Some factors affecting fat content in cacao beans (**Theobroma cacao L.**), with emphasis on the effect of the pollinator parent*[1/]

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RESUMO

As sementes obtidas em dois experimentos de polinização controlada foram utilizadas no estudo de variação da porcentagem de gordura nas sementes de cacau. A análise dos resultados não permitiu detectar diferenças significativas entre as plantas de um mesmo clone. Uma correlação positiva e significativa (r = 0,56 a 0,66) foi encontrada entre o peso seco das sementes e a porcentagem de gordura, calculado com base nos totais das amostras de cada um dos clones. As determinações em sementes individuais mostravam correlações baixas e muitas vezes insignificativas. A variabilidade observada, com respeito a porcentagem de gordura, nas sementes obtidas nos diferentes cruzamentos e mesmo dentro de um único fruto, chegou a um máximo de 8 por cento.

Tanto o efeito do polem, como do clone materno, resultaram em diferenças significativas com relação a porcentagem de gordura. As determinações em sementes individuais mostraram diferenças consideráveis entre os diversos cruzamentos no que respeita a varição do caráter, enquanto que os recíprocos revelaram amplitude de variação semelhante. Estas observações indicam que o genotipo do zigoto poderá ser o fator principal na determinação da porcentagem de gordura nas sementes.

Introduction

ALTHOUGH the cacao crop is not a typical oil crop, its seeds contain about 50 per cent of a valuable fat. The demand for cacao butter has always been high, due to its various industrial applications e.g. in farmaceutical and cosmetical products. Already various researchers have paid attention to this important character. Vello (7), when measuring the fat content of hybrid progenies in Bahia, Brazil, discovered differences up to three per cent, suggesting however that many uncontrolled factors might have affected the differences. Alvarado and Bullard (1) indicated intermediate inheritance, when comparing three hybrid populations to their parent clones. Toxopeus and Wessel (6) describe how the season con affect fat content and showed furthermore a positive correlation between bean weight and fat content within a West-African farmers population. As first reported by Toxopeus and Jacob (5), pollination studies indicate that both the female and male parent might influence the fat content of the beans.

This study was aimed to further analyse the factors affecting fat content and to determine the variation between individual beans. Special attention was paid to the effect of the pollinator parent.

Material and methods

The beans analysed resulted from pollination experiments carried out in the clonal garden of the Cacao Research Center, CEPEC, in Itabuna, Bahia, Brazil. Eight different clones were crossed in a 4 by 4 reciprocal cheme (Table 4). Because little was known about the fat content of the clones, the choice of the parents was mainly based on variability in pod characters and bean weight. IMC 67, SCA 12, ICS1 and UF677 are important clones used for hybrid production and have a different parental background. The SIAL and SIC

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clones are selections from the local Amelonado population. The SIC clones belong to the local white beaned cultivar 'Catongo', wich reportedly contains 2 per cent more fat than the local purple population (personal communication, F. Vello). The success of the reciprocal part of the pollination scheme has been small, due to a heavy wilting of young fruits. This was notably more severe on the SIAL and SIC clones, probably because they had been heavily hand-pollinated for hybrid production in the years before, so that their physiological condition was poor

The pollinations were carried out in a few weeks of July 1970, so as to avoid seasonal influences. To protect the flowers from insect visits, glass tubes were placed on them before and after pollination. Each cross was made on one or more different mother trees. The fruits were all harvested in the same phase of ripening, *i.e.* when the colour changed clearly from green or red to yellow. Very irregular or damaged fruits were excluded from the experiment. Immediately after harvest the beans were peeled off and dried for 48 hours at 105°C, and then stored until fat analyses were done in the summer of 1972.

The possible effect of diffeernt female trees of one clone on the fat content of its beans was studied separately. Two or three different trees of SCA 12 were pollinated with each of three male parents. Pollinations of this experiments were performed in December 1970 and the fruits harvested in June 1971.

All fat analyses reported here were done by the low resolution nuclear magnetic resonance method (NMR). The equipment used was the Newport Instruments Quantity Analyser MK 1A of the Central Institute for Nutrition and Food Research T.N.O. in Zeist, Holland. The N.M.R. appeared to be a quick and accurate method for fat determinations in chocolate and related products (8). The method has also been used before for oil determinations of individual seeds of many oil crops (3). In our random samples of beans from the same population the NMR detected constantly about 1 per cent more fat than the traditional soxhlet method. This difference is most probably due to fat remaining in undestroyed cells, that is not extracted by the soxhlet metod (8). The NMR apparatus was equiped with two measure units: the 40 ml unit was used for the determinations on the random samples from the crosses, and the 2 ml unit was used for the individual bean measurements.

When sampling for fat analyses, the beans of each cross were first divided in 5 weight classes, each representing 20 per cent of the total variation in bean weight of the cross. Afterwards, two subsamples were chosen randomly within each weight class. Thus fat content and bean weight could be related as well as the average fat content per cross determined. Each subsample contained 7 to 16 whole peeled and dried beans, weighing together 12 to 16 grams, which was about the maximum to enter in the 40 ml measure unit. Between every 10 samples two controls containing pure cacao butter were measured for calibration purposes and to determine the accuracy of the method. The average difference for the two control samples was 0.36 per cent with a standard deviation of 0,33 per cent.

Individual beans were sampled from 4 crosses and their reciprocals. For the fat determinations they had to be crumbled to enter in the 2 ml measure unit. Beans with a bigger weight than 1 gram did not enter completely in the unit and in those cases a random sample from the crumbs was taken. The average difference between the two control tubes was for the individual bean measurements 0.50 with a standard deviation of 0.30.

Processing of the results was done by hand calculations and by computer, using programs for analyses of regression, correlation and variance.

Results

The effect of the tree on fat content

The possible difference between one tree and another of the same clone was studied by crossing randomly choosen SCA 12 trees with three different male parents. From each tree two to five fruits were harvested and the beans mixed together, from which two subsamples were taken for fat analyses (Table 1-a). The statistical analysis, as shown in Table 1-b, indicates the effect of the trees not to be significant. The P value for trees within polinator parents exceeds by far the 10 per cent level of significance. Notable is, that the effect of the pollinator is significant at the 5 per cent level.

Correlation between bean weight and fat content

Based on the records of 10 samples per cross, the correlation between dry peeled bean weight and fat content was established. The 10 samples were taken 2 by 2 from 5 bean weight classes, representing the whole range of variation in bean weight of the particular cross. Table 2-a shows a positive and significant correlation for nearly all crosses, existing however important differences between crosses. In order to calculate the overall correlation within crosses, the records of each sample were expressed as percentages of the average of each cross. Based on these relative figures of all 160 samples, the correlation appeared to be significant with $r = 0.56$ and $b = 0.06$ (Table 2-b). This indicates that within each cross an increase of 100 per cent in bean weight was accompanied by an average increase of 6 per cent in fat (absolute figures about 3 per cent fat content).

Table 1-b: *Analysis of variance*

source	*df*	*ms*	*F*	*P*
Pollinators	*2*	*9.498*	*14.47*	*0.027*
Trees within pollinators	*4*	*0.933*	*1.63*	*0.267*
Error	*7*	*0.570*		

Table 1-a —Fat analyses of some crosses, using 2 or 3 different trees for each combination

Cross	SCA 12 x IMC 67			SCA 12 x UF 613		SCA 12 x SIAL 98	
Tree code	a	b	c	d	e	f	g
Number of harvested beans	178	164	182	144	17	140	179
Fat content subsamples	51.0 54.4	54.2 54.1	56.5 54.4	53.2 53.7	54.3 54.7	51.9 52.6	50.7 52.2
Average per tree	54.2	54.1	55.4	53.5	54.5	52.2	51.4
Average per cross	51.6			54.0		51.8	

Table 2-b furthermore shows the correlation calculated for the average records of each cross being insignificant (correlation 1). However, when by subtraction the figures were made relative to the averages of each clone, the correlation becomes significant again (correlation 3). This difference disappears when excluding from the first calculation those clones with particular high bean weights, ICS 1 and UF 677 (correlation 2). This indicates, that the observed relation between bean weight and fat content may exist within certain populations, but not neccssarely between populations.

Table 2-a: Correlation and regression coefficients between fat content (in %) and peeled dry bean weight (in grams), as measured by 10 samples per cross, divided over 5 classes for bean weight. Model for linear regression is $y = a + bx$, where y is fat content and x is bean weigt. The significance of the correlation (r) and regression (b) coefficients is indicated by 1 or 2 dots (5 and 1% level respectively). The figures under "total" indicate the values as calculated over all subsamples per clone

		SIAL 105	SIAL 169	SIC 802		Total SIC 806
IMC 67	b	5.84	3.50	0.95	3.22	3.45
	r	0.93 ··	0.85 ·	0.57 ·	0.83 ·	0.56 ··
SCA 12	b	1.93	6.19	2.40	2.04	4.15
	r	0.40	0.91 ··	0.66 ·	0.67 ·	0.56 ··
ICS 1	b	x	x	2.48	0.04	3.00
	r			0.66 ··	0.01	0.57 ··
UF 677	b	2.63	2.98	x	4.17	3.48
	r	0.63 ·	0.75 ··		0.81 ··	0.66 ··

		IMC 67	ICS 1	UF 677	Total
SIAL 105	b	3.31	10.88	—2.44	10.34
	r	0.69 ·	0.75 ··	—0.15	0.65 ··

Fat analyses on individual beans.

From four crosses and their reciprocals, individual beans were sampled at random for fat determinations. The standard deviation for fat content within each cross varied from 0.8 to 2.2, showing the absolute variation for some crosses to be as high as 8 per cent (see Table 3). Even within single fruits, like in the cross SIC 806 × UF 677, we can observe this magnitude of variation. The beans of this cross varied from 49.3 to 56.9 in fat content. In general all crosses with UF 677 showed relatively high variation.

The correlation coefficient between dry bean weight and fat content appeared to be low and nearly always non-significant.

Effect of the pollinator on fat content and bean weight

Table 4-a shows the results as averages of the 10 samples per cross. The variation in fat content between both male and female parents was relatively small, but equal in magnitude. The maximum difference between females was 1.86 per cent and between males 1.77 per cent fat. The statistical analysis (Table 4-b) shows the effect of the females to be significant at the 5.5 per cent level. The effect of the pollinator parent was significant at the 10.3 per cent level.

Notwithstanding the positive correlation between bean weight and fat content as observed for the average figures per cross (Table 2-b), the effect of the pollinator parent on bean weight did not show to be significant.

Conclusions and discussion

The absolute variation in fat content between the crosses of our experiment was about 6 per cent, SIAL 105 × ICS 1 being lowest with 50.3 per cent and IMC 67 × SIAL 105 highest with 56.3 per cent. However even greater variation can be expecetd within the cacao crop. Alvarado and Bullard (1) showed differences from 47.9 to 55.4 per cent. Toxopeus (4) demonstrated a variation of 48.9 to 62.3 per cent in fat content when analysing bean samples of 26 crosses, all harvested within a few days time so as to avoid seasonal in-

fluence This variation, relatively being about 25 per cent, should be of interest to the cacao breeders since chocolate manufacturers use to pay more for a higher fat content (1).

For the individual bean measurements the correlation coefficients between bean weight and fat content appeared to be low and nearly always non-significant. However when samples were analysed consisting of several beans and chosen from different weight classes, the correlation becomes in general positive and significant (Table 2). Toxopeus and Wessel (6) showed a similar correlation to exist within a West-African farmers population. From our results it cannot be deducted if this correlation is mainly based on physiological or genetical factors. Table 4-b shows a positive and significant correlation for the average records per cross, when related to the average of each female clone. The pollinators inducing high fat content generally caused a higher bean weight too, indicating a genetical effect on the correlation. The differences in bean weight, however, were not such as to result in a significant effect of the pollinator parent on bean weight.

Important differences in variation of fat content of individual beans were observed between crosses, the reciprocals of wich showed similar magnitudes of variation (Table 3). This indicates that differences in variation of fat content may be determined genetically and that selection for genotypes inducing high variation should be possible. In our experiment UF 677 showed, either used as male or female parent, to induce the highest variability.

Our results confirm the effect of the pollinator on the fat content of the beans as first reported by Toxopeus and Jacob (5). Differences of 3-4 per cent in fat content were observed between male parents. The statistical analysis in one case detected significance at 2.7 per cent and in the other case at 10 3 per cent (Table 1-b and 4-b respectively). The SIC and SIAL clones, used as male parents in Table 4-a, are all selections from the local Amelonado population. We consider those clones to be alike in their inherent fat content in such a degree as to diminish the apparent effect of the pollinator parent

Table 2-b.—Overall correlation and regression coefficients for dry bean weight versus fat content. For the correlations from 1 to 4 the absolute fat content and bean weight were used, for the 5th correlation, however, the data were transferred into percentages of the averages per cross.

	r	b
1 — for the averages of all crosses (see table 4-a)	0.44	1.58
2 — idem 1, excluding the crosses on ICS 1 and UF 677	0.81 ··	17.71
3 — for all crosses, but related to the average per female clone by subtraction	0.76 ··	11.04
4 — idem 3, excluding the crosses on ICS 1 and UF 677	0.76 ··	12.57
5 — for all 160 samples, but expressed as percentages of the average records of each cross (= 100)	0.56 ··	0.06

Observing the results some indications for practical use might be considered. First, when screening trees or clones on fat content, the effect of the pollinator will have to be taken in account and preferably all material should be crossed with the same male parent. Second, we may conclude from the significant effect of the pollinator, that the genotype of the bean might be the main factor in determining its fat content, indicating that differences between individual beans may also be inheritable. For the maize *(Zea mays L.)* crop this has been demonstrated, and Bauman (2) achieved important progress by single kernel selection for higher oil content. To investigate this possibility for cacao, a non destructive method for individual bean determinations will have to be developed. The NMR is a destructive one, because the beans have to be dried under 5 per cent humidity wich will kill the seed. Possibly indirect measurements using differences in specific gravity or

Table 3.—Fat analyses on individual beans of four crosses and their reciprocals.*

Crosses	SIAL 105 x IMC 67	reciprocal	SIAL 105 x UF 677	reciprocal	UF 677 x SIC 806	reciprocal	IMC 67 x SIC 806	reciprocal
Number of fruits sampled	13	15	9	6	7	1	13	1
Number of beans analysed	30	30	30	29	31	14	30	18
$\bar{y}$	53.4	55.3	53.6	54.3	55.9	54.1	55.8	54.2
S_y	1.33	1.27	2.19	2.21	2.13	2.01	0.84	1.17
r	0.43 ·	0.18	0.05	0.39 ·	0.29	—0.07	0.20	—0.21

* $\bar{y}$ = average fat content of all beans measured

s_y = standard deviation of fat content and r = the correlation coefficient between fat content and dry bean weight (significance at 5% indicated with one dot)

Table 4-a.—Results of the pollinations and fat determinations. Top figure: number of fruits harvested, middle figure: fat content and lower figure: dry bean weight per cross. The fat content and bean weight were determined as averages of the records of 10 samples per cross, each of which contained 7 to 16 beans

	SIAL 105	SIAL 169	SIC 802	SIC 806	average fat content and bean weight
IMC 67	15 56 39 0 91	15 54 09 0 82	6 56 07 0 98	13 55 81 0 77	55 59 0 87
SCA 12	13 52 97 0 76	15 52 92 0 79	14 54 78 0 87	13 54 28 0 81	53 73 0 81
ICS 1	0 (54 32)* (1 76)*	0 (53 52)* (1 70)*	9 54 41 1 70	14 55 87 1 88	54 53 1 76
UF 677	6 54 52 1 73	14 54 48 1.62	0 (55 66)* (1.76)*	7 56 11 1 78	55 19 1.72
Average fat content	54 54	53 76	55.23	55 52	
Average bean weight	1.29	1.23	1 33	1.31	

reciprocals	IMC 67	ICS 1	UF 677	average fat content and bean weight
SIAL 105	13 54 38 0 87	11 50 32 0 68	9 52 29 0 77	52 33 0 77

* Figures calculated by the missing plot technique

the half bean technique will prove to be adequate. If indeed single beans transmit their fat content to the trees growing from them, this will be of substantial aid in accelerating breeding progress in the perennial cacao crop.

Summary

The beans resulting from two pollination experiments were used for the study of some sources of variation on the fat content of cacao beans. No significant differences between trees of the same clone were found. A possitive and significant correlation ($r=0.56$ to 0 66) between bean weight and fat content existed within the bean populations as a total for female clones. For individual bean measurements, however, this correlation was low and often insignificant. Absolute differences in fat content of individual beans within a cross or even within one fruit could be high as 8 per cent.

Female and male parents both affected significantly the fat content of the beans. Also the fat determinations on individual beans showed considerable differences in variation of fat content between crosses, the reciprocals of which always showed the same magnitude of variation. So it would seem, that the genotype of the zygote may be a main factor in determining its fat content.

Table 4-b.—The analysis of variance for parental effects on fat content (the reciprocals are excluded).

source	df	ms	F	P
Female parent	3	2 495	4 55	0 055
Male parent	3	1.770	3 23	0 103
Error	6	0.548		

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Resumen

Las semillas obtenidas en dos experimentos de polinización controlada fueron utilizadas en el estudio de la variación de porcentajes de grasa en las semillas de cacao. El análisis de los resultados no permitió detectar diferencias significativas entre las plantas de un mismo clon. Se encontró una correlación positiva y significativa ($r = 0,56$ a $0,66$) entre el peso seco de las semillas y los porcentajes de grasa, calculada basándose en los totales de las muestras de cada uno de los clones. Las determinaciones en semillas individuales mostraban correlaciones bajas y muchas veces sin significación. La variabilidad observada, en lo que se refiere a porcentajes de grasa en las semillas obtenidas en los diferentes cruzamientos, y también dentro de un solo fruto, llegó a un máximum de 8 por ciento.

Tanto el efecto del polen, como el del clon materno, resultaron en diferencias significativas con relación a porcentajes de grasa. Las determinaciones en semillas individuales mostraron diferencias considerables entre los diversos cruzamientos en lo que respecta a la variación del carácter, mientras que los recíprocos revelaron una amplitud semejante de variación. Estas observaciones indican que el genotipo del zigote podrá ser el factor principal en la determinación de los porcentajes de grasa de las semillas.

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