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Heterosis and Heritability of Chemical Quality of Butter Derived from Some Cocoa (*Theobroma Cacao* L.) Clones

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Abstract: Butter from dry cocoa beans of five cocoa (Theobroma cacao L.) clones and their intercrossed hybrids were analyzed for chemical quality (acid, peroxide and iodine) in order to gain insight on the heterosis and broadsense heritability values for these traits. Standard procedures were used for estimation of the lipid content and the indices. Hybrid F20 had the highest lipid content (59.7%) while the parent SCA12 and hybrid F12 had the lowest with 44.5 and 45% respectively. The acid index varied between 19.4 and 28.8 mg KOH/g, iodine index from 10.2 to 30.5 g/100g and peroxide index from 0.3 to 7.3 meq.g O₂/kg. The acid index was higher than recommended limits for edible oils, iodine index lower and peroxide index within the international norms for conventional oils. Hybrids F12, F40 and F79 presented a positive mid-parent heterosis for acid index while F14, F13, F20 and F79 showed it for peroxide index. With regards to best-parent heterosis, only F40, F13 and F79 for acid index and F14 and F13 for peroxide index showed positive heterosis effect. For iodine index, about 71 % of hybrids showed a positive midparent heterosis. The acid and peroxide indices showed high heritability (while the iodine index had moderate heritability) estimates and this heritability could be said to be cytoplasmic except for the reciprocal hybrids F13 and F79 (acid index). The Principal component analysis categorized two distinct groups of six individuals each. Quality characteristics were moderate for butter obtained from the first cluster and lower for the second one. When all these quality parameters are taken into account, F79 and its reciprocal hybrid F13 showed the highest hybrid vigour. Their parents can be therefore considered as the best combination for producing hybrids with high nutritional oil quality vigour.

Keywords: Cocoa butter, parameters of qualities, heterosis, heritability, Theobroma cacao L.

Introduction

Theobroma cacao L. (cocoa tree) is native to the Amazon region and cultivated in tropical regions throughout the world (Beckett, 1994; Ardhana and Fleet, 2003). This crop provides a substantial income for small holders in the tropics. Most of the cultivated cocoa trees in Cameroon were originally derived from old varieties introduced by German colonialists, and from second generation seeds obtained from new hybrid cultivars.

From the last decade and up till now, the trading of cocoa beans depends on the physical characteristics of the cocoa beans (*cut test*). Agro and pharmaceutical industries are interested on bioactive

compounds present in cocoa beans such as polyphenols and alkaloids (Effa et al., 2015). Compounds such as fatty acids and tocopherols (vitamin E) present in cocoa beans play a key role in the anti-oxidation process. Cocoa beans provide butter that is stable to oxidation because it is rich in antioxidant compounds. Cocoa butter is widely used in pharmacological and cosmetic products. It has an increasing international demand by cosmetic and pharmaceutical industries, and it is also used in chocolate manufacture.

Fats from cocoa butter are associated with a decrease in low-density lipoprotein (LDL) oxidation, oxidative stress, platelet activation, platelet function, and an

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increase in high-density lipoprotein (HDL) concentration, antioxidant status, together with an improvement in endothelial function (Andres-Lacueva et al., 2008).

One of the typical properties of cocoa butter is the occurrence of substantial quantities of 2-oleyl glycerides of palmitic and stearic acids. These triglycerides are mainly responsible for providing the valuable crystallization and melting characteristics so essential in providing sharp melting at body temperature in chocolate confectionery (Lipp and Anklam, 1998).

Cocoa beans used in the confectionery industry come from different geographical areas, and may have different chemical and organoleptic properties. The geographical influence on fatty acid composition had been shown by Kanematsu et al. (1978b) and Klagge and Sen Gupta (1990). Cocoa butter from Africa (Ghana, Ivory Coast) contains a significantly lower amount of oleic acid than that from South America (Ecuador, Brazil) and South East Asia (Malaysia, Java). Chocolate producers must therefore select and combine these beans in various proportions in order to meet certain quality standards and economic specification. This task can be avoided if a screening of some cocoa clones in terms of their butter quality can be done. So, some clones with high potential of butter quality can be exhibited and exploited at industrial levels. Since it is known that there are at least two parental clones in cocoa plantations, uncontrolled pollination will increase the number of unidentified hybrids. It will be very interesting then, to know if the quality of butter from cocoa hybrids can be predicted on the basis of those of its parental clones.

The aim of this study therefore is to estimate the heterosis and heritability of the chemical quality of butter derived from five cocoa (Theobroma cacao L.) clones and their intercrossed hybrids under the same environmental conditions in Cameroon, in order to acquire information needed for breeding.

Materials and Methods

Cocoa plant materials

Height Clones: Three local Trinitario (SNK13, SNK16 and SNK413), one Trinitario introduced from Trinidad (ICS40), and four Forestero (SCA12, UPA134, T79/467 and T79/501) from the gene banks of the Cameroon Cocoa Development Corporation (SODECAO) at the Mengang Station (South Cameroon) were used to create progenies. Crossings were done (Table 1) at the Mengang Station of SODECAO in May, June and July 2013 by manual pollination (Cilas, 1991).

Post-harvest treatment of cocoa beans

One thousand ripe cocoa pods from different parental cocoa clones and hybrids were harvested from the experimental plots of the SODECAO at Mengang Station. The ripe pods were split and beans obtained were fermented using the traditional heap method. The fermentation was done by putting the extracted cocoa beans in heaps on the fermenting surface covered with banana leaves. The heaps of beans were again covered with banana leaves and fermented for six days with opening and overturning every two days. The fermented cocoa beans were then dried under the sun on bamboo mats for twelve days.

Lipid extraction

Lipid extraction was carried out by the method described by Folch (Folch, 1957) modified by Xanthopoulou et al. (2009). After removing solvent using a rotatory evaporator, the oil obtained was dried under a stream of nitrogen and then stored in a freezer (-20° C) for chemical analyses.

Oil content

The oil content was determined by hot continuous extraction of the oil in a Soxhlet apparatus using hexane as solvent, according to AOAC (1980).

Parameters of oil quality

Standard procedures of French Association of Normalization (AFNOR, 1981), were used for the determination of the quality indices of the oils. These procedures were applied for the acid index (NFT60-204), iodine index (NFT60-203) and peroxide (NFT60-220).

Heterosis

The methods for calculating heterosis were according to Tang and Xiao (2013) as follows:

Mid-parent heterosis (MPH) = $[F1 - MP] \ge 100 / \mu$ Best-parent heterosis (BPH) = $[F1 - BP] \ge 100 / \mu$ Where , F1 is the hybrid value; MP is the mid-parent value of both parents; BP is the value of the best parent and μ denote is the average value of all parents and F1 combinations in the factorial mating design.

Heritability

Broad-sense heritability (h_{bs}^2) estimates of each cross were calculated for traits related to quality parameters (acid, iodine and peroxide indices) according to Phudenpa et al. (2004), using the following formulae: $\mathbf{Vp} = \mathbf{V}_{G} + \mathbf{V}_{E}$

$$\mathbf{h}^2_{\rm bs} = \mathbf{V}_{\rm G} / \mathbf{V}_{\rm G} + \mathbf{V}_{\rm E}$$

 $h^{2}_{bs} = [V_{F1} - (V_{P1} + V_{P2})/2]/V_{F1}$

Where: V_{F1} = the variance of any cross; V_{P1} = the variance of female parent and V_{P2} = the variance of male parent; V_G = genetic variance

Estimation of environmental variance (V_E) for any cross was calculated as $(V_{P1} + V_{P2})/2$. In each cross, variances between F1 and parents were obtained from the analysis of variance following a completely

randomized design, assigning replication as classes

Statistical analyses

The values are means of three replications. Where appropriate, the data were tested by one-way ANOVA using the software SPSS 18.0 for windows, followed by Tukey post hoc test. Principal component analysis (PCA) was performed to establish associations among chemical parameters by using the SPAD 5.5 statistical software package.

Results and Discussion Hand pollinisation tests

Height parental clones were used and 24 hybrids were expected to be obtained. Crossings yielding SNK16 and Forastero clones gave a percentage of success null. The same observation is noticed in SNK16-ICS40 crossing. Those clones coupled with Forastero or ICS40 are incompatible. This incompatibility can be due to genetic factors. Five of the 24 crossings were successful but pods obtained were lost due to Black Pod Disease or destroyers. undergoes Hybrid number 12 the 'wilt phenomenon". This phenomenon is characterized by the freeze of the development of young pods that yellowed and dried. The latter failed down onto the wind action and other physical reasons. Physiological (deficiency of organic material) and hormonal reasons have been advanced to explain the untimely drop (Mbondji, 2010). The development of his reciprocal hybrid (hybrid number 11) failed. Crossings number 9 and 10 gave low percentage of success of hand pollinisation. Furthermore, seven hybrids according to their percentage of success of hand pollinisation have been considered in this study (Table 2).

Total lipid contents

The lipid contents of the different cocoa clones and hybrids are presented on Table 3. Hybrid F20 had the highest fat content (59.7%) among all individuals while SCA12 parental clone (44.5%) and F12 hybrid (45%) had the lowest. These results were similar to those obtained in Cucurbitaceae seeds (42-57.3%) by Fokou et al. (2004). Very low oil yields (2-16.9%) were obtained with the cold extraction method, using methylene chloride/methanol (2/1 V/V) solvents. These yields were lower than those obtained by Idah et al. (2014) (25.5%) for the extraction of cashew nut oil seed using the same solvent mixture. In families B, C and D the offsprings had higher oil contents than those of their parents.

Parameters of Oil Quality

The acid index gives an overview of the amount of free fatty acids in the oil or butter which are responsible for the acidity and oxidability of oils to produce unpleasant odours. It indicates the degree of stability of vegetable oils. Parent SNK13 (28.3) and hybrid F20 (28.8 mg KOH/g of butter) had the highest acid values while the lowest was in parent SCA12 (19.4) (Table 4). The offsprings of group B showed acid values lower than their parents SNK13 and T79/501. The contrary was observed in family C. The acid indices of these samples (19.4-28.8 mg KOH/g) were higher than those of peanut oil (5.99) (Atasie et al., 2009) and some Cucurbitaceae seed oils (1.76-22.23mg KOH/g) (Fokou et al., 2009). According to Krishnamurthy (1982), the maximum value of acid index of edible oils is 15mgKOH/g of oil. The high values obtained in our samples can be due the process of oil production (fermentation, drying and extraction). In fact, during fermentation, fatty acids are released under the action of bacterial lipase oxidation.

The Iodine index indicates the degree of unsaturation of oils. It gives an idea on how the oil needs to be stored. The higher the iodine index, the higher the degree of unsaturation, and the shorter the shelf-life of the oil (Hui 1996). In our samples, this parameter varied from 10.2 to 30.5 g/100g of oil while red oils obtained from Amaranthus hybridus seeds have a high iodine value (100 g/100g) (Dhellot et al., 2006). The iodine index of our samples was lower than the norms (58-72 mgI2/100g) (NBF 01-005, 2006), indicating that these oils have low levels of unsaturated fatty acids.

In the cocoa butter from families A and B, the parents had iodine values lower than their offsprings. The iodine values of the hybrids from these 2 families were 1.4 times higher than those of their parents (Table 3). When compared to frying oils whose iodine indices are between 80-100g/100g of oil (Atasie et al.., 2009), cocoa butter cannot be considered as frying oil.

The peroxide index reflects the level of oxidation of fatty acids and indicates the degree of degradation of the butter. The lower the peroxide value, the better the quality of the butter. The lowest peroxide index was found in the hybrid F14 (0.3) while the highest was found in the parent ICS40 (7.3 meq.g O2/kg of butter) These peroxide values were lower than the international norms for conventional oils where the maximal value is 10 meq.02/kg (Codex Alimentarius, 1992; NBF 01-005, 2006). This shows that these oils are relatively more stable to oxidation, may be due to the lower content of unsaturated fatty acids in cocoa butter. The lower peroxide values can also be due to the high level of polyphenols and other antioxidant compounds (Vit E and K) in this butter (Kiritsakis, 1989). However, F13 and F25 hybrids showed higher peroxide values than those of their respective parents.

Heterosis effect of the parameters of quality of cocoa butter

The negative values observed for acid and peroxide indices expressed a positive heterosis. In fact, the nutritional quality of oil is inversely proportional to these two indices. The hybrids concerned therefore show heterosis effect and are said to be considered more vigourous than their parents. Hybrids F12, F40 and F79 showed positive mid-parent heterosis for acid index while F14, F13, F20 and F79 showed it for peroxide index (Table 5). Only F79 showed hybrid vigour for the two parameters. According to the bestparent heterosis, only F40, F13 and F79 for acid index and F14 and F13 for peroxide index showed positive heterosis effect.

On the other hand, the positive values observed for iodine Index indicate a positive heterosis effect because the nutritional quality of oil is proportional to the number of unsaturated fatty acids in the oil. The hybrids, F12, F13, F79, F20 and F40 therefore showed positive heterosis effect with F40 showing the highest hybrid vigour. When all these quality parameters are taken into account, F79 and his reciprocal one F13 showed the highest hybrid vigour. Their parents could therefore be considered as the best combination for producing hybrids with high nutritional oil quality vigour.

Heritability estimates

Heritability (h^2) is the percentage or proportion of a character transmitted from a parent to its offspring. It is considered as low (h2 < 0.1), moderate (0.1 < h2 < 0.3) or high (h2 > 0.3) (Lynch and Walsh (1998). Broad-sense heritability estimates for the different indices are presented on Table 6. According to classification of Lynch and Walsh (1998), the acid index showed high heritability for all the hybrids of the different families except F25 with had moderate heritability. Moreover, the reciprocal hybrids, F13 and F79 showed high and similar heritability estimates suggesting a nuclear heritability.

The heritability of iodine index is moderate in F12, F14, F25, F40 and F79. An investigation of the heritability of polyphenolic, anthocyanin and antioxidant capacity of cocoa beans derived from four cocoa clones and their offprings by Effa et al. (2015) found that no maternal effect was detected in the transmission of these metabolites. The iodine index was highly inherited in F13 (0.83) and F20 (0.44). There is a great difference between heritability of iodine index in F13 and F79, indicating maternal heritability.

The Peroxide index parameter was not heritable for F12, F20 and F25 hybrids. Their heritability estimates were zero. On the contrary, F79 showed moderate heritability while F13, F14 and F40 showed high heritability (Table 5). There was a great difference between heritability for F12 and F40 while

family C showed no heritability of peroxide index.

Principal component analysis was used to visualize the variations in the samples. The first three principal components generated from all the data represented 93.5% of the total variability. The acid and peroxide indices were the dominating features in the first principal component (54.0% of the total variability), while iodine index was the feature with the highest weight in the second principal component (28.9% of the total variability). The Peroxide index had the highest weight in the third principal component (10.6% of the total variability).

The analysis of two-dimensional scores plot in the space defined by PC1 and PC2 showed that the distribution of samples followed a pattern. The first group with three parents (SNK413, ICS40 and SCA12) and three hybrids (F40, F13 and F25) had high acid index. The second group mostly consisted of reciprocal hybrids (F12, F79 and F20) of the first group with two parents (SNK13 and T79/501) showed high iodine index and low peroxide index (Fig. 1). Therefore, the quality characteristics were low for butter obtained from six individuals of the first cluster and moderate for individuals of the second one.

Conclusion

This work whose aim was produce hybrids and evaluate the quality of the butter from these hybrids and their parents, taken from the clones of the cocoa farm of SODECAO at the Mengang station, and to study the heritability of these parameters of quality showed that, from the height parental clones, only three reciprocal families gave satisfactory results. Incompatibilities were observed in some of the clones. The different clones were rich in butter. The acid index was higher, iodine index lower than limits and peroxide index within the international norms for conventional oils. The butter from these samples is therefore not advisable for frying. The different hybrids had relative heterosis. The parameters of quality were highly inherited and their heritability was of nuclear origin for most hybrids. For the acid index, the parents T79/501 and SNK13 were the best parental combination. Heterosis values for acid index showed that F79 from this combination had higher hybrid vigour than the other offsprings. The couple ICS40 and SCA12 had the best parental combination for iodine index. Despite the low hybrid vigour of F13, this character is highly inherited maternally. The hybrid F79 had a high hybrid vigour for peroxide index but higher in F40 and F14. This character was the least transmitted.

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Heterosis and Heritability of Chemical Quality of Butter Derived from Some Cocoa (Theobroma Cacao L.) Clones

<u>List</u> Tab	of Tables le 1. Manual pollinisation realized	l in this study at	the Mengang	SODECAO stati	on.	
N°	Crossings / Back-crossings	Crossings realized	Nodes obtained	Mature pods obtained	% of success	Behaviour
1	(♀)SCA12×SNK13 (♂)	50	16	05	14.3%	Viable
2	(\bigcirc) SNK13× SCA12(\bigcirc)	26	09	00	0%	Failed
3	$(\stackrel{\bigcirc}{_+})$ ICS40×SNK16 ($\stackrel{\nearrow}{_{\bigcirc}}$)	25	-	-	0%	incompatible
4	(\bigcirc) SNK16×ICS40 (\bigcirc)	26	-	-	0%	incompatible
5	(♀) UPA134× SCA12 (♂)	50	16	03	6%	unavailable
6	(\bigcirc) SCA12×UPA134 (\bigcirc)	26	05	05	14.3%	unavailable
7	(♀)SCA12×SNK413 (♂)	50	16	07	10.8%	Viable
8	(\bigcirc) SNK413× SCA12 (\bigcirc)	35	13	06	17.6%	Viable
9	(♀) SNK16×SNK13 (♂)	65	09	01	2.7%	unavailable
10	(\bigcirc) SNK13×SNK16 (\circlearrowright)	34	06	02	5.9%	unavailable
11	(♀) SNK413×ICS40 (♂)	37	6	-	0%	Failed
12	(♀) ICS40×SNK413 (♂)	65	9	-	0%	Burned
13	(♀)SCA12×ICS40 (♂)	28	10	05	12%	Viable
14	(\bigcirc) ICS40× SCA12 (\bigcirc)	40	14	08	30.8%	Viable
15	(♀) SNK16×T79/467 (♂)	41	-	-	0%	incompatible
16	(♀) T79/467×SNK16 (♂)	26	-	-	0%	incompatible
17	(♀) UPA134×T79/467(♂)	25	05	01	4%	unavailable
18	(♀) T79/467×UPA134(♂)	25	03	-	0%	Failed
19	(♀) T79/501×SNK13 (♂)	25	10	06	24%	Viable
20	(♀) SNK13×T79/501 (♂)	50	06	03	12%	Viable
21	(♀) UPA134×SNK16 (♂)	25	-	-	0%	incompatible
22	(\bigcirc) SNK16×UPA134 (\circlearrowright)	25	-	-	0%	incompatible
23	(♀) SNK13×ICS40 (♂)	25	05	01	4%	Viable
24	(\bigcirc) ICS40×SNK13(\bigcirc)	25	-	-	0%	incompatible

Table 2. (Feneral	descripti	ion of	cocoa	crossings	retained	in this	study
Tuble 4. C	Jonorai	acourpu	ion or	cocou	crossings	retuined	in uno	bluuy.

Families	Crossings	Families	Back-crossings
	(♀) SNK 13 × T79/501 (♂)		(♀) T79/501 × SNK 13 (♂)
F13		F79	
F25	$(\stackrel{\bigcirc}{_+})$ SNK 413 × SCA12 ($\stackrel{\frown}{_{\circ}})$	F20	(\bigcirc) SCA 12 × SNK 413 (\bigcirc)
F12	$(\stackrel{\bigcirc}{_+})$ SCA 12 × ICS 40 $(\stackrel{\frown}{_{\bigcirc}})$	F40	(\bigcirc) ICS 40 × SCA 12 (\bigcirc)
F14	(\bigcirc) SCA 12 × SNK 13 (\bigcirc)		

	Cocoa clones/hybrids	Lipids (%)
Groups		
	ICS 40	54.05 ± 0.37^{de}
	SCA 12	44.54 ± 1.37^{a}
А	F12	44.98 ± 2.05^{a}
	F40	48.81± 0.71 ^{bc}
	T79/501	46.68 ± 0.93^{ab}
	SNK 13	50.82 ± 0.52^{cd}
В	F13	53.68 ± 1.41^{de}
	F79	$59.32 \pm 1.83^{\rm fg}$
	SNK 413	53.80 ±1.38 ^{de}
	SCA 12	44.54 ± 1.37^{a}
С	F25	55.74 ± 0.52^{ef}
	F20	59.70 ± 1.81^{de}
	SCA 12	44.54 ± 1.37^{a}
D	SNK 13	50.82 ± 0.52^{cd}
	F14	52.11 ± 5.04^{g}

Table 3. Lipid	contents of	different	cocoa	clones	and h	ybrids.

*Means with the same letter superscripts in the same column are not significantly different (P < 0.05).

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Groups	Cocoa	Acid index	lodine index	Peroxide index (magO ₂ /kg)	Saponification
	ciones/nybrius	(ingKOII/g)	(g12/100g)	(meqO2/kg)	(mgKOH/g)
	ICS 40	$24.78{\pm}0.41^{bc}$	$23.03{\pm}2.56^{cde}$	7.27± 0.31 °	171.71±16.05°
٨	SCA 12	19.40± 1.07 ^a	17.61 ± 4.80^{a}	3.97± 0.31 ^b	158.46±13.75 ^a
A	F12	25.45 ± 1.00^{bc}	$24.38{\pm}~2.04^{\text{ cde}}$	1.53± 0.21 ^b	188.49±21.45 ^{cd}
	F40	19.64± 0.71 ^a	30.48 ± 1.76 ^e	$4.07\pm0.31^{\text{ c}}$	168.53±22.56ª
	T79/501	$26.65{\pm}~1.40^{\rm~cd}$	11.51± 3.1 ^a	$0.40\pm0.40~^{a}$	194.41±20.4 ^d
р	SNK 13	$28.28{\pm}0.40^{de}$	21.00 ± 2.34 ^{cd}	1.033 ± 0.25 ^{ab}	189.43±23.4 ^d
D	F13	23.84 ± 0.70^{b}	18.29 ± 6.18^{abc}	4.37± 0.21 °	159.43±14.43 ^{bc}
	F79	$24.08 \pm 0.40^{\text{ b}}$	29.13±1.17 ^{cd}	0.80 ± 0.20 ^{ab}	175.43±22.22°
	SNK 413	25.25 ± 0.00^{bc}	10.16± 1.02 ^a	3.87± 0.50 °	142.43±19.22 ^b
C	SCA 12	19.40± 1.07 ^a	17.61 ± 4.80^{a}	3.97± 0.31 ^b	158.46±13.75 ^a
C	F25	$25.25{\pm}0.00^{bc}$	11.18± 2.03 ^{ab}	5.01 ± 0.25 ^d	179.43±15.41 ^{ab}
	F20	28.75± 0.00 ^e	19.30 ± 3.04^{bc}	1.09 ± 0.30 ab	185.43±21.42 ^e
	SCA 12	19.40± 1.07 ^a	17.61± 4.80 ^a	3.97± 0.31 ^b	158.46±13.75 ^a
D	SNK 13	$28.28 \pm 0.40^{\text{ de}}$	21.00 ± 2.34 ^{cd}	1.033± 0.25 ^{ab}	189.43±23.4 ^d
	F14	$28.05 \pm 0.70^{\text{ de}}$	16.59± 1.55 ^{abc}	0.30 ± 0.20 ^a	191.33±14.21 ^e

Table 4. Acid, iodine and peroxide indices of cocoa butter.

*Means with the same letter superscripts within the same column are not significantly different (P < 0.05).

Famille	HF(%)	Acid index	Peroxide index	Iodine index
	Hydrides		MPH/BPH	MPH/BPH
		MPH/BPH		
Α	F12	-13.2 /1.9	509.3/5.9	12.5/- 78.9
	F40	-12.3/- 20.7	11.6/32.3	79.2/- 44.0
В	F13	14.3/-15.7	-72.7/- 12.9	20.0 / <i>323</i>
	F79	-11.1/- <i>14</i> .8	-27.6 /38.7	50.0/- 22.6
С	F25	13.1/-0.4	28.0/10.0	-19.5/29.5
	F20	28.8/13.9	-72.2 /90.0	39.0/- 71.8
D	F14	17.7/-0.8	-88.0/-21	-14.0/-71

Table 5. Mid-parent heterosis (MPH) and Best-parent heterosis (BPH) of hybrids derived from crossings of five cocoa clones.

Table 6. Broad-sense heritability estimates for different index values.

Groups	h² Hybrids	Acid index	Peroxide index	Iodine index
Α	F12	0.32	0.22	0.00
	F40	0.50	0.17	0.43
В	F13	0.54	0.83	0.32
	F79	0.52	0.15	0.13
С	F25	0.27	0.26	0.00
	F20	0.34	0.44	0.00
D	F14	0.34	0.14	0.43
h ² n	nean	0.40	0.31	0.19

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