Parental selection for the formation of interspecific hybrid populations of oil palm

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ABSTRACT. The objective of this work was to select families with superior performance and large genetic variability for diallel crosses for potential use in the pre-breeding of oil palm. The experiment consisted of 42 full-sib families divided into three trials with 16 families and 3 witnesses in common in randomized blocks, with four blocks and 12 plants per block. The characteristics evaluated in the experiment were number of bunches per plant (NBP), bunch weight per plant (BWP) and bunch average weight per plant (BAW). The estimation of variance components showed that there was greater genetic variance within families than between families. The heritability for all traits was high, above 0.75, and the coefficient of environmental variation was low to moderate for all traits (between 4 and 13). Tocher's method separated families into seven groups, whereas the UPGMA method identified six groups. To use the best families in diallel crosses, families 15, 14, 22, 21, 28, 37, 33 and 39 were selected based on individual performance as determined by the Scott-Knott test and genetic diversity by grouping.

Keywords: *Elaeis guineensis*, cluster analysis, genetic diversity.

Seleção de genitores para formação de população de híbridos interespecíficos de dendê

RESUMO. O objetivo deste trabalho foi selecionar famílias com desempenho superior e ampla variabilidade genética para cruzamentos dialelos e com potencial utilização no pré-melhoramento de dendê. O experimento consistiu em 42 famílias de irmãos completos, divididas em três ensaios com 16 famílias e 3 testemunhas comuns, sendo estes em delineamento em blocos ao acaso com quatro blocos, e 12 plantas por bloco. As características avaliadas no experimento foram número de cachos por planta (NCP), produção de cachos por planta (PCP) e peso médio de cacho (PMC). Com estimação dos componentes de variância verificou-se que existe uma maior variância genética dentro de famílias do que entre famílias. A herdabilidade para todas as características foram altas, acima de 0,75 e o coeficiente de variação ambiental encontrado para todas as características foi de baixo a moderado (entre 4 e 13). As famílias foram separadas em sete grupos pelo método de Tocher e em seis grupos com o método UPGMA. Visando a utilização das melhores famílias em cruzamentos dialelos, as famílias 15, 14, 22, 21, 28, 37, 33 e 39 foram selecionadas com base na performance individual, através do teste de Scott-Knott, e diversidade genética através do agrupamento.

Palavras-chave: *Elaeis guineensis*, análise de agrupamento, diversidade genética.

Introduction

Oil palm (*Elaeis guineensis*) is native to Africa and has been considered a prospective feedstock for biodiesel production (PLEANJAI; GHEEWALA, 2009). This tree is largely produced in the world's tropical zone (YUSOF; CHEN, 2003) and could supply two large industrial sectors: the food industry and the oleochemical industry.

Oil palm cultivation is an important industrial and agro-ecological activity that has been commercially exploited for approximately 25 years. Oil palm is cultivated for mesocarp-derived crude palm oil (CPO) and palm ernel-derived palm

kernel oil (PKO). CPO and PKO may be used alone or in blends with other oils in food and other applications. Furthermore, palm oil is an optimal source of energy and a clean, renewable biofuel (BAKOUMÉ et al., 2010). Commercial production can begin three year after planting but the maximum yield capacity of the plants is realized after seven years when the plant enters the productive phase.

The oil palm breeding program is relatively new compared to other industrial species, such as coffee and coconut (CEDILLO et al., 2008). Oil palm plantations have been continuously

expanding since the first commercial plantations were introduced in 1969 in Malaysia (TAN et al., 2009).

The oil palm breeding program has focused on oil production (COCHARD et al., 2005; SILALERTRUKSA et al., 2012). Oil production is correlated with the oil content of the bunch as well as the number of bunches produced. The number of bunches is influenced by many factors, including the proportion of female flowers in relation to the total number of flowers, the fraction of aborted inflorescences and defects in mesocarp formation, and environmental factors, such as soil, humidity and temperature (KALLARACKAL et al., 2004; HENSON; HARUN, 2005).

One strategy that can be used in the oil palm breeding program is family selection. Selection of families at the initial stage, before clones are obtained, may reduce the time spent developing a new cultivar (BARBOSA et al., 2005). The progenies or families are units that can be selected according to their average phenotypic values. A family is chosen primarily when the selected trait presents low heritability because they are greatly affected by the environment (PEDROSO et al., 2011).

Several authors have shown that the use of family selection can contribute to parental selection and to crosses aimed at obtaining improved populations in several cultures (ANDRADE et al., 2011; FERREIRA et al., 2010; ARNHOLD et al., 2009). However, little work on family selection in the initial generation in a breeding program has been reported in the palm oil literature (BAKOUMÉ et al., 2010; CEDILLO et al., 2008; SOH et al., 2003). Thus, the objective of this study was to select families as potential parents for oil palm breeding programs aimed at obtaining higher genotypes to be used for establishing cultivars.

Material and methods

Conduction of the experiment

The experiment consists of the first trial competitions of oil palm interspecific hybrids in an area in Brazil in which fatal yellowing occurs. The experiment was performed in 2007 in an area with incidences of fatal yellowing (FY) in Marborges Agroindústria S.A., located in Moju, state of Pará. The treatments consisted of 42 interspecific hybrids of oil palm (*Elaeis guineensis* x *Elaeis oleifera*), of which 41 hybrids from *E. oleifera* originated from Manicoré and one from Coari. The pre-germinated hybrid seeds were supplied by Embrapa Amazônia Ocidental from an experimental area in Rio Urubu, located in Rio Preto da Eva in the state of Amazonas.

The experiment was composed of three trials in a completely random block design, with 16 treatments and 4 replicates each. Of the 16 treatments, three shared a common control with the three trials (progenies 40, 41 and 42) (Table 1). The treatments consisted of full-sib families. The experimental plot was composed of 12 plants spaced 9 m apart in a triangular arrangement, with 7.8 m between lines. The experimental stand had 2,302 plants. Practices of management, fertilization, control of pests and diseases, and harvest were conducted according to company procedures, which used a production system similar to that for African oil palm with few nutritional adjustments and the use of assisted pollination throughout the productive period. Evaluations of bunch weight per plant (BWP) have been conducted since 2010, and a patrol is performed every 20 days to harvest the heavy, ripened bunches. The number of bunches per plant (NBP) are also recorded to obtain the bunch average weight per plant (BAW). All plants were harvested but the evaluation was carried out only for the six plants central considered in this study.

Table 1. Progeny characterization with their respective parents.

Progeny	Parents	Trial	Progeny	Parents	Trial
1	RU 2839 D x RU 56 P	1	22	RU 2901 D x RU 2693 P	2
2	RU 78 D x RU 53 P	1	23	RU 1588 D x RU 2730 P	2
3	RU 2846 D x RU 2692 P	1	24	RU 101 D x RU 2710 P	2
4	RU 92 D x RU 56 P	1	25	RU 1578 D x RU 2710 P	2
5	RU 3079 D x RU 2749 P	1	26	RU 2845 D x RU 2729 P	2
6	RU 3101 D x RU 56 P	1	27	RU 3795 D x RU 2749 P	3
7	RU 3189 D x RU 2710 P	1	28	RU 2841 D x RU 2692 P	3
8	RU 3856 D x RU 2691 P	1	29	RU 2842 D x RU 2730 P	3
9	RU 1605 D x RU 2730 P	1	30	RU 2842 D x RU 2707 P	3
10	RU 1578 D x RU 2691 P	1	31	RU 3099 D x RU 2693 P	3
11	RU 1578 D x RU 2692 P	1	32	RU 3089 D x RU 2730 P	3
12	RU 1586 D x RU 2730 P	1	33	RU 3089 D x RU 2710 P	3
13	RU 92 D x RU 2692 P	1	34	RU 1608 D x RU 2749 P	3
14	RU 2787 D x RU 2733 P	2	35	RU 1778 D x RU 2698 P	3
15	RU 3308 D x RU 2691 P	2	36	RU 3170 D x RU 2710 P	3
16	RU 3111 D x RU 2693 P	2	37	RU 3123 D x RU 2700 P	3
17	RU 3111 D x RU 2700 P	2	38	RU 3169 D x RU 2700 P	3
18	RU 2914 D x RU 2700 P	2	39	RU 2905 D x RU 2693 P	3
19	RU 3359 D x RU 2700 P	2	40	RU 3791 D x RU 2692 P	1. 2 .3
20	RU 1578 D x RU 2730 P	2	41	RU 1604 D x RU 56 P	1. 2 .3
21	RU 2900 D x RU 2693 P	2	42	RU 1724 D x RU 2710 P	1. 2 .3

Statistical analysis

To test the hypothesis of significant genetic variation among the means of full-sibling families, each characteristic was analyzed for variance using the Genes software (CRUZ, 2006) with data from individuals within the plots. Random block design was used according to the following statistical model:

$$Y_{ijk} = \mu + G_i + L_k + B/L_{jk} + T_l + LT_{kl} + TG/L_{lik} + E_{ijkl},$$

where i = 1, 2, ... g families; j = 1, 2, ... b blocks; l = 1,2, … T witness; and k = 1, 2, ... n_{ij} plants per plot. Y_{ijk} is the observation in the kth plant, in the ith family of the jth block; μ is the population overall mean; G_i is the effect of the genotype i; L_k is the effect of the trial k; B/L_j is the effect of the block j within trial k; T_l is the effect of the witness l; LT_{kl} is the effect of the interaction trial x witness; and E_{ijkl} is the experimental error; and δ_{ijk} is the variation effect among plants within families, where $\delta_{ijk} \sim$ NID $(0, s^2_{fd})$.

The components of variance for each trait were estimated according to Gelman (2005), in which variances of the following were estimated: block variances; phenotypic variance among the means of families, within families and among plants in the experiment; environmental variance among plots; and genotypic variance among the means of families, within families or among plants within families and additive genetic variance.

Heritability coefficients for individual plants within families in the block, in the experiment and for the means of families were all estimated according to Gelman (2005), as were the phenotypic, genetic, environmental and experimental variation coefficients.

The Scott-Knott test was used at 5% probability to form groups between progenies and Pearson's correlation was calculated among traits and to determine the contribution of traits to genetic diversity.

Cluster analysis

Cluster analysis was performed to optimize the selection of families for diallel cross. Estimates of the family genotypic values obtained for each trait under study (NBP, BWP and BAW) were considered to be variables in the cluster process.

The Tocher cluster method and the average linkage hierarchical method, also known as the unweighted pair-group method using arithmetic averages (UPGMA), were used for family clustering (JOHNSON; WICHERN, 2007).

The Tocher method is based on a matrix of dissimilarity where the average dissimilarity of the group must be less than the average distances between other groups. The distance among the individual k and the group formed by individuals ij is given by:

$$d_{(ij)k} = d_{jk} + d_{ik}.$$

The inclusion or exclusion of k at group θ assumed that the mean increase promoted by inclusion of k in a predetermined group is less than θ, where:

if $d_{(group)k}/n < \theta$, k must be included in the group, or

if $d_{(group)k}/n > \theta$, k must be excluded from the group,

where n is the number of individuals in the original group.

In the UPGMA method, the dendogram is constructed based upon the individual with the greatest similarity and by the distance from individual k to the cluster formed by the individuals i and j. This is given by the following:

$$d_{(ij)k} = \frac{d_{ik} + d_{jk}}{2},$$

that is, the set of means of the distance between pairs of individuals.

The optimum number of clusters (partition) for the hierarchical cluster method used in this study was obtained using the method proposed by Mojema (1977), in which the number of clusters is given by the first stage in the dendogram. Here,

$$\alpha_j > \bar{a} + S_a\theta,$$

where j=1,2, ..., n; α_j is the distance for joint stage corresponding to n-j+1 clusters; $\bar{a}$ and S_a are the mean and standard deviation, respectively; and θ is a constant. The value of θ in this study was set at 1.25 according to the suggestion of Milligan and Cooper (1985).

Results and discussion

In the analysis of variance, the variation in source of treatments was found to be significant (Table 2). This shows the existence of genetic variability in the population and that the selection is effective in generating selection gains.

Table 2. Joint analyses of variance of the traits NBP (number of bunches per plant). BWP (bunch weight per plant), BAW (bunch average weight).

SV	DF	MS			F		
		NBP	BWP	BAW	NBP	BWP	BAW
Block	9	14,35	480,83	6,21			
Experiment	2	75,79	3239,09	18,81	4,56★	7,43★★	4,82★★
Control	2	16,33	392,83	1,39	0,96	0,9	0,36
C x Exp	4	22,29	661,78	4,78	1,34	1,52	1,23
G/EXP	36	31,07	1450,97	8,29	1,87★★	3,33★★	2,13★★
(CxG)/T	3	54,80	1238,76	19,18	3,3★	2,84★	4,92★★
Residue	135	14,60	435,58	3,9			
Total	191						

★and★★ significance level of 5 and 1%, respectively. C – control, Exp – experiment, G – genotype, T – trial.

Genetic variance (σ^2) within families was greater than among families for all traits evaluated (Table 3). This indicates that selection within families may be better than selection among families due to the greater variability within families. The block effect and the environmental effect among plots tended to zero for NBP and BAW; thus, the experimental precision was high, and the block effect was small (ROSADO et al., 2009).

Table 3. Variance estimates for bunch weight per plant (BWP). number of bunches per plant (NBP), and bunch average weight (BAW) in oil palm full-sibling families.

Trial	Variance	NBP	BWP	BAW
1	σ^2_{gm}	1.59	100.44	0.6
	σ^2_{gd}	4.77	301.33	1.81
	σ^2_{fd}	9.55	440.11	1.33
	σ^2_{ft}	11.88	575.7	2.05
	σ^2_{b}	0.16	4.13	0.02
	σ^2_{e}	0.58	31	0.1
2	σ^2_{gm}	3.93	228.48	0.63
	σ^2_{gd}	11.78	685.44	1.91
	σ^2_{fd}	13.68	371.99	1.45
	σ^2_{ft}	18.55	646.38	2.13
	σ^2_{b}	0.24	4.77	0
	σ^2_{e}	0.69	41.15	0.04
3	σ^2_{gm}	2.41	58.49	0.56
	σ^2_{gd}	7.24	175.48	1.69
	σ^2_{fd}	10.93	455.45	1.48
	σ^2_{ft}	14.01	559.48	2.27
	σ^2_{b}	0.06	3.47	0.02
	σ^2_{e}	0.73	49.01	0.21

[1]σ^2_{gm}, genotypic variance among family means; σ^2_{gd}, genotypic variance within the family; σ^2_{fd}, phenotypic variance within families; σ^2_{ft}, total phenotypic variance; σ^2_{b}, phenotypic variance due to the block effect; and σ^2_{e} environmental variance among plots.

Heritability among and within families varied for all traits (Table 4). Heritability was high (0.75, 0.83 and 0.86 for NBP, BWP and BAW, respectively), showing that there is high genetic control for those traits. Selection among families is more advantageous for NBP; although σ^2 is greater within families, heritability is higher among families. In other words, the environmental effect is lower and the genetic gain is greater among families (Table 5). However, heritability for BWP and BAW was greater within families than among families. Overall, heritability among families was higher than within families,

as shown in Martins et al. (2005) and Paula et al. (2002). As a consequence, we can select among and within families to exploit variability and increase total genetic gain (ROSADO et al., 2009).

Table 4. Estimates of heritability coefficients for bunch weight per plant (BWP), number of bunches per plant (NBP) and average bunch weight (BAW) in oil palm full-sibling families.

Trial	Heritability coefficient[1]	NBP	BWP	BAW
1	h^2_{m}	0.81	0.85	0.91
	h^2_{d}	0.5	0.68	0.95
	h^2_{ex}	0.53	0.7	0.9
	h^2_{b}	0.54	0.7	0.92
2	h^2_{m}	0.89	0.92	0.94
	h^2_{d}	0.86	0.98	0.99
	h^2_{ex}	0.85	0.95	0.97
	h^2_{b}	0.86	0.96	0.96
3	h^2_{m}	0.85	0.72	0.87
	h^2_{d}	0.66	0.38	0.99
	h^2_{ex}	0.69	0.42	0.98
	h^2_{b}	0.69	0.42	0.99
Joint	h^2_{m}	0.47	0.7	0.53

[1]h^2_{m}, heritability coefficient of family means; h^2_{d}, heritability coefficient within families; h^2_{ex}, heritability coefficient of the experimental plants; h^2_{b} heritability coefficient of plants in the block.

Table 5. Estimated selection gain (GS) for number of bunches per plant (NBP), bunch weight per plant (BWP), bunch average weight (BAW) and families selected with a selection intensity of 11.90%.

Selection	SG			Total SG	Selected families
	NBP	BWP	BAW		
NBP[1]	1.44	19.42	0.55	21.41	15, 5, 14, 19, 12
BWP	1.31	22.3	0.75	24.36	15, 5, 14, 21, 13
BAW	0.8	13.13	1.02	14.95	21, 15, 20, 22, 14

★Columns represent selected traits, and rows represent selection gain for each trait when only the column trait is selected.

A significant genetic variability (p < 0.001) was found in the joint analysis of the evaluated traits. As such, this population was able to be used for the selection and recombining of genetic material aimed at obtaining a new generation consisting of the greatest number of favorable alleles for certain traits. The results of the joint analysis showed that selection among families is important for bunch weight per plant because there is a high variability among them; this may generate a high selection gain. Because heritability is high, the selection is efficient on those traits

with high heritability because the environmental effect upon this expression is small. To perform cluster analysis, the control used must not interact with the different set trials. This criterion was checked, and the three controls used in the study did not show significant interaction with the trials; therefore, they were useful in this analysis and did not affect the results.

Another parameter used to compare genetic variability among families was the coefficient of variation. When CV_g/CV_e approaches 1, selection gain is higher. CV_g/CV_e was greater than 1 for all traits in all trials in this work (Table 6). As a consequence, we concluded that genetic gain will be high for all variables. Thus, genetic gain can be achieved over several selection cycles.

CV_{gd} was higher than CV_{gm} for all evaluated traits except for BAP in trial 3. Thus, selection within and among families is expected to promote greater advances when compared to selection among families (ROSADO et al., 2009).

Table 6. Estimates of the coefficients of variation for bunch weight per plant (BWP), number of bunches per plant (NBP) and bunch average weight (BAW) in oil palm full-sib families.

Trial	CV (%)	NBP	BWP	BAW
1	CV_{gm}	10.84	16.07	14.4
	CV_{gd}	18.78	27.83	24.94
	CV_{ex}	10.16	13.29	8.51
	CV_e	6.54	8.93	5.76
	CV_{gm}/CV_e	1.65	1.79	2.5
	CV_{gd}/CV_e	2.86	3.11	4.33
2	CV_{gm}	19.48	31.7	17.23
	CV_{gd}	33.74	54.9	29.84
	CV_{ex}	13.63	18.1	8.96
	CV_e	8.19	13.45	4.44
	CV_{gm}/CV_e	2.37	2.36	3.88
	CV_{gd}/CV_e	4.12	4.08	6.72
3	CV_{gm}	15.52	15	14.79
	CV_{gd}	26.88	25.98	25.62
	CV_{ex}	13.01	18.54	11.49
	CV_e	8.51	13.73	9
	CV_{gm}/CV_e	1.82	1.09	1.64
	CV_{gd}/CV_e	3.16	1.89	2.84
Joint	CV_g	17.87	29.12	20.80

CV, coefficient of variation; CVgm, genetic coefficient of variance among families; CVgd, genetic coefficient of variance within family; CVex, phenotypic coefficient of variance among plants in the experiment; CVe, environmental coefficient of variance; CVgm/ CVe e CVgd/CVe, relationship between the genetic coefficient of variance within and among families and the environmental coefficient of variance.

After ANOVA analysis, in which families were considered random effects, found the existence of genetic variability for all target traits and the possibility of gain with selection, the effect of fixed treatments was considered to identify the best families to constitute the new generation from crosses in the palm oil breeding program. Thus, the Scott-Knott test was performed ($p < 0.05$), and significant differences were found among families.

Families 5 and 15 were those which presented the highest mean values for all traits. For NBP, families 14 and 19 can be selected for use in pre-breeding programs. Families 22 and 23 can be selected for bunch average weight. The Scott-Knott test formed 2 groups for NBP and 3 groups for BWP and BAW. Using the Scott-Knott test, the controls (families 40, 41 and 42) were significantly inferior to the best families for the traits evaluated. This shows that the experimental families presented many alleles which can be introduced into varieties increase productivity, or they can be crossed among each other to generate new cultivars with a much higher yield than the current oil palm cultivars. The Scott-Knott test showed that the best families were 15, 5 14, 19, 22, 21, 28, 4, 37, 13, 3, 2, 11, 1, 36, 41, 9 and 32 for NBP; 5 and 15 for BWP; and 5, 15, 33, 22, 8, 39, 31, 2, 32 and 30 for BAW.

By using the Pearson correlation at 5%, positive correlations of 0.88 among NBP and BWP, of 0.35 between NBP and BAW, and of 0.72 between BWP and BAW were observed. These positive correlations are very important for selection because when families with high NBP are selected, families with higher values for other traits will be selected as a consequence. The closer the correlation is to one, the easier the selection of those joint traits is in a given family.

The Tocher's test cluster showed the formation of 7 groups (Table 7). Group 6 is formed by families 5 and 15, which were the best for the three traits in the Scott-Knott test. If the target of the breeder is to make converging crosses, the selection of those families is interesting because the population mean will be high and will generate a high mean in the next generation. However, a breeding program prioritizing crosses among materials with high means and presenting genetic diversity among individuals is desirable. Thus, to cross good and diverging genetic material, materials that complement each other are identified, and the non-additive fraction present in the genetic variance is utilized.

The UPGMA cluster method formed 6 groups (Figure 1) that are very similar to those formed by Tocher's cluster method (Table 7). Those groups were set by the local criterion and separated at a distance of 3.84, as suggested by Mojema (1977).

The dissimilarity measure (SINGH; SINGH, 1981) showed that BWP provided 71.26% of the genetic diversity among populations, BAW provided 22.48%, and NBP provided 6.25%. This shows that most genetic diversity among families results from BWP.
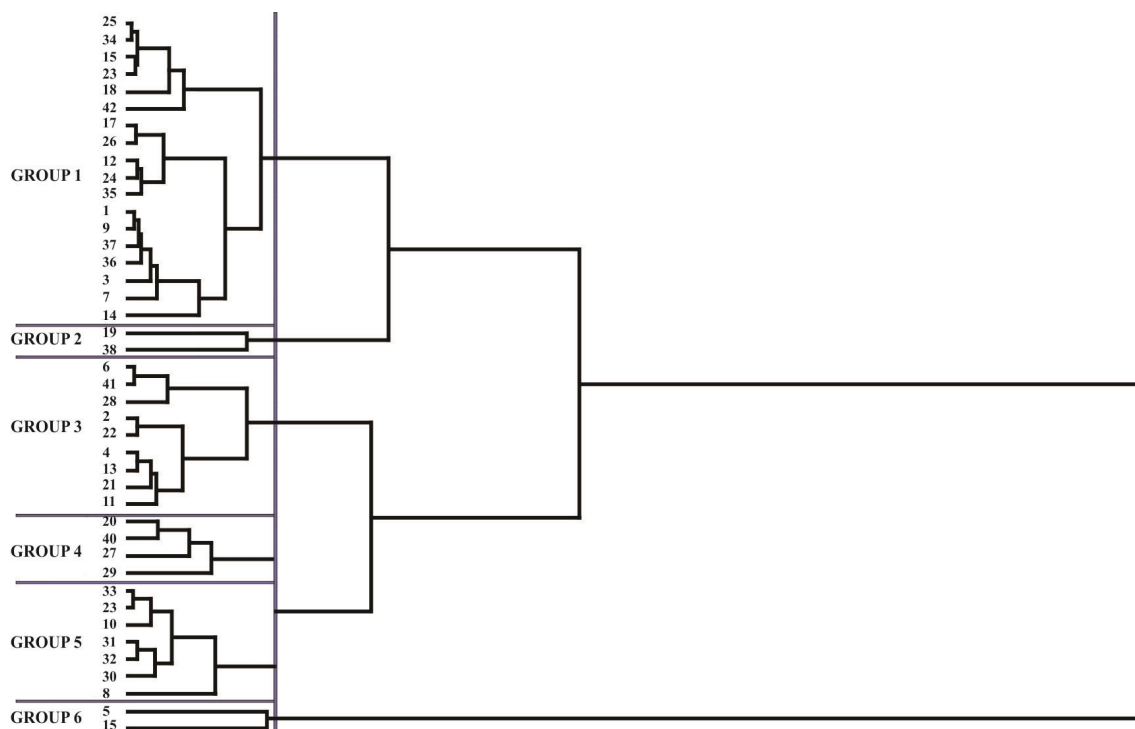
Figure 1. Dendogram by cluster method: average linkage among clusters (UPGMA).

Table 7. Cluster formation by Tocher's method and by UPGMA.

	TOCHER		UPGMA
CLUSTER	FAMILIES	CLUSTER	FAMILIES
1	30, 32, 40, 39, 41, 9, 34, 42, 28, 7, 6, 11, 1, 2, 4, 31, 3, 12, 19, 18, 25, 17	1	30, 32, 40, 39, 9, 41, 28, 34, 7, 42, 3, 37, 1, 6, 11, 4, 12
2	35, 38, 37	2	17, 25, 10, 23, 26
3	16, 24, 23, 20, 29, 10, 27, 22, 8	3	14, 19
4	13, 33, 21	4	2, 13, 8, 31, 33, 21
5	5, 15	5	20, 22, 27, 16, 24, 10, 29
6	14	6	35, 38
7	26	7	36
8	36	8	5, 15

Thus, if those families in a pre-breeding program are to be used to generate variability, selection has to be performed specifically for BWP because this is the trait with the greatest genetic variability. Moreover, BWP is the most agronomically important trait among the three analyzed traits.

Therefore, this study found that there is great diversity in interspecific hybrids; thus, a very high genetic gain can be expected for this culture with a breeding program. This work is important for showing that families may be used in pre-breeding programs aimed at achieving higher germplasm for the evaluated traits. Because the experimental families were generated from diverging crosses, most of the genes are in heterozygosis. In an oil

palm study, Luyindula et al. (2005) found that there is a high inbreeding depression rate for the number of bunches per plant and bunch weight.

Crosses between diverging parents with diverse genetic material are needed for generating variability in pre-breeding. This will generate a high mean of values in the progeny, thus facilitating the selection of higher plants.

Crosses among parents of families 15, 14, 22, 21, 28, 37, 33 and 39 could be potential crosses to generate variability in the next generation. These families could also provide high means of the traits evaluated in this study because they presented the highest means in the Scott-Knott test and belong to different groups by cluster analysis.

An individual with higher values than their parents can be generated in the next generation from crosses between families of different groups because there is a combination of alleles controlling each trait.

Following the identification of higher families, diallel crossing is an interesting method to discover which potential crosses will improve the mean values for all target traits of the next generation.

Conclusion

Families with great potential for diallel crosses among families 15, 14, 22, 21, 28, 37, 33 and 39 were identified, as cluster analysis grouped these families

into different groups and each have high means as shown by the Scott-Knott test. The existing genetic variability in the oil palm crop is important for breeding this culture, and the possibility of gain is high if divergent potential parents are used to provide a high mean in the next generation.

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