

# Estimation of genetic parameters for *in vitro* oil palm characteristics (*Elaeis guineensis* Jacq.) and selection of genotypes for cloning capacity and oil yield



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## ABSTRACT

Oil palm has stood out among the promising species for biofuel production and plant improvement through cloning superior individuals is the technology that contributes most to increase oil production. However, there are no studies on the genotypes behavior regarding both *in vitro* performance and yield potential simultaneously. By this mean, the objective of this paper was to study the genetic control of the related characteristics to the oil palm cloning process, and to identify responsive genotypes within a collection of 32 elite materials of a commercial planting in order to select the superior genotypes for the formation of a clonal garden. Callogenesis and production of embryogenic lines, which are main characteristics related to the cloning process, presented genetic control, verified through estimates of genetic parameters: heritability, coefficient of relative variation, and selective accuracy; also indicating efficiency in the selection of superior genotypes within the evaluated set. Genotypes A-13, A-14, A-18, A-20 and A-21 were selected as superior genotypes for both characteristics, such as oil yield and *in vitro* performance, due to formation of embryogenic lines. This has been the first study investigating the genetic control of the cloning capacity aiming to the selection of genotypes for a clonal garden formation.

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## 1. Introduction

The increase in oil prices and the intention of reducing the emission of greenhouse gases has boosted the demand for biofuels (Gan and Li, 2014). Oil palm (*Elaeis guineensis* Jacq.) has stood out due to its high oil yield if compared to other biofuel producing plants, such as soybean, sunflower and rapeseed (Gan and Li, 2014; Lam et al., 2009; Mielke, 2013). The global dependence for oil, extracted from oil palm tends to increase in the coming years: it is estimated that the production should meet a demand of 26.6 million tons by 2035, due to technological investments (Gan and Li, 2014).

One of the technologies that have contributed the most to the real increase of oil production is the plant breeding (Nugroho et al., 2014). The planting of improved oil palm varieties is critical to long-term high production efficiency and also to the sustainabil-

ity of the crop. Current commercial oil palm planting materials are composed by a mixture of *dura* (D) × *pisifera* (P) or *tenera* (T) hybrids from non-fully inbred parents, thus presenting considerable genetic variability between and within the hybrids, depending on the relatedness and inbreeding status among their D and P parents. Conventional hybrids breeding methodology would require at least three generations or over 20 years to achieve the superior yield of these individuals (Wong and Bernardo, 2008; Soh, 2005). As strategy, the development of commercial plantations of elite clones can offer advantages such as harvest uniformity, facilities in management practices and optimization of the oil production (Malike et al., 2012). Success in cloning would short-circuit the process (Soh et al., 2011) and maximizing oil yielding in up to 18 t/ha, improving both production quality and quantity (Nugroho et al., 2014).

Clonal propagation has an important role through tissue culture, where the goal is to select multiple elite genotypes containing desirable characteristics, and then propagate them in mass, forming uniform commercial plantations (Nugroho et al., 2014). Associated with clonal propagation, palms ortet selection from commercial fields is an indispensable step in the cloning process. Ortets are selection from the best (but not limited to) families within the

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established progeny test trial. Particular emphasis is given to every trait related to oil productivity and crop management, as oil-to-bunch and related component traits like: mesocarp-to-fruit, oil-to-wet/dry mesocarp and palm stature (short, light petioles and canopy) (Soh et al., 2011).

The technique used for oil palm clonal propagation is somatic embryogenesis, being the stages of callogenesis and production of embryogenic lines, fundamental for its success. Orbits which are responsive during the process to these stages can produce more somatic embryos and consequently more ramets or clones. Added to this, the estimation of genetic parameters for these two *in vitro* traits can promote early selection of responsive genotypes, increasing the commercial propagation, once only genotypes that produce callus and embryogenic lines are selected, reducing costs, space and human resources. However, it is important to follow a cloning protocol that ensures efficiency in these two steps, and the use of genotypes that respond to this protocol. Therefore, it is essential to study the genetic parameters involved in callogenesis and embryogenic lines production in order to select genotypes that add superior characteristics to the establishment of clonal gardens.

The majority of the studies that determine genetic parameters in oil palm aim only to agronomic characteristics related to oil yield (Lopes et al., 2012; Soh et al., 2003). To date, there are no studies with the objective of determining ideal genotypes, as for oil yield characteristics and for cloning *in vitro* performance, through the study of genetic parameters.

This work had as objective to study the genetic control of the main characteristics related to oil palm cloning process, and to identify responsive genotypes within a collection of 32 elite materials of a commercial planting, in order to select genotypes superior in both, oil productivity and propagation ability, for clonal garden formation.

## 2. Materials and methods

### 2.1. Selection of genotypes for cloning

For the experiment implementation, thirty-two genotypes of oil palm (*E. guineensis* Jacq.) 'Tenera' hybrids *dura* (D) × *psífera* (P) were selected from a commercial plantation company Agropalma S.A. located in of Tailândia, Pará, Brazil.

The selected materials are elites from different research centers and exhibit considerable genetic variation. The individual selection of plants elites to be cloned was performed systematically and accurately considering yield, vegetative and plant health aspects. The yield characteristics used in the selection process of the genotypes came from six years of field evaluations, and are reported to have great heritability, and thus, suffering few environmental influence (Lopes et al., 2012; Soh et al., 2003), what made of these characteristics important for selection in breeding process.

As productive aspects the elite plant to be cloned should provide: good yields as a fresh bunch production (200–250 kg), fresh bunch number, oil potential in the bunch (28%), average weight of fresh bunch and annual production at least 10 t for year. As for the vegetative aspects were considered the plants that showed slow growth (0.25–0.50 m/year). Finally, as phytosanitary aspects were considered free arrays of diseases and symptoms of nutritional deficiency.

### 2.2. Obtainment and preparation of the foliar explants

For the induction of the somatic embryogenesis process, immature leaflets of the heart of the palm region were used as explants, which were extracted from the donor matrix plant with a chainsaw.

After extraction, each heart of the palm was sent to the Vegetal Cell and Tissue Culture Laboratory of the Universidade Federal de Viçosa, in Viçosa—Minas Gerais, 72 h after removing material.

As soon as the vegetal material arrived in the laboratory, the heart of the palm were externally sanitized with alcohol 70% and the external leaf layers were carefully removed, being the immature leaves separated in seven layers: leaf-1 (most external) to leaf-7 (most internal) (Corley and Tinker, 2003). Next, the leaflets of each leaf were detached from the rachis, being 1/3 of the apex of the leaflet eliminated. The leaflets were then submitted to disinfection in aseptic solution of sodium hypochlorite (1% of active chlorine) for 20 min and rinsed successively eight times in sterile deionized water in laminar flow chamber. After disinfection, the leaflets were sectioned transversely into 1 cm segments of length, which were used as explants to induce the somatic embryogenesis.

### 2.3. Callogenesis induction

The Basal Medium (MB) used was composed by salts and Y3 vitamins (Eeuwens, 1978), 30 g L<sup>-1</sup> of sucrose, 1 g L<sup>-1</sup> of casein hydrolyzed, 1000 mg L<sup>-1</sup> of myo-inositol, 100 mg L<sup>-1</sup> of arginine, 100 mg L<sup>-1</sup> of asparagine, 100 mg L<sup>-1</sup> of glutamine and jellied with 2.5 g L<sup>-1</sup> of Phytigel® (Sigma, USA). The pH was 5.7 ± 0.1 and the mediums were autoclaved for 20 min at 121 °C to 1.5 atm.

For the callus induction, the medium culture used was consisted of MB, supplemented with 800 μM of acid 2,4-dichlorophenoxyacetic (2,4-D) and 3 g L<sup>-1</sup> of active charcoal (Sigma, USA). The explants were inoculated into Petri dishes of polystyrene (90 × 15 mm) containing 30 mL of this medium culture. Five explants were inoculated per dish and 500 dishes were sealed with PVC film (Rolopac®) and kept in the growing room, at a temperature of 27 ± 1 °C in light absence for 90 days.

### 2.4. Multiplication of the callus and achievement of the embryogenic lines

The obtained callus of each genotype were inoculated into multiplication culture, constituted by MB with 9 μM of 2,4-D and 1000 μM of putrescine added. After inoculation of the callus, the dishes were sealed with PVC film (Rolopac®) and kept in the growing room, at a temperature of 27 ± 1 °C in light absence. After 60 days, callus that formed embryogenic lines were separated, subdivided and subcultivated in the same multiplication medium.

Lines that presented granular aspect, yellow coloration, multiplication capacity (increased five times or more from the original size), were considered as embryogenic lines. To confirm their embryogenic capacity, other than those described characteristics, these lines were placed on regeneration medium to determine their ability of somatic embryos production. The regeneration medium was constituted by MB added of 0,1 μM of 2,4-D, active charcoal (Sigma, USA) and 1000 μM of putrescine.

The non-embryogenic lines showed low or no ability to multiply and when placed on regeneration medium also showed no ability in somatic embryos formation.

The genotypes classified as responsive were those which had *in vitro* performance regarding to embryogenic lines formation, enabling the somatic embryos formation.

### 2.5. Estimation of genetic parameters

Estimates of genetic parameters were obtained by the mixed model methodology, REML (Restricted Maximum Likelihood)/BLUP (Best Linear Unbiased Prediction) (Resende, 2007), following the model below.

**Table 1**  
Oil palm 'Tenera' hybrids (*Elaeis guineensis* Jacq.) used in adult plants cloning.

Tenera hybrids	Seed supplier	Number of accessed matrices	Age (years)
Deli × Ekona	ASD	2	9
Deli × Ghana	ASD	3	11
Deli × La Mé	Murrin corporation	9	9–26
Deli × La Mé	Embrapa manaus	4	11–13
Deli × Yangambi	Univanich palm oil	8	10–27
Deli × Avros	ASD	2	13
Kigoma	ASD	2	13
Deli × Dami	Dami las flores	2	14–15

### 2.5.1. Model

$$y = Xb + Zg + e \quad (1)$$

where  $y$ ,  $b$ ,  $g$  and  $e$  are the vectors of data, of fixed effects (overall average), total genotypic effects (random) and random errors, respectively; and  $X$  and  $Z$  are the incidence matrices for  $b$  and  $g$ , respectively.

### 2.5.2. Mixed model equations

$$\begin{bmatrix} X'X & X'Z \\ X'X & Z'Z + A^{-1} \left( \frac{(1-h^2)}{h^2} \right) \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix} \quad (2)$$

where  $h^2 = \hat{\sigma}_g^2 / (\hat{\sigma}_g^2 + \hat{\sigma}_e^2)$  is the individual heritability in the broad sense.

### 2.5.3. Estimators of variance components by EM algorithm

$$\hat{\sigma}_e^2 = \frac{[y'y - \hat{b}'X'y - \hat{g}'Z'y]}{[N - r(X)]} \quad (3)$$

$$\hat{\sigma}_g^2 = \frac{[\hat{g}'A^{-1}\hat{g} + \hat{\sigma}_e^2 \text{tr}C^{22}]}{N_g} \quad (4)$$

where  $r(X)$  is the number of columns linearly independent of  $X$  and  $C^{22}$  is represented by

$$\begin{bmatrix} C^{11} & C^{12} \\ C^{21} & C^{22} \end{bmatrix} = \begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + A^{-1} \left( \frac{\sigma_e^2}{\sigma_g^2} \right) \end{bmatrix}^{-1} \quad (5)$$

where  $N_g$  is number of random elements (individuals);  $A$  is the additive genetic relationship matrix;  $\text{tr}$  is the matrix trace operator, given by the sum of the diagonal elements of the matrix; and  $N$  is the total number of data.

The predicted genotypic values were used to calculate the selection index, based on the rank-summation index (Mulamba and Mock, 1978) of genotypes that formed embryogenic lines.

For the selection of superior genotypes, regarding yield, the following characteristics were evaluated on field for at least five years of production: fresh bunch yield (t/ha), estimated oil potential (t/ha), industrial extraction rate (t/ha), which is given by the ratio of fresh bunch yield and estimated oil potential, and the annual average height increment (m).

Selegen-Remi/Blup (Resende, 2007) software was used to perform the statistical analyses (Table 1).

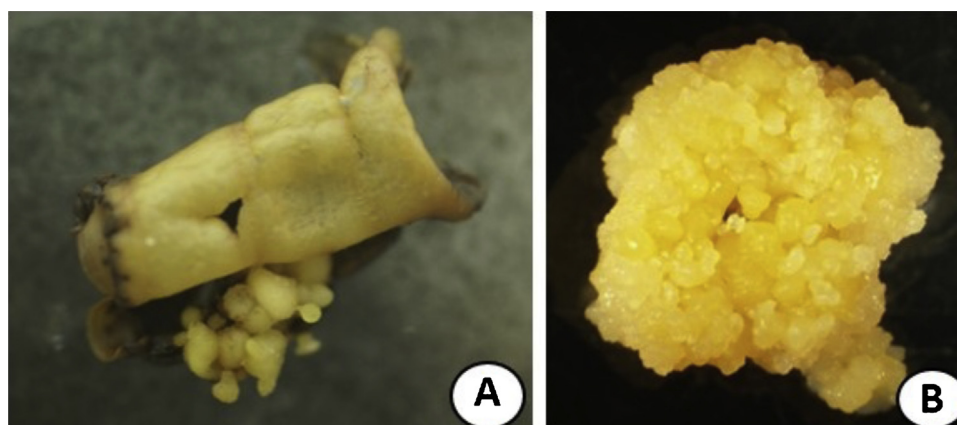
## 3. Results

### 3.1. Genetic parameters: callogenesis and embryogenic lines production

According to Resende (2002), heritability values between 0.15 and 0.50 are considered "moderate". In this study, the heritability of individual plants in the broad sense ( $h^2g$ ) were considered 'moderate' for CAL and LIN (Table 2), which characterizes a considerable genetic control of these characteristics. The heritability at the level of clone average of the current study were of high magnitude, corresponding to 0.94 for CAL and LIN (Table 2). These high values are due to the high number of replications used (500 dishes).

As for the selective accuracy, CAL and LIN (Fig. 1A and B) characteristics were considered "very high" (Table 2), according to the classification of Resende and Duarte (2007). This result indicates trustworthiness in the estimated genetic values for those characteristic, and therefore, reliability in selection.

The coefficient of relative variation (CVr) is the parameter that helps detecting genetic variability in a population, obtained by the relation between the coefficients of genetic and experimental variations, not influenced, therefore, by the character average. In this study, CVr for CAL and LIN were 0.42 and 0.49, respectively. Accord-



**Fig. 1.** (A) Callus with nodular and defined globular structures, beige color to light yellow, formed at the end of the leaf explants after 90 days of cultivation *in vitro* through induction medium. (B) Embryogenic lineage with nodular and yellowing after 60 days of *in vitro* culture on multiplication medium.

**Table 2**

Estimates of genetic parameters obtained from the analysis of 32 oil palm genotypes (*Elaeis guineensis* Jacq.), after 90 days of *in vitro* cultivation, in relation to explants with callus formation (CAL) and embryogenic lines formation (LIN).

Parameters	CAL	LIN
Vg	0.011	0.012
Ve	0.063	0.049
Vf	0.075	0.061
CVr	0.4231	0.4939
h <sup>2</sup> <sub>g</sub>	0.1518 ± 0.0098	0.1961 ± 0.01
Acaccuracy	0.97	0.97
h <sup>2</sup> <sub>mc</sub>	0.94	0.94
M	8.06	6.1

Vg: genetic variance among accessions; Ve: residual variance; Vf: individual phenotypic variance; CVr: coefficient of relative variation; h<sup>2</sup><sub>g</sub>: heritability of individual plants in the broad sense; h<sup>2</sup><sub>mc</sub>: heritability in the broad sense at cloning means level; M: overall mean.

**Table 3**

Ranking of 32 oil palm genotypes (*Elaeis guineensis* Jacq.) based on genotypic and phenotypic means for induction of callogenesis, after 90 days of *in vitro* cultivation.

Rank	Genotype	Genotypic means (u + g)	Phenotypic means	Embriogenic lines
1	A-04	51.47	52.11	Y
2	A-05	34.95	35.27	Y
3	A-06	21.36	21.56	N
4	A-31	16.90	17.03	Y
5	A-07	16.81	16.91	Y
6	A-28	9.95	9.98	Y
7	A-17	9.43	9.45	Y
8	A-03	8.12	8.12	N
9	A-02	7.07	7.06	Y
10	A-25	6.63	6.57	N
11	A-15	6.24	6.22	Y
12	A-23	6.12	6.09	N
13	A-16	5.91	5.88	Y
14	A-09	5.84	5.80	Y
15	A-01	5.49	5.44	N
16	A-08	4.76	4.72	Y
17	A-32	4.65	4.61	N
18	A-30	4.60	4.53	Y
19	A-14	4.12	4.07	Y
20	A-11	3.91	3.85	N
21	A-10	3.88	3.83	Y
22	A-22	3.61	3.55	Y
23	A-13	3.50	3.44	Y
24	A-18	2.97	2.91	Y
25	A-21	2.61	2.54	Y
26	A-19	1.70	1.62	N
27	A-26	1.32	1.11	Y
28	A-20	1.08	0.99	Y
29	A-29	0.99	0.90	Y
30	A-12	0.98	0.89	N
31	A-24	0.72	0.60	N
32	A-27	0.47	0.33	N

u + g: predicted genotypic value; u = 8.06: overall mean; Y: yes; N: no.

ing to Resende and Duarte (2007), the quality of the experiment is evaluated by the accuracy, which is a function of CVr and replications number, this way, the found values, demonstrated high experimental quality and reliability for the practical selection of both characteristics.

Due to the importance of the variable CAL in the cloning process, the genotypic values (u + g) for this characteristic were obtained. Out of the 32 evaluated genotypes, the first eight presented genotypic values above the overall average. Among these, only two did not form embryogenic lines. The genotypes A-04 and A-05 occupied the positions 1 and 2 in the ranking, which depicts high percentages of callogenesis. The last two positions were occupied by genotypes A-24 and A-27. It was observed that the genotypic values are very close to the phenotypic average values (Table 3). This is due to the high estimates (0.94) of heritability based on the clones' average, which were close to 1.

**Table 4**

Classification based on average index of ranks, fresh bunch yield (FBY), estimated oil potential (EOP) and mean annual increment (AAI) of 21 oil palm genotypes (*Elaeis guineensis* Jacq.) that formed embryogenic lines.

Rank	Genotype	Rank-FBY	Rank-EOP	Rank-AAI	Rank-mean
1	A-20	1	2	3	2.00
2	A-14	5	3	5	4.33
3	A-13	7	4	4	5.00
4	A-21	3	5	11	6.33
5	A-18	2	1	20	7.67
6	A-15	4	7	12	7.67
7	A-04	11	10	2	7.67
8	A-16	8	9	10	9.00
9	A-30	6	8	15	9.67
10	A-02	13	11	6	10.00
11	A-05	12	6	13	10.33
12	A-07	9	17	9	11.67
13	A-29	20	18	1	13.00
14	A-28	10	12	19	13.67
15	A-08	16	13	14	14.33
16	A-10	14	15	17	15.33
17	A-22	19	19	8	15.33
18	A-17	17	14	16	15.67
19	A-31	21	20	7	16.00
20	A-09	15	16	18	16.33
21	A-26	18	21	21	20.00

### 3.2. Productive potential of 32 oil palm genotypes

Using the Average index of Ranks of Mulamba and Mock (1978), based on genotypic values, the genotypes that formed embryogenic lines were classified according to the characteristics related to oil yield and annual increase average in favorable improvement order. The lowest value indicates a more favorable combination among the established characters, and the highest an unfavorable combination. Among the genotypes that produced embryogenic lines, A-20, A-14, A-13, A-21 and A-18 occupied the first five positions in the Average index of Ranks, making them suitable for selection, as they bring together the ideal characteristics for cloning and oil production (Table 4).

Table 5 shows the results of the main characteristics targets of the selection of potential genotypes, which formed embryogenic lines. They are: fresh bunch yield, estimated oil potential, and annual average increment. Based on the relationship between the estimated oil potential and fresh bunch yield, it was estimated the industrial extraction rate of each genotype (Table 5). Genotypes A-14, A-18 and A-20 showed high values for estimated potential oil (up to 10 t/ha). For fresh bunch yield characteristic, genotypes A-18, A-20 and A-21 presented the highest values (above 40 t/ha). On the other hand, genotypes A-04, A-20 and A-29 had the lowest values for annual average increment (less than 0.27 m). Genotype A-20 stood out for its good performance for fresh bunch yield, estimated oil potential and annual average increment.

## 4. Discussion

The knowledge of genetic control of the main characteristics related to oil palm cloning is indispensable for obtaining gains with selection, when the objective is to establish a clonal garden with superior individuals aiming at clonal planting. In the present study, it was possible to determine the genetic control of CAL and LIN through their heritability values.

Heritability is the genetic parameter of greatest importance and application in plant breeding programs. Its relevance is related to the fact of being able to show how the genetic effects are presented in the individual's phenotype, since, it is the genotypic value that interests and influences the next planting (Falconer and Mackay, 1996).

**Table 5**

Fresh bunch yield (FBY), industrial extraction rate (IER), estimated oil potential (EOP) and annual average increment (AAI) of oil palm genotypes (*Elaeis guineensis* Jacq.) that produced embryogenic lines.

Genotype	Background	FBY (t/ha)	IER (t/ha)	EOP (t/ha)	AAI (m)
A-02	Deli × Ekona	24,53	28,1	6,89	0,32
A-04	Deli × La Mé	26,25	27,2	7,14	0,26
A-05	Deli × La Mé	25,01	35,6	8,91	0,34
A-07	Deli × Ghana	27,51	17,8	4,89	0,33
A-08	Deli × Ghana	20,92	32,2	6,74	0,35
A-09	Deli × Yangambi	20,92	24,8	5,19	0,46
A-10	Deli × Yangambi	22,26	24,3	5,42	0,44
A-13	Deli × La Mé	32,71	30,4	9,96	0,30
A-14	Deli × La Mé	33,81	31,0	10,48	0,31
A-15	Deli × Avros	35,86	23,2	8,31	0,34
A-16	Deli × Avros	29,15	24,6	7,18	0,33
A-17	Kigoma	19,66	27,8	5,47	0,39
A-18	Kigoma	43,8	28,3	12,39	0,50
A-20	Deli × La Mé (Embrapa)	45,04	23,8	10,72	0,27
A-21	Deli × La Mé	40,89	23,5	9,61	0,33
A-22	Deli × La Mé	17,21	23,5	4,04	0,33
A-26	Deli × Yangambi	17,43	19,2	3,35	0,53
A-28	Deli × Yangambi	27,09	25,2	6,84	0,48
A-29	Deli × La Mé (Embrapa)	15,21	29,4	4,47	0,25
A-30	Deli × Ghana	33,51	22,2	7,43	0,37
A-31	Deli × La Mé	12,91	27,3	3,53	0,33

Estimates of heritability in the broad sense for CAL and LIN were 15% and 19%, respectively. In peach palm, [Farias Neto et al. \(2013\)](#) found heritability values for bunch yield of 21.3%, and classified it as ‘intermediate magnitude’. According to [Resende \(2002\)](#), the majority of quantitative characters of economic importance has individual heritability of approximately 20%.

In oil palm, heritability estimates are scarce for *in vitro* characteristics, and studies of morphological and agronomic characters are more common. [Soh et al. \(2003\)](#) found heritability values ranging from 22 to 36% for yield characters. [Soh \(1994\)](#) reported individual heritability values in the narrow sense of 35% for number of bunches, 20% for bunch weight, and 15% for oil yield in oil palm. In interspecific hybrid ‘caiaué’, derived from the crossing of *E. guineensis* and *Elaeis oleifera*, the heritability for morphological and agronomic characters ranged from 24.5 to 30.4% ([Lopes et al., 2012](#)). [Farias Neto et al. \(2008\)](#), in acai berry, report heritability ranging from 12 to 44%. These results demonstrate that in palms, the heritability values found for most characters range from 12 to 44%, and are considered favorable for selection.

The selective accuracy shows a correlation between the real and predicted genetic values, and the higher the value, the more reliable the individuals’ evaluation. This genetic parameter is closely linked to heritability at the means level ([Resende, 2002](#)). According to the classification of heritability and accuracy in terms of magnitude and their associations, proposed by [Resende \(1997\)](#), the values found in this study were considered of ‘high’ magnitude for CAL and LIN, indicating advantages when using these two traits in selection.

Another genetic parameter of high importance in plant breeding studies is the coefficient of relative variation (CVr). [Resende and Duarte \(2007\)](#) suggest that each experiment has a particular value, and not a generalized CVr. Fixing the number of replications, the magnitude of CVr can be used to infer the accuracy and precision in genetic evaluation. Therefore, appropriate CVr values should be inferred together with the number of replications, since, values lower than the unit may also provide high accuracy and precision. Coefficient of relative variation values found in this experiment, together with a high number of replications (500), provided high selective accuracy, which indicated good precision and experimental quality.

The predicted genotypic value is important, since, it reveals the genetic potential of the individual in the selection without the

environmental effect, resulting in greater reliability and genetic gain. The rank of the predicted genotypic values showed that eight genotypes presented high values for callogenesis in relation to the overall mean (8.06%), indicating superiority of these materials for this character.

Callogenesis is an important step for cloning success; however, for this technique to be successful, the formation of embryogenic lines is essential. Among the eight outstanding genotypes in the rank, only two did not form embryogenic lines. For [Silva et al. \(2012\)](#) and [Thawaro and Te-chato \(2009\)](#), the success of somatic embryogenesis depends on the genotype on which the technique is applied. The obtained results showed that these eight genotypes are the most efficient in callogenesis and, in most cases, allow the formation of embryogenic lines.

To date, there are no studies in which the selective accuracy, the coefficient of relative variation and the predicted genotypic value have been described for traits related to oil palm cloning.

The Average index of Ranks of [Mulamba and Mock \(1978\)](#), ranks the genotypes, initially, for each character, by assigning lower absolute values to those of better performance. Finally, the values assigned to each character are summed to obtain the average of ranks, which indicates the classification of genotypes.

The genotypes that formed embryogenic lines were selected in favorable order for characteristics related to bunch and oil yield, and annual average increment. Genotypes A-20, A-14, A-13, A-21 and A-18 occupy the top positions in the rank of averages, and are suitable for selection, since, they meet the ideal characteristics for both oil production and cloning. The mentioned characteristics are the main ones to the ideotype proposed for oil palm.

For industry, oil palm genetic materials with high oil yield are those that produce over 8.6 t/ha/year ([Corley et al., 1976](#)), and it is expected that selected genotypes produce 12.2 t/ha/year ([Rajanaidu et al., 1990](#)), and there is a hypothesis that yield of selected plants may be up to 18.2 t/ha/year ([Corley, 1998](#)). Allied to this performance, bunch yield should also be taken into account, as it correlates with the increase in the yield per area. The annual average increase in height indicates the plant lifetime, so, the lower the value, the greater the plant’s lifetime. However, even if the genotype presents high annual average increase in height, such as A-18 (0.5 m/year), and oil yield and high bunch yield are compensatory, a management strategy may be adopted so that it allows the efficient exploitation of this material.

This was the first study that investigated the relationship between the genetic parameters and the selection of elite genotypes for the formation of an oil palm clonal garden, evaluating important characteristics for cloning (callus and embryogenic lines), and its productive potential. In previous study, [Ooi et al. \(2012\)](#) correlated somatic embryogenesis in oil palm with changes in hormone-responsive genes, in particularly the putative Aux/IAA gene, EglIAA9, reported that this marker has predictive power for selected ortet.

For future studies will be necessary to evaluate the performance of these materials in field, as for the appearance of the “mantled flower” and an analysis of the relationship between the cloning capacities of these genotypes with the ramets production. For the efficiency of oil palm breeding programs and clonal garden formation, heritability of clonability and sensitivity to clonal abnormality (*i.e.*, oil palm mantling phenomenon) and their repeatability in clones and re-clones are considered important for selection of oil palm genotypes. Selection of superior genotypes for the cloning process may optimize breeding programs, since it saves resource, time and space.

## 5. Conclusion

Callogenesis characteristics and embryogenic lines formation showed genetic control that allows selection of responsive genotypes to cloning.

All evaluated genetic parameters: heritability, coefficient of relative variation, and accuracy, indicated efficiency in the selection of superior genotypes within the evaluated set.

Genotypes A-13, A-14, A-18, A-20 and A-21 were selected as the superior genotypes for both oil yield and *in vitro* performance, due to embryogenic lines formation.

In this context evaluation of the performance of these genotypes in field, together with phenotypic analysis for “mantled flower”, are necessary and suggested.

## References

- Corley, R.H.V., 1998. What is the upper limit to oil extraction ratio? In: Proceeding Oil and Kernel Production in Oil Palm—A Global Perspective Palm Oil Research Institute, Malaysia, pp. 256–269.
- Corley, R.H.V., Tinker, P.B., 2003. *The Oil Palm*, fourth ed. Blackwell Science Ltd, Great Britain, pp. 562.
- Corley, R.H.V., Wood, B.J., Hardon, J.J., 1976. Future developments in oil palm cultivation. In: *Oil Palm Research*. Elsevier, Amsterdam.
- Euwens, C.J., 1978. Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palms (*Cocos nucifera*) and cultured *in vitro*. *Physiol. Plantarum* 36, 23–28.
- Falconer, D.S., Mackay, T.F.C., 1996. *Introduction to Quantitative Genetic*, fourth ed. Longman, Harlow, England.
- Farias Neto, J.T., Resende, M.D.V., Oliveira, M.S.P., Nogueira, O.L., Falcão, P.N.B., Santos, N.S.A., 2008. Estimativas de parâmetros genéticos e ganhos de seleção em progênies de polinização aberta de açaizeiro. *Rev. Bras. Frutic.* 30, 1051–1056.
- Farias Neto, J.T., Clement, C.R., Resende, M.D.V., 2013. Estimativas de parâmetros genéticos e ganho de seleção para produção de frutos em progênies de polinização aberta de pupunheira no estado do Para, Brasil. *Bragantia* 32, 122–126.
- Gan, P.Y., Li, Z.D., 2014. Econometric study Malaysia's palm oil position in the world market to 2035. *Renew. Sustain. Energy Rev.* 39, 740–747.
- Lam, M.K., Tan, K.T., Lee, K.T., Mohamed, A.R., 2009. Malaysian palm oil: surviving the food versus fuel dispute for a sustainable future. *Renew. Sustain. Energy Rev.* 13, 1456–1464.
- Lopes, R., Cunha, R.N.V., Resende, M.D.V., 2012. Produção de cachos e parâmetros genéticos de híbridos de caiaué com dendezeiro. *Pesqui. Agropecu. Bras.* 47, 1496–1503.
- Malike, A.F., Abdulah, N., Amiruddin, M.D., 2012. Selection of oil palm clones with high bunch index for Recloning at Malaysian Palm Oil Board (MPOB). In: Paper Presented at the International Annual Symposium on Sustainability Science and Management, Terengganu, Malaysia.
- Mielke, T., 2013. Palm oil the leader in global oils and facts supply. In: Paper Presented at the Malaysia/Myanmar Palm Oil Trade Fair and Seminar, Yangon, Myanmar, 28 June (Available at: [http://www.mpoc.org.my/upload/Plenary\\_Paper-Thomas-Mielke.pdf](http://www.mpoc.org.my/upload/Plenary_Paper-Thomas-Mielke.pdf) accessed 11.09.14).
- Mulamba, N.N., Mock, J.J., 1978. Improvement of yield potential of the Eto Blanco maize (*Zea mays* L.) population by breeding for plant traits. *Egypt. J. Genet. Cytol.* 7, 40–51.
- Nugroho, Y.A., Sumertajaya, I.M., Wiendi, N.M.A., Toruan-Mathius, N., 2014. Estimation of genetic parameters for *in vitro* culture traits and selection best progenies for Tenera oil palm tissue culture. *Energy Procedia* 4, 316–322.
- Ooi, S.E., Choo, C.N., Ishak, Z., Ong-Abdullah, M.O., 2012. A candidate auxin-responsive expression marker gene, *EglAA9*, for somatic embryogenesis in oil palm (*Elaeis guineensis* Jacq.). *Plant Cell Tiss. Org.* 110, 201–212.
- Rajanaidu, N., Rao, V., Halim, A.H., Ong, S.H., 1990. Genetic resources: new developments in oil palm breeding. *Elaeis* 1, 1–10.
- Resende, M.D.V., 1997. Avanços da genética biométrica Florestal. In: encontro sobre temas de genética e melhoramento. Piracicaba Anais. Piracicaba ESALQ-USP, 150–158.
- Resende, M.D.V., 2002. Genética biométrica e estatística no melhoramento de plantas perenes. Embrapa Informação Tecnológica, Brasília, Brazil, pp. 975.
- Resende, M.D.V., 2007. Matemática e estatística na análise de experimentos e no melhoramento genético, first ed. Embrapa Informação Tecnológica, Brasília, Brazil, pp. 561.
- Resende, M.D.V., Duarte, J.B., 2007. Precisão e controle de qualidade em experimentos de avaliação de cultivares. *Pesqui. Agropecu. Trop.* 37, 182–194.
- Silva, R.C., Gomes Luis, Z., Scherwinsky-Pereira, J.E., 2012. Differential responses to somatic embryogenesis of different genotypes of Brazilian oil palm (*Elaeis guineensis* Jacq.). *Plant Cell Tiss. Org.* 111, 59–67.
- Soh, A.C., 1994. Ranking parents by best linear unbiased prediction (BLUP) of breeding values in oil palm. *Euphytica* 76, 13–21.
- Soh, A.C., 2005. Super yielding oil palm strategic breeding plan. Advances and breeding and clonal technologies for super yielding for planting materials. In: 2005 National Seminar, Kuala Lumpur 7–8 March, 2005.
- Soh, A.C., Gan, H.H., Wong, G., Hor, T.Y., Tang, C.C., 2003. Estimates of within family genetic variability for clonal selection in oil palm. *Euphytica* 133, 147–163.
- Soh, A.C., Wong, G., Tang, C.C., Chew, P.S., Chong, S.P., Ho, Y.W., Wong, C.K., Cho, C.N., Nor Azura, H., Kumar, H., 2011. Commercial-scale propagation and planting of elite oil palm clones: Research and development towards realization. *J. Oil Palm Res.* 23, 935–952.
- Thawaro, S., Te-chato, S., 2009. Effect of genotypes and auxins on callus formation from mature zygotic embryos of hybrid oil palms. *J. Agric. Technol.* 5, 167–177.
- Wong, C., Bernardo, R., 2008. Genome wide selection in oil palm: increasing selection gain per unit time and cost with small population, *Theor. Appl. Genet.* 116, 815–824.