.058171	0.1610606	-6.57	0.000
	Length	2.791197	0.6269517	4.45	0.000
	Constant	0.8389767	1.156791	0.73	0.468
2	Age	-1.058171	0.1610606	-6.57	0.000
	Length	1.450985	0.2098531	6.91	0.000
	Constant	-1.190313	0.7095598	-1.68	0.093
3	Age	-1.058171	0.1610606	-6.57	0.000
	Length	1.10248	0.1651023	6.68	0.000
	Constant	-4.705674	1.171882	-4.02	0.000
4	Age	-1.058171	0.1610606	-6.57	0.000
	Length	0.6590793	0.1079739	6.10	0.000
	Constant	-6.322021	1.509297	-4.19	0.000

Log-likelihood = -87.496548.
Likelihood-ratio χ^2 = 321.63 (p = 0.0000)
Pseudo-R^2 = 0.6476.

TABLE 5. FLOWER STAGE PREDICTION FOR INFLORESCENCES BASED ON AGE AND INFLORESCENCE LENGTH DATA

Inflorescence sample	Age of palm	Frond No.	Length (cm)	Pheno-type*	Event probabilities for stages (*p*-value)				
					1	2	3	4	5
U1	10	12	6.5	M	0.0002 (0.72)	0.9120 (0.00)	0.0875 (0.09)	0.0003 (0.38)	3.31e-06 (0.58)
U2	10	14	9.0	M	2.10e-07 (0.81)	0.2164 (0.10)	0.7790 (0.00)	0.0046 (0.24)	1.72e-05 (0.52)
U3	10	12	7.0	N	5.57e-05 (0.74)	0.8341 (0.00)	0.1654 (0.06)	0.0005 (0.35)	4.60e-06 (0.57)
U4	10	11	7.0	N	5.57e-05 (0.74)	0.8341 (0.00)	0.1654 (0.06)	0.0005 (0.35)	4.60e-06 (0.57)

Note: * M - mantled; N - normal.

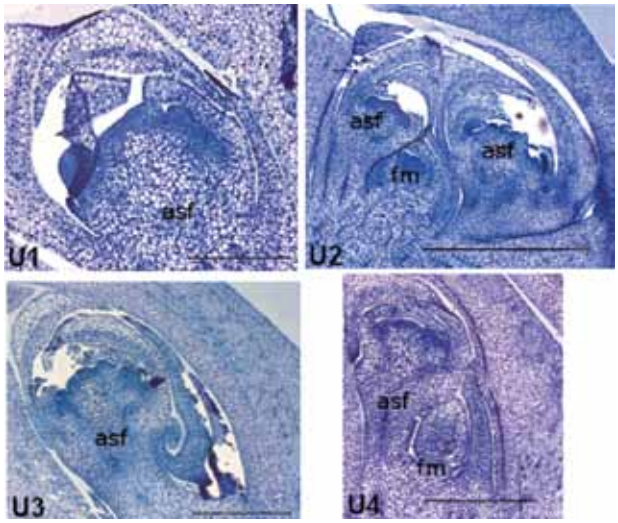

Figure 5. Histological observations from inflorescences U1 to U4. Bar = 400 µm. asf - Accompanying staminate flower, fm - floral meristem.

Inflorescences in younger palms, such as three-year old palms, exhibited smaller inflorescences at similar frond numbers as older palms. This is supported by observations that the fruit bunch sizes in young palms are smaller (Huth *et al*., 2014). This indicated that the maturity of flowers in younger palms is associated with inflorescences of higher frond numbers compared to older palms. Hence, age of the sampled palm influences the size of the inflorescences.

The options in the statistical modelling for the data in this study was limited to non-parametric tests as the dependent variable was categorical. Initially, two approaches were suitable, *i.e.* logistic regression or discriminant analysis. Discriminant analysis is able to predict group membership from a set of predictors (Tabachnick and Fidell, 1996). Assumptions for discriminant analysis would need to be met, such as for the independent variables

to be normally distributed, linearly related and equality of variance-covariance within the group. The inflorescence length data is of a non-normal distribution. Data transformation would be required before discriminant analysis can be carried out as an alternative modelling approach. Various transformations were attempted on the inflorescence length data but only Johnson transformation could generate normally distributed data from it. However, using the transformed data still did not meet the assumptions for parametric tests. Hence, ordinal logistic regression was conducted instead.

The final reduced generalised ordered logit model generated in this study indicated that both the inflorescence length and age of the sampled palm contributed significantly to the flower stage determination of the sample. Moreover, the model was used to predict four inflorescence samples based on their lengths and age of their respective sampled palms. The estimated flower development stage of the inflorescence was close to the true stage determined by histological analysis. Although this was a small test set, this indicated that the model may be useful for the prediction of flower development stage for palms within the range of three to 10 years of age. Thus, this predictive model based on age of sampled palm and inflorescence length can be used to estimate the flower developmental stage of sampled inflorescences.

CONCLUSION

The generalised ordered logit model generated from this study was able to predict the flower development stages of four inflorescence test samples based on the two predictor variables and the prediction was verified by histological analysis. Thus, this model would help in the selection of inflorescence samples for histological verification before proceeding to downstream experiments. This would therefore reduce the costs and time needed for extensive histological analyses prior to the selection of target inflorescence samples from every sampled palm.

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REFERENCES

ADAM, H; JOUANNIC, S; ESCOUTE, J; DUVAL, Y; VERDEIL, J L and TREGEAR, J W (2005). Reproductive developmental complexity in the African oil palm (*Elaeis guineensis*, Arecaceae). *Am. J. Bot.*, *92*: 1836 -1852.

ADAM, H; JOUANNIC, S; MORCILLO, F; VERDEIL, J L; DUVAL, Y and TREGEAR, J W (2007). Determination of flower structure in *Elaeis guineensis*: do palms use the same homeotic genes as other species? *Ann. Bot.*, *100*: 1-12.

BEULÉ, T; CAMPS, C; DEBIESSE, S; TRANCHANT, C; DUSSERT, S; SABAU, X; JALIGOT, E; SYED ALWEE, S S R and TREGEAR, J W (2011). Transcriptome analysis reveals differentially expressed genes associated with the mantled homeotic flowering abnormality in oil palm (*Elaeis guineensis*). *Tree Genet. Genomes*, *7*: 169-182.

BREURE, K (2003). The search for yield in oil palm: basic principles. *Oil Palm: Management for Large and Sustainable Yields* (Fairhurst, T H and Hardter, R eds.). PPI, PPIC and IPI, Singapore, Canada, Switzerland. p. 59 - 91.

CORLEY, R H V; LEE, C H; LAW, I H and WONG, C Y (1986). Abnormal flower development in oil palm clones. *Planter*, *62*: 233 - 240.

CORLEY, R H V and TINKER, P B (2003). *The Oil Palm*. 4th Edition, Blackwell Publishing Company, UK. p. 27.

FISHER, D B (1968). Protein staining of ribboned epon section for light microscopy. *Histochemie*, *16*: 92-96.

HUTH, N I; BANABAS, M; NELSON, P N and WEBB, M (2014). Development of an oil palm cropping systems model: lessons learned and future directions. *Environ. Model. Softw.*, http://dx.doi.org/10.1016/j.envsoft.2014.06.021.

JALIGOT, E; ADLER, S; DEBLADIS, É; BÉULE, T; RICHAUD, F; ILBERT, P; FINNEGAN, E J and RIVAL, A (2011). Epigenetic imbalance and the floral developmental abnormality of the *in vitro*-regenerated oil palm *Elaeis guineensis*. *Ann. Bot.*, *108*: 1453-1462.

KONAN, K E; DURAND-GASSELIN, T; KOUADIO, Y J; FLORI, A; RIVAL, A; DUVAL, Y and PANNETIER, C (2010). *In vitro* conservation of oil palm somatic embryos for 20 years on a hormone-free culture medium: characteristics of the

embryogenic cultures, derived plantlets and adult palms. *Plant Cell Rep.,* 29: 1-13.

LOH, H P and SHARMA, M (1999). Short-term yield forecasting using Ulu Bernam method. *Yield Potential and Forecasting Symposium*. Subang Jaya, Malaysia.

MGBEZE, G C and ISERHIENRHIEN, A (2014). Somaclonal variation associated with oil palm (*Elaeis guineensis* Jacq.) clonal propagation: a review. *African J. Biotech.,13*: 989-997.

MONTOYA, C; COCHARD, B; FLORI, A; CROS, D; LOPES, R; CUELLAR, T; ESPEOUT, S; SYAPUTRA, I; VILLENEUVE, P; PINA, M; RITTER, E; LEROY, T and BILLOTTE, N (2014). Genetic architecture of palm oil fatty acid composition in cultivated oil palm (*Elaeis guineensis* Jacq.) compared to its wild relative *E.oleifera* (H.B.K.) Cortés. *PLoS One, 9(5)*: e95412. DOI:10.1371/journal.pone.0095412.

MURPHY, D J (2014). The future of oil palm as a major global crop: opportunities and challenges. *J. Oil Palm Res.Vol. 26(1)*: 1-24.

TABACHNICK, B G and FIDELL, L S (1996). *Using Multivariate Statistics*. NY: Harper Collins.

VON UEXKULL, H R and FAIRHURST, T H (1991). *IPI Bulletin 12: Fertilizing for High Yield and Quality: The Oil Palm*. Intl. Potash Inst., Switzerland.

WILLIAMS, R (2006). Generalized ordered logit/ partial proportional odds models for ordinal dependent variables. *The Stata J., 6(1)*: 58-82.

YONG, K K and WONG, M K (2012). Statistical modeling of weather-based yield forecasting for young mature oil palm. *APCBEE Procedia*, *4*: 58-65.