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Diversity of cacao fat in germplasm from Rondônia, Brazil

Caio Márcio Vasconcellos Cordeiro de Almeida[1], Valdir Alves Facundo[2], Henrique Otávio Braga Brazil[2], Luiz Antônio dos Santos Dias[3], José Raymundo Pires Medeiros[4], and Jorge Luiz Andrade Costa[3]

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ABSTRACT - *Chocolate is food of high nutritional value made from the fat extracted from cacao beans. Fat content and melting point are features of utmost importance for the chocolate, pharmaceutical and cosmetics industries. This paper quantified the cacao germplasm diversity from Rondônia for these two attributes. Expressive diversity was detected among the 102 evaluated clones in April/May 2003 for fat content and melting point. The mean fat content was 55.53% (51.04 to 58.19%) and the mean fat melting point 29.55 ºC (25.60 to 32.53 ºC). The accessions from wild populations in Rondônia were outstanding for both attributes. The two periods (September/October 2001 and April/May 2003) affected the evaluated clones differently. The plot of principal components analysis showed expressive variability and was, to a certain extension, able to separate wild from domesticated accessions.*

Key words: cacao germplasm, diversity, fat content and melting point, genetic breeding.

INTRODUCTION

Cacao (*Theobroma cacao* L.) for chocolate fabrication has been cultivated in the Brazilian Amazon region since 1670 (Alden 1974). It is highly nutritive food owing to its high sugar, fat and protein contents. Today's cacao agribusiness in the Amazon is represented by around 106 thousand hectares of cacao stands, of which 75% are concentrated in Pará and Rondônia according to Mendes (1998). It was first planted in 1971, in the state of Rondônia, which is third in dry bean yield (17.8 thousand t year^{-1}). Around 615 accessions, mostly wild populations from the state itself, are maintained in the genebank of the Experimental station of Ouro Preto. Their qualitative characteristics still need to be evaluated.

Cacao fat, extracted from the beans, is used predominantly in chocolate production. It is the most valuable product of the fruit of the cacao. Fat of cocoa is the most relevant feature for food, pharmaceutical and cosmetics industries. It is therefore of economic interest to deepen the understanding of the chemical composition and physical characteristics of cacao fat, particularly in the case of chocolate bars. When made of fat with a low melting point the bars soften up on warm days. Different genotypes and seasonal climatic variations can additionally affect the fat composition, whose content in the beans is fairly variable. Pires et al. (2003), evaluated 490 cacao accessions and observed great variability in fat content (45.4 to 60.3%) and possibilities of progress with selection. In the south of Bahia, higher temperatures during fruit development

[1] CEPLAC/SUPOC, Avenida Governador Jorge Teixeira, 86, Bairro Nova Porto Velho, 78.906-100, Porto Velho, RO, Brasil
[2] Departamento de Química, Universidade Federal de Rondônia (UFR), BR 364, Km 9,5, 78.900-500, Porto Velho, RO, Brasil
[3] Departamento de Fitotecnia, Universidade Federal de Viçosa (UFV), 36.570-000, Viçosa, MG, Brasil. *E-mail: lasdias@ufv.br
[4] Estação Experimental Ouro Preto - ESTEX-OP/CEPLAC, BR 364, Km 325, 78.950-000, Ouro Preto do Oeste, RO, Brasil

contributed to a higher saturation and, consequently, hardness of the fat. Fruits that developed in the colder months had a lower fat melting point than fruits grown under higher temperatures. Powell (1984) observed that the climatic conditions can be determinant for the chemical and physical properties of cacao fat. Indeed, the fat content is strongly affected by environmental influence, presenting low narrow-sense heritability (12.5%), as estimated by Pardo and Enriquez (1988).

In the Brazilian Amazon region, studies showed the effect of the genotype on fat content and melting point. Lambert et al. (1999a), analyzing cacao Amazon genotypes, evidenced a great variability in fat content (49.1 to 59.4%). The studies further showed that commercial cacao from the Amazon region meets the chocolate industry's standards (Ribeiro 1988, Ávila and Dias 1993). The present study aimed at a quantification of the diversity in germplasm from Rondônia for cacao fat content and melting point. This information is crucial for the generation of superior clonal varieties and as a guideline in genetic improvement of the species.

MATERIALS AND METHODS

Clone Accessions

The evaluated clonal accessions were from the cacao genebank Experimental Station ESTEX (lat 10º 44' S, long 62º 13' W, and alt 280 m asl), in Ouro Preto do Oeste, Rondonia. According to the Köppen classification, Rondonia has a rainy tropical climate of the Aw type–with mean temperatures above 18 ºC in the coldest month and a well-defined dry period. The region of Ouro Preto do Oeste presents mean annual precipitation values of 1940 mm, a mean temperature of 24.6 ºC and relative air humidity of 81%. The soil is predominantly podzolic mesotrophic and of intermediate natural fertility.

Of the 615 accessions established, 207 were selected for fruit and seed characterization Almeida et. al (2005). One hundred and two of these accessions were evaluated in the present study, of which 2 were accessions of series BE, 81 of series CAB, 14 of series EEOP, 2 of series MA, 2 of series POUND and IMC 67 (Table 1). The criterion used for the selection of these accessions was the presence of ripe and healthy fruits, that is yellow and free-diseased synthom fruits, for the characterization of defined periods regarding the water availability: April/May – period in which fruits developed without water stress and; September/October – when fruits developed under occurrence of water stress Almeida et. al (2005). Samples of September/October 2001 from 36 accessions and samples of April/May 2003 from 102 accessions were evaluated for fat content (%) and fat melting point (ºC), resulting in 29 common clones in the two periods.

Laboratory analyses

For each sample, 50 normal unfermented seeds were taken randomly from the fruits. The pulp was extracted in a blender and dried to constant weight at 105 ºC. The beans were then peeled and their cotyledons sent to the Chemistry Department of the Federal University of Rondônia - UNIR, in Porto Velho, for laboratory analyses. Each sample was ground to homogenous cacao powder and thereafter dried at 105 ºC for 20 hours. A sub-sample of 5 g was digested in HCL 4 N solution, filtered and washed with warm water. The digested material was placed in a Soxhlet flask and subjected to extraction with petroleum ether under reflux for 5 hours in a Soxhlet extractor in three replications per sample. The results were expressed in percentage of fat of dry matter. The melting point was determined by the open capillary method (BSI 1958), with three replications per sample.

Statistical analyses

The two evaluated characteristics were analyzed for normal distribution. The existing slight deviations from normality would not justify the use of transformation functions. Consequently, the mean original data were subjected to analyses of variance in a factorial scheme, considering the interaction clones x periods as error. The accession means were compared by the Scott-Knott test (Scott-Knott1974). This kind of mean test allows to analyse a great number of treatment and clustered them in groups of means similar statistically. Analyses of principal components were also performed with the mean original data. A plot was constructed with the two components for the multivariate analysis of diversity Dias (2006). All analyses were run on softwares SAS (1989) and Statistica (1997).

RESULTS AND DISCUSSION

Highly significant ($P<0.01$) differences were detected among the 102 evaluated clones for fat content

Table 1. Code and origin of the accessions

Code	Origin
BE	Belém, district. Accessions collected in 1965 in the surroundings of Belém, PA
CAB	Cacao from the Brazilian Amazon: i) CAB 8 to 13 - accessions collected in 1976 in the Ji-Paraná river basin amongst wild populations on the Muqui farm, Presidente Médici, RO; ii) CAB 35 to 42 - accessions collected in the region of Santarém, PA; iii) CAB 218 to 411 - accessions collected in 1981 and 82 in the Jamari river basin, amongst wild populations in the district of Ariquemes, RO; iv) CAB 417 to 473, accessions collected in 1983 in the Ji-Paraná river basin, amongst wild populations in the districts of Jaru, Ouro Preto do Oeste, Ji-Paraná, and Mirante da Serra, RO
EEOP	Experimental station in Ouro Preto. Accessions selected in 1977, on a plantation of hybrid varieties in José Wensing' farm, Ouro Preto do Oeste, RO, considering the criterion witches' broom tolerance
IMC	Iquitos Mixed Calabacillo. Accession selected in 1938 by JF Pound, in Iquitos, Peru
MA	Manaus, district. Accessions collected in 1965 on the Careiro island, Careiro, AM
POUND	Family name of JF Pound. Accessions selected in 1943 by JF Pound in Iquitos, Peru

and melting point collected in the period April/May 2003, indicating a significant diversity in the accessions (Table 2) The coefficients of variation (CV) presented very low values (0.21 and 0.22% for fat content and melting point, respectively), as expected in a laboratory study under uniform experimental conditions.

The mean fat content was 55.53% (Table 2) with an amplitude of 51.04% (clone CAB 394) to 58.19% (clones CAB 424 and CAB 453) (Table 3), expressing considerable variability. The Scott-Knott test at 5% probability (Table 3) detected 23 groups. The largest group of best-performing accessions consisted of the clones CAB 424, CAB 453, CAB 245, and CAB 452, all with over 58.0% fat content from the Jamari and Ji-Paraná river basins, RO. This set can be amplified by clones with means over 56.9%, which are CAB 226, CAB 261, POUND 7, CAB 237, POUND 12, CAB 429, CAB 443, CAB 378, CAB 253, CAB 242, EEOP 15, CAB 219, EEOP 6, CAB 427, and EEOP 13. The pattern established by the chocolate industry however requires 56 to 58% (Anonymous 1984). In this case, considering the minimum content of 56.0%, 36 other clones can be added to the afore-mentioned: BE 10, MA 11, 7 clones of the series EEOP and 27 others of the CAB series. Of the outstanding 55 clones, 39 (BE 10, MA 11, CAB 40, and CAB 42 - 70.9%) are originated from wild populations from the state of Rondonia (series CAB), four from populations of the Brazilian Amazon with a certain domestication level, 10 of selections made on a plantation of hybrid varieties in Ouro Preto do Oeste, Rondonia (series EEOP) and two from selections (POUND 7 and POUND 12) made in Iquitos, Peru.

Table 2. Summary of the analyses of variance for fat content and melting point in 102 cacao accessions, evaluated in April/May 2003

Sources of variation	df	Mean Squares	
		Fat content (%)	Melting point (ºC)
Clones (C)	101	8.6382**	5.8089**
Error	204	0.0152	0.0042
Mean (C)		55.53	29.55
CV (%)		0.22	0.21

** $P < 0.01$ by F test

In a comparison of the contents observed for clones BE 9, BE 10, IMC 67, MA 11, MA 14, POUND 7, and POUND 12 with those obtained by Pires 2003 in southern Bahia, the contents of the present study are predominantly superior, with variations of nearly 4%, especially for BE 10 and MA 11. In the Ribeira valley, São Paulo, Tucci et al. (1999) observed contents of 55.3 and 51.7% for clones IMC 67 and POUND 7, respectively, while in our study these contents were 55.3 and 57.4%. These differences may be ascribed to the climatic differences between the evaluated regions and the effect of pollen, as observed by Pires (2003), since the sampled fruits were open-pollinated. Summing up, data indicate that these clones have a good genetic potential as genepool for cacao improvement programs.

For the fat melting point a mean of 29.55 ºC (Table 2) and amplitude of 25.6 ºC (clone CAB 13) to 32.53 ºC (CAB 372) (Table 3) were observed. Thirty-five groups were formed in the comparison of the clone means (Table 3). The group of best-performing clones contained CAB 372, with melting point at 32.53 ºC, followed by four

Table 3. Means of fat content and melting point evaluated in April/May 2003, in 102 cacao accessions

Number	Clones	Fat Content (%)	Fat Melting point (°C)	Number	Clones	Fat Content (%)	Fat Melting point (°C)
1	BE 9	53.30 q[1]	29.33 t	52	CAB 431	54.24 o	31.67 e
2	BE 10	56.49 f	29.63 r	53	CAB 432	54.90 m	31.20 h
3	CAB 8	51.87 u	28.07 D	54	CAB 433	55.53 j	30.80 j
4	CAB 9	55.15 l	27.73 E	55	CAB 434	55.15 l	29.63 r
5	CAB 10	51.10 x	31.13 h	56	CAB 437	54.34 o	32.27 b
6	CAB 11	56.80 e	30.50 k	57	CAB 439	56.53 f	30.30 m
7	CAB 12	54.33 o	30.93 i	58	CAB 443	57.20 c	30.53 k
8	CAB 13	51.73 u	25.60 K	59	CAB 224	56.09 h	29.83 q
9	EEOP 10	56.61 f	30.00 o	60	CAB 444	56.32 g	30.03 o
10	EEOP 11	54.71 n	29.30 t	61	CAB 447	52.70 r	32.00 d
11	EEOP 12	55.90 i	28.10 D	62	CAB 450	56.10 h	28.27 C
12	EEOP 13	56.95 d	28.70 z	63	CAB 225	54.52 n	32.13 c
13	EEOP 15	57.04 d	28.43 B	64	CAB 452	58.14 a	28.70 z
14	EEOP 16	56.18 g	30.20 n	65	CAB 453	58.19 a	29.70 r
15	EEOP 19	56.88 e	29.03 v	66	CAB 456	56.29 g	29.10 v
16	EEOP 21	56.10 h	28.83 x	67	CAB 226	57.76 b	29.23 u
17	EEOP 22	56.57 f	30.20 n	68	CAB 465	56.51 f	30.10 o
18	EEOP 4	55.55 j	29.50 s	69	CAB 470	55.43 k	27.80 E
19	EEOP 5	54.50 n	29.70 r	70	CAB 473	55.37 k	29.40 t
20	EEOP 6	56.98 d	29.23 u	71	CAB 228	52.16 t	28.70 z
21	EEOP 7	56.68 f	32.40 b	72	CAB 229	55.27 l	30.87 i
22	EEOP 8	56.70 f	29.90 p	73	CAB 230	55.54 j	27.03 G
23	IMC 67	55.29 l	29.30 t	74	CAB 231	56.85 e	28.63 A
24	MA 11	56.82 e	28.20 C	75	CAB 232	51.56 v	26.90 H
25	MA 14	55.11 l	30.83 i	76	CAB 233	52.22 t	29.57 r
26	POUND 12	57.30 c	29.50 s	77	CAB 234	55.56 j	31.37 g
27	POUND 7	57.41 c	29.30 t	78	CAB 237	57.35 c	27.60 F
28	CAB 389	56.21 g	30.07 o	79	CAB 240	54.62 n	26.70 I
29	CAB 390	56.29 g	30.30 m	80	CAB 242	57.10 d	29.63 r
30	CAB 392	56.07 h	29.60 r	81	CAB 245	58.15 a	27.83 E
31	CAB 218	56.15 h	29.17 u	82	CAB 246	56.00 h	29.80 q
32	CAB 394	51.04 x	29.10 v	83	CAB 248	51.81 u	26.37 J
33	CAB 397	56.40 g	29.67 r	84	CAB 249	56.13 h	30.77 j
34	CAB 399	54.30 o	32.37 b	85	CAB 250	55.23 l	31.27 g
35	CAB 219	56.99 d	30.40 l	86	CAB 252	55.91 i	27.53 F
36	CAB 400	56.31 g	29.77 q	87	CAB 253	57.13 d	28.47 B
37	CAB 402	56.24 g	30.70 j	88	CAB 261	57.64 b	27.77 E
38	CAB 403	56.41 g	29.20 u	89	CAB 371	55.33 k	30.23 n
39	CAB 409	53.93 p	29.40 t	90	CAB 372	52.56 s	32.53 a
40	CAB 410	56.83 e	30.83 i	91	CAB 373	53.15 q	28.23 C
41	CAB 411	52.43 s	30.50 k	92	CAB 374	54.51 n	30.10 o
42	CAB 417	56.43 g	31.50 f	93	CAB 375	55.20 l	29.37 t
43	CAB 418	55.76 i	30.30 m	94	CAB 376	55.99 h	28.07 D
44	CAB 419	52.21 t	31.20 h	95	CAB 378	57.16 d	28.83 x

to be continued

Number	Clones	Fat Content (%)	Fat Melting point (°C)	Number	Clones	Fat Content (%)	Fat Melting point (°C)
45	CAB 420	56.04 h	28.30 C	96	CAB 379	54.02 p	28.03 D
46	CAB 424	58.19 a	28.60 A	97	CAB 380	56.24 g	31.10 h
47	CAB 425	55.25 l	29.17 u	98	CAB 381	56.66 f	29.37 t
48	CAB 426	56.10 h	28.83 x	99	CAB 35	55.01 m	28.23 C
49	CAB 427	56.95 d	32.33 b	100	CAB 37	55.38 k	30.53 k
50	CAB 428	53.19 q	28.23 C	101	CAB 40	56.15 h	28.80 x
51	CAB 429	57.29 c	29.77 q	102	CAB 42	56.62 f	28.70 z

[1] Means following by the same letter do not differ from each other by Scott-Knott (1974) test at 5% of probability

groups comprising six accessions: EEOP 7, CAB 399, CAB 427, CAB 437, CAB 225, and CAB 447, all with values equal to or more than 32.0 ºC. Considering randomly those of the upper third, i.e., over 30.1 ºC, ten other groups with 26 clones were included: MA 14, EEOP 16, EEOP 22, and 23 clones of series CAB. An analysis of these 33 clones of the upper third show that 28 (84.8%) of them were originated in equal shares from wild populations of the state of Rondônia (series CAB) of the Jamari and Ji-Paraná river basins, two from populations of the Brazilian Amazon with some degree of domestication (MA 14 and CAB 37) and three from selections realized on a plantation of hybrid varieties in Ouro Preto do Oeste (series EEOP). Highly significant differences (P<0.01) were also detected among the 29 clones evaluated simultaneously in September/October 2001 and April/May 2003 for fat content and melting point as well as a differentiated performance in the periods (Table 4). The periods influenced the evaluated clones differently. The coefficient of variation also presented low and close values.

Table 4. Summary of the joint analyses of variance considering periods of time, for fat content and melting point, in 29 cacao accessions

Sources of variation	df	Mean Squares: Content (%)	Mean Squares: Melting point (ºC)
Clones (C)	28	8.8710 **	4.5753**
Periods (P)	1	475.8294 **	369.6147**
CxP	28	4.4640 **	2.2364**
Clones/P	56	6.6675 **	3.4059**
Clones/P1	28	9.3310 **	3.1026**
Clones/P2	28	4.0040 **	3.7092**
Pooled error	116	0.0183	0.0062
Mean		53.78	28.17
CV (%)		0.25	0.28

**P < 0.01 by F test

The mean fat content of the 29 evaluated clones was 52.12% in September/October 2001 and 55.37% in April/May 2003, while the melting point of the fat presented means of 26.71 and 29.79 ºC, respectively, in the same periods. It is known that a substantial part of cacao fat is synthesized from 120 days after pollination on (Lehrian and Keeney 1980) and that variation in the air temperature during fruit development affects the chemical fat composition and consequently the physical traits (Lambert et al. 1999b). Based on the fact that July/August 2001 corresponds to a period of over 120 days after pollination of the fruits sampled in September/October and that February/March 2003 corresponds to the same period for those sampled in April/May, we observe mean minimum temperatures of 19.6 and 22.6 ºC, respectively, in the cited periods. This information suggests that variations in the environmental temperature during the phase of fruit maturation affect the chemical composition of the fat (content and melting point) and seems to indicate that reductions in the mean minimum temperature, in the period of more than 120 days after pollination, reduce the fat content and melting point of the evaluated clones. Lambert et al. (1999b) made similar observations in southern Bahia.

Mean minimum temperatures below 19-20 ºC are frequent in the western Amazon region in the months June to August, owing to the occurrence of the phenomenon of chills, cold spells coming from the Antarctic regions. Under these circumstances, we assumed that cacao beans from harvests realized between July and October presented, predominantly, lower values of fat content and melting point compared to those harvested in other periods, so the end product is of low quality. Data of the cacao yield distribution of ESTEX-OP regarding the accessions selected on plantations of hybrid varieties (series EEOP) show that the harvest in the cited period can reach between 13 and 38% of the annual yield volume.

The analysis of the means of the effect of periods also allowed the visualization of variations between 0.6 and 5.6% for fat content and 2.2 and 4.5 ºC for fat melting point of the 29 evaluated clones. Four accessions (CAB 08 and 10, EEOP 5 and 19) stood out since they presented a lower sensitivity to temperature variations for fat content. The variations observed in the contents were less than 1.0%. EEOP 19 attained the level required by the chocolate industry, that is, 56%. For the fat melting point, CAB 08, 10, 12, 410 and EEOP 22 stood out, with variations between 2.2 and 2.6 ºC. The values for fat content in the accessions CAB 08 and 10, despite present in both groups, was little attractive (< 52.0%) and the fat melting point low to intermediate (< 31.2 ºC).

The plot of the principal components analysis (Figure 1), with 102 accessions evaluated in April/May 2003, revealed two large groups: the first consisted of the wild populations of the state of Rondônia (series CAB) and the second of half-wild populations from different origins (series BE, EEOP, IMC, MA, POUND, and CAB 35 to 42). The wild accessions of Rondônia comprise the Jamari and Ji-Paraná river basins. Several wild subpopulations found in upland areas in today's districts Ariquemes, Jaru, Ouro Preto do Oeste, Ji-Paraná, Mirante da Serra and Presidente Médici were collected in 1976, 1981, 1982 and 1983. Due to the similarities among these subpopulations and the absence of geographical or ecological barriers between them, Almeida et al. (1995) suggested the possibility that they could belong to one and the same genepool.

In the present study, the accessions appear dispersed in the plot but form a cloud of points concentrated basically in the squares I, II and IV. A clear distinction between the basins of the rivers Jamari and Ji-Paraná is not possible, while there are, on the contrary, areas of interconection. Some subgroups are

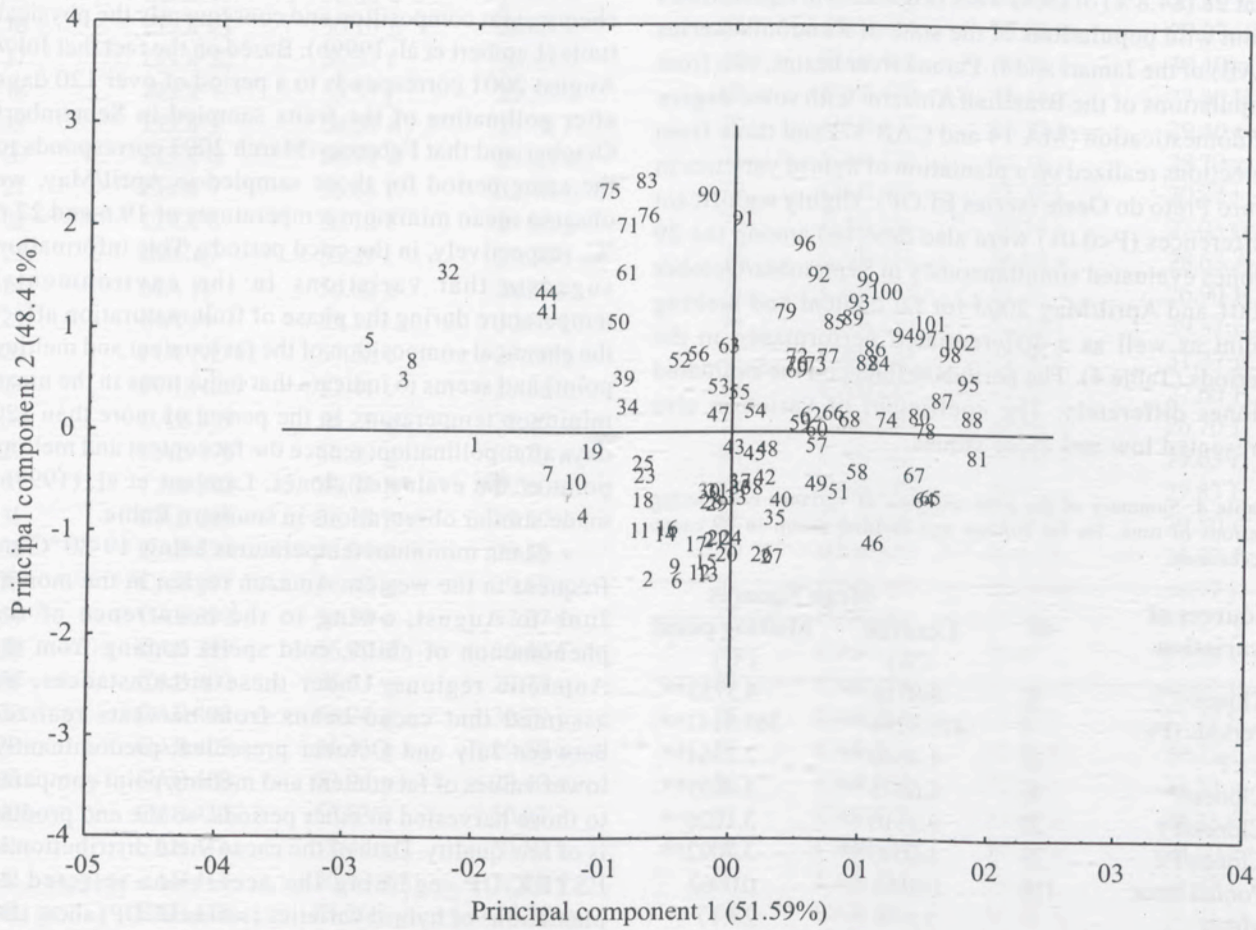

Figure 1. Plot of principal components of 102 cacao accessions (coded as in Table 3) evaluated in April/May 2003, for fat content and melting point

however formed by the closeness of locations. For example, the accessions CAB 228, 232, 233, 248, 372, and 373 (points 71, 75, 76, 83, 90, and 91) in square II, collected in areas close to each other in Ariquemes, form a subgroup. It is further worth mentioning that the reason why the subgroup formed by accessions CAB 08, 10 and 13 (points 3, 5 and 8) in square II appears most far away from the others could be that they had been collected in the upper course of the river Ji-Paraná, in a region further away from the others. The same was not true for the regionally similar CAB 09, 11 and 12 (points 4, 6 and 7), collected on the same farm, but located in square III amongst the domesticated accessions. Similarly, CAB 394 (point 32), in square II was also somewhat dissociated from the regionally similar CAB 389, 390 and 392 (points 28, 29 and 30), located in square III. This great dispersion of genotypes of the several wild subpopulations of Rondonia reflects the great variability in the two study traits and among the accessions collected from an wide area (about 210 x 100 km) between the cities Ariquemes and Presidente Médici, along the highway Marechal Cândido Rondon - BR 364.

The accessions of the half-domesticated populations were predominantly concentrated in square III, for the most part dispersed along the axis of the first principal component. Series EEOP stood out, selected in 1977 on a plantation of hybrid varieties in Ouro Preto do Oeste, which formed a compact subgroup, as observed by Almeida et al. (2005). Its accessions are also located relatively close to those of series BE (points 1 and 2), MA (points 24 and 25) and IMC 67 (point 23). POUND 7 and 12 (points 26 and 27) are located in square IV, though very close to those cited above. Only CAB 35, 37, 40, and 42 (points 99, 100, 101 and 102) collected in Santarém, Pará, a longstanding cultivation region, are located far away from these, in square I, where they form a different subgroup.

Figure 1 further provides a guideline for crossings between accessions for the generation of heterotic hybrids. In this sense, the subgroup with CAB 08, 10, 13, and 394 (points 3, 5, 8 and 32) in square II seems to be more distant from CAB 35, 37, 40 and 42 (points 99, 100, 101, and 102) in square I, or from POUND 7 and 12 (points 26 and 27) in square IV, and can generate more heterotic hybrids. Such information along with yield components will offer more efficiency in the planning of crossings. Summing up the study points out the existence of multivariate divergence in the target genebank, which can be exploited to realize more promising crosses, as argued by Pires et al. (1998). In conclusion, this study revealed the existence of significant diversity in cocoa fat content and melting point among the accessions conserved in the ESTEX genebank, which can be exploited in future germplasm enhancement and breeding programmes.

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Diversidade para manteiga de cacau no germoplasma de Rondônia, Brasil

RESUMO - *Chocolate é um alimento de alto valor nutricional produzido a partir de manteiga extraída de amêndoas de cacau. Conteúdo e ponto de fusão de manteiga são características importantes para as indústrias chocolateiras, farmacêuticas e de cosméticos. Este trabalho quantificou a diversidade do germoplasma de cacau da Rondônia para estes dois atributos. Expressiva diversidade foi detectada entre os 102 clones avaliados em Abril/Maio de 2003, para conteúdo e ponto de fusão de manteiga. O conteúdo médio de manteiga foi de 55,53% (51,04 a 58,19%) e o ponto médio de fusão de 29,55 °C (25,60 a 32,53 °C). Os acessos de populações silvestres da Rondônia foram superiores em ambos os atributos. Os dois períodos (Setembro/Outubro de 2001 e Abril/Maio de 2003) afetaram diferentemente os clones avaliados. A análise do gráfico de componentes principais revelou expressiva variabilidade e foi, em certa medida, suficiente para separar acessos silvestres de domesticados.*

Palavras-chave: germoplasma de cacau, diversidade, conteúdo e ponto de fusão de manteiga, melhoramento genético.

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