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Variability in genetic resources of cacao in Rondônia, Brazil

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ABSTRACT - *The limited genetic variation exploited in improvement results in a narrowing of the genetic base and vulnerability of the crop. Breeders should therefore be provided with information on the variability available in germplasm banks. In this study, 207 clone accessions of the cacao genebank were evaluated for five periods for the mean fruit weight, mean weight of wet seeds fruit^{-1}, mean number of normal seeds fruit^{-1}, mean individual wet and mean individual dry seed weight. Data means were subjected to uni- and multivariate analyses. The accessions presented highly significant differences for the five traits, indicating expressive variability. Genotypes of interest were identified for the improvement program of cacao in Rondônia, especially to raise the yield. The accessions EEOP 3, EEOP 5, MA 12, RO 263, RO 265, and STM 66/04 were outstanding for most traits.*

Key words: *Theobroma cacao*, genetic resources, characterization, variability.

INTRODUCTION

Genetic resources represent the raw material used to create novel varieties that are more productive, better adapted to the regions of cultivation and more resistant to pests and diseases of economically interesting plants. Together with the related wild species, they represent the repository of potential genetic variability for improvement programs of the cultivated plants.

It is known that only a small quantity of the genetic variation of the species *Theobroma cacao* L. that represents cacao has been used in improvement programs of producer countries. Such programs are mostly based on few parents, so reduced genetic gains in the long run can be foreseen (Bartley 1981). On the other hand, this bottle-neck at the genetic base increases the crop's vulnerability. A proof thereof is the limited progress obtained towards the control of the main cacao diseases. It is therefore necessary to provide breeders with information on the genetic diversity contained in the germplasm banks, which is still little known and exploited. This can and must be done by means of the characterization

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of these genetic resources.

The characterization consists in the systematic and scheduled achievement of data based on the evaluation of traits able to describe and differentiate the existing accessions. In general, traits of the plant, leaf, flower, fruit and seed are considered. Almeida and Dias (2001) present an analytical approach to the genetic resources of cacao, including evaluation and characterization.

In the Amazon region of Brazil, the activity of characterization of genetic resources of cacao began in the 1970s. Fourteen clones derived from wild and half-domesticated populations from the Amazon were described by means of nine quantitative and qualitative traits (Carletto and Costa 1977). Later, Barriga et al. (1985), Bartley et al. (1988) and Kobayashi et al. (1998) extended this characterization to accessions from different watersheds in the Brazilian Amazon region. The presumed genetic structuring of natural cacao populations per watersheds (Barriga et al. 1985, Almeida et al. 1987) was recently reinforced (Dias et al. 2003). All these studies have evidenced the existence of great genetic variability to be explored in improvement programs.

In Rondônia (RO), the Brazilian state with the third-highest production of dry cacao beans - 17.8 mil ton year^{-1}, the activities of characterization of genetic resources were never continuously carried forward as it would be desirable. Still, the Comissão Executiva do Plano da Lavoura Cacaueira – CEPLAC has a genebank at the Experimental station of Ouro Preto holding around 615 cacao clones that represent a repository of important genes, despite to date lacking due characterization. The present study aimed at a characterization of part of this genetic stock, using main agronomical descriptors of cacao as a support for the creation of a superior clonal variety for future distribution to cacao farmers and to orient research on genetic improvement of the species.

MATERIAL AND METHODS

Plant material

The study was realized at the Genebank of cacao of the Experimental station in Ouro Preto – ESTEX-OP (lat 10º 44’ 30" S, long 62º 13’ 30" W, alt 280 m asl), which belongs to CEPLAC in Ouro Preto do Oeste, RO. This region has a mean annual rain precipitation of 1940 mm, a temperature mean of 24.6 ºC and air relative humidity of 81% (Scerne et al. 2000). The soil in the study area is of a podzolic mesotrophic type of mean natural fertility.

This Genebank was planted in August 1977. It comprises accessions from diverse origins, especially wild populations from the state of Rondônia. At that time banana plants (*Musa* sp.) were used as temporary shading in the same 3.0 x 3.0 m spacing as the cacao trees and *Clitoria racemosa* as permanent shade in a 12.0 x 12.0 m spacing. Maintenance in the last years was realized according to the technological information for cacao cultivation in the Brazilian Amazon.

Of the 615 installed accessions 207 were selected for characterization, as follows: 2 accessions of series BE, 6 of series CAM, 20 of EEOP, 2 of ICS, 4 of MA, 2 of POUND, 163 of series RO and 6 of STM, besides IMC 67 and RB 39, all listed in Table 1.

Evaluated traits

The criterion used for the selection of these accessions was the presence of ripe and healthy fruits to realize a characterization in five sampling periods (Apr-May/2001, Sep-Oct/2001, Apr-May/2002, Sep-Oct/2002 and Apr-May/2003), determined according to the water availability for plants aiming at an evaluation of the seasonal variations: April/May – period when fruits developed without occurrence of water stress and; September/October – period of water stress.

Significance and description of the genetic origin of the accessions under study are listed in Table 1. The following traits were evaluated in the characterization: mean fruit weight (MFRW in g), mean wet seed weight fruit^{-1} (WSWF in g), mean number of normal seeds fruit^{-1} (NNSF), mean individual wet seed weight (IWSW in g), and mean individual dry seed weight (IDSW in g). MFRW, WSWF and NNSF were estimated based on samples formed by the minimum of 15 fruits each collected in one to three harvests realized in each period, amounting to up to 45 fruits. In each sample, based on 50 normal seeds randomly picked from among the harvested fruits, IWSW and thereafter IDSW were estimated after pulp extraction and drying at 105 ºC for 48 hours. IWSW and IDSW may therefore have been sampled in up to 150 normal seeds. Three hundred and two data per trait were processed, since not all clones had fruits in the five evaluation periods.

Univariate analyses

The five evaluated traits were analyzed for normal distribution. Although only WSWF presented normality, the slight deviations of the others was not sufficient to justify the use of transformation functions. Consequently, the mean original data were subjected to analyses of variance in a simple factorial design (clones x periods, considering the interaction clones x periods as error) as suggested by Pimentel Gomes (1990). The accession means were compared by the test of Scott-Knott.

Multivariate analyses

Analyses of main components were also processed using the original data means. A biplot was constructed for the multivariate graph analysis of variability with the two first components (Dias 1998). All analyses were processed on software SAS (SAS Institute Inc. 1989) and Statistica (StatSoft 1997).

RESULTS AND DISCUSSION

Univariate analyses

Highly significant differences ($P < 0.01$) were detected between clones and periods (Table 2) evidencing expressive variability in clones and period for all traits. The coefficients of variation (CV) varied from 9.1% for NNSF to 19.4% for MFRW. The expected reduction of values in the period from September-October 2001 was

Table 1. Significance and genetic origin of the accessions evaluated

BE – Belém, township. Accessions collected in 1965 in the surroundings of Belém, PA

CAM – Cacao Amazônico. Accessions collected in 1976 in the watershed of the Ji-Paraná river from wild populations on the Fazenda Muqui, Presidente Médici, RO

EEOP – Experimental station Ouro Preto. Accessions selected in 1977 on a plantation of hybrid varieties, on the estate of José Wensing, Ouro Preto do Oeste, RO, considering the criterion of witches' broom tolerance

ICS – Imperial College Selections. Accessions selected in 1931 by JF Pound, in Trinidad

IMC – Iquitos Mixed Calabacillo. Accession selected in 1938 by JF Pound, in Iquitos, Peru

MA – Manaus, township. Accessions collected in 1965 on the Ilha do Careiro, Careiro, AM

POUND – Family name of JF Pound. Accessions selected in 1943 by JF Pound in Iquitos, Peru

RB – Rio Branco. Accession collected in 1965 in Rio Branco, AC

RO – Rondônia. RO 12 the RO 148: accessions collected in 1981 and 1982 in the Jamari river basin, from wild populations in the county of Ariquemes. RO 153 to RO 798, except for RO 263 and RO 265: accessions collected from 1983 to 1985 in the watershed of the Ji-Paraná river, from wild populations in Jaru, Ouro Preto do Oeste, Ji-Paraná, Presidente Médici, Urupá, Mirante da Serra, Cacoal and Rolim de Moura. RO 263 and RO 265: accessions collected in 1983 on commercial plantations, based on improved seeds

STM – Santarém. Accessions collected in the region of Santarém, PA

Table 2. Summary of the analyses of variance and means of five traits evaluated in 207 cacao accessions

		Mean squares				
Sources of variation	**df**	**MFRW**[1]	**WSWF**[2]	**NNSF**[3]	**IWSW**[4]	**IDSW**[5]
Clones (C)	206	35522.80*	1321.26*	52.31*	0.55*	0.08*
Periods (E)	4	148910.29*	3855.68*	52.71*	2.59*	0.18*
Error	91	6957.86	119.13	10.70	0.06	0.01
Mean (C)		430.4	91.0	36.0	2.5	0.8
CV (%)		19.4	12.0	9.1	10.0	13.4
Means (E)		**MFRW**	**WSWF**	**NNSF**	**IWSW**	**IDSW**
Apr-May/2001		353.2	78.6	35.8	2.3	0.80
Sep-Oct/2001		424.8	89.0	35.9	2.4	0.88
Apr- May /2002		530.0	106.9	42.3	2.4	0.84
Sep- Oct/2002		423.7	87.9	34.5	2.5	0.80
Apr- May /2003		428.4	99.8	38.9	2.5	0.90

*$P < 0.01$ by the F test

[1]Mean fruit weight; [2]Mean weight of wet seeds $fruit^{-1}$; [3] mean number of normal seeds $fruit^{-1}$; [4]mean individual wet seed weight; [5]Mean individual dry seed weight

not verified. Since the data of rain precipitation at ESTEX-OP showed that water stress occurred during part of the development period of the sampled fruits, a plausible explanation for this fact could be that MFRW was higher than 550g in 50% of accessions in the sample, which resulted in an elevation of the mean values of the other analyzed traits. In 2002, on the contrary, a reduction of the mean values of up to 20.1% was verified when compared with April-May of the same year. Such observations can help to plan studies on irrigation of cacao plantations by indicating the most critical periods for supplementary irrigation.

Significant differences between groups of means of the 207 clones were verified for all five traits (Table 3), indicating the potential of these clones as source of genes for genetic improvement programs of cacao. MFRW presented an amplitude of variation from 194.9 g for clone RO 582 to 960.7 g for STM 66/04, evidencing high variability. Based on the means, the clones were classified in six groups, significantly different from each other by the Scott-Knott test. The group with most expressive MFRW values contained clones IMC 67, BE 9 and STM 66/04, with means varying from 922.9 to 960.7 g. Still, considering the three most expressive groups, i.e., those whose clones presented means of over 638.0 g (fruits of intermediate to large size), the set was enriched with clones EEOP 16, MA 11, BE 10, EEOP 5, EEOP 3, RO 73, RO 265, POUND 12, EEOP 18, MA 12, RO 263, and EEOP 9. Of the 15 aforementioned clones, 14 (93.3%) belong to the series BE, EEOP, IMC, MA, POUND, and STM, besides clones RO 263 and RO 265. Series BE, MA and STM were selected in the surrounding areas of Belém, Manaus and Santarém, respectively. These regions have a history of cacao cultivation since the 18^{th} century. Reports mention river people practicing selection by selecting the largest fruits that would provide seeds for the next planting and, therefore, represent genetic material with a certain level of domestication. The series EEOP represents clones selected on a plantation of hybrid varieties in Ouro Preto do Oeste, similar to clones RO 263 and RO 265. Finally, the series IMC and POUND also constitute selections realized in Iquitos, Peru. We therefore conclude that the more expressive values for MFRW must have been result of a selection for increase of the fruit size in the outstanding clones. RO 73 is the only descent from a wild cacao population. It was found on the right-hand banks of the Jamari river, approximately 18 km from the city of Ariquemes, RO, in northeastern direction.

WSWF also showed broad variation from 35.6 g for RO 44 to 169.8g for RO 263. The clones were allocated in eight different groups. The most outstanding group consisted of STM 66/04, MA 12, POUND 12, EEOP 5, IMC 67, MA 15, EEOP 3, EEOP 18, EEOP 17, and RO 263, all with WSWF higher than 140.0 g. This set may be amplified with eight other clones (STM 61/01, STM 66/05, RO 265, BE 9, ICS 100, STM/CASA, EEOP 9, and STM 62/03), seen as of interest for productivity increase of WSWF to over 130.0 g. Similar to trait MFRW, the outstanding clones come from regions of the Brazilian Amazon (series MA, BE and STM) where they probably underwent some selective process by locals or represent products of genetic improvement programs (series ICS, IMC, POUND and EEOP, and clones RO 263 and RO 265). Once again, one may conclude that these more expressive values for WSWF can be ascribed to a selection for fruit size increase.

An analysis of trait NNSF showed a variation ranging from 15.3 for RO 507 to 52.1 for MA 15. The clones were divided in six distinct groups. The most outstanding group consisted of clones EEOP 9, RO 168, RO 192, POUND 7, EEOP 7, EEOP 14, and MA 15, all with NNSF above 46 units. Still, considering cacao clones with NNSF above 40 as of interest for improvement programs, the following were also noteworthy: RO 579, IMC 67, EEOP 4, RO 321, RO 616, POUND 12, EEOP 19, RO 265, RO 59, EEOP 18, EEOP 12, EEOP 21, MA 12, EEOP 8, RB 39, EEOP 3, EEOP 5, EEOP 15, EEOP 10, EEOP 20, EEOP 6, RO 22, and STM 66/04. Of these 30 accessions, only eight (RB 39, RO 22, RO 59, RO 168, RO 192, RO 321, RO 579, and RO 616) are derived from wild cacao populations, while the others are products of improvement programs or of selection realized by locals.

For IWSW the range was also great; from 1.6 g for RO 616 to 4.8 g for ICS 9. Seven clone groups were formed. The most outstanding group consisted of only three clones: EEOP 17, RO 263 and ICS 9 with values between 4.3 and 4.8 g, followed by a group with variation from 3.8 to 4.1 g, composed by clones STM 66/04, ICS 100 and STM/CASA. However, the third group presented values above 3.1 g, attaining a level that is also of interest for improvement programs, and comprised: RO 53, EEOP 4, BE 10, MA 11, RO 265, POUND 7, RO 95, RO 96, EEOP 5, STM 63/01, RO 435, EEOP 11, STM 66/05, STM 62/03, EEOP 3, RO 306, BE 9, IMC 67, STM 61/01, MA 12, and EEOP 18. One notes that of the 27 outstanding clones only five (RO 53, RO 95, RO 96, RO 306, and RO 435) are

Table 3. Means of five traits evaluated in 207 accessions of cacao

Moments	Traits[1]				
	MFRW[1]	WSWF	NNSF	IWSW	IDSW
Mean	430.38	90.98	35.95	2.45	0.82
Range	1262.00	144.77	39.00	3.55	2.03
Skewness	1.53	0.75	0.05	1.11	1.55
Curtosis	4.66	-0.04	-0.003	1.33	4.27
C.V. (%)	38.50	34.48	18.64	25.61	29.57
Variance	27456.27	984.35	44.93	0.39	0.05

[1]Acronyms listed in Table 2

derived from wild cacao populations and the others from selective processes of anthropic nature.

The range for IDSW ranged from 0.4g for RO 32 to 1.8 g for ICS 9, and eight clone groups were formed. The four most expressive groups presented means of over 1.0 g, which is the standard required by the chocolate industry. These are: EEOP 11, IMC 67, EEOP 16, STM 61/01, STM 66/05, RO 263, STM 63/01, EEOP 18, STM 62/03, STM/CASA, EEOP 5, ICS 100, EEOP 17, and ICS 9. As mentioned above, all these 14 genotypes originated from selective processes with human participation. We may therefore conclude that the most expressive values for IDSW resulted from selection for increase of fruit size in the outstanding clones.

Multivariate analysis

In the analysis of main components the two first components absorbed 92% of the total variation. The visualization of the biplot (Figure 1) evidences a clear distinction between genotypes derived from wild populations from the state of Rondônia and domesticated ones from different origins, especially those from Trinidad (clones 29 and 30 - series ICS). One can also observe the formation of subgroups among the domesticated accessions. For example, of the 20 evaluated clones of the series EEOP, selected on a plantation of hybrid varieties for tolerance to witches' broom (*Crinipellis perniciosa*), 11 (clones 9, 11, 13, 14, 18, 19, 20, 23, 25, 26, and 27) were relatively close, forming a subgroup; six others (clones

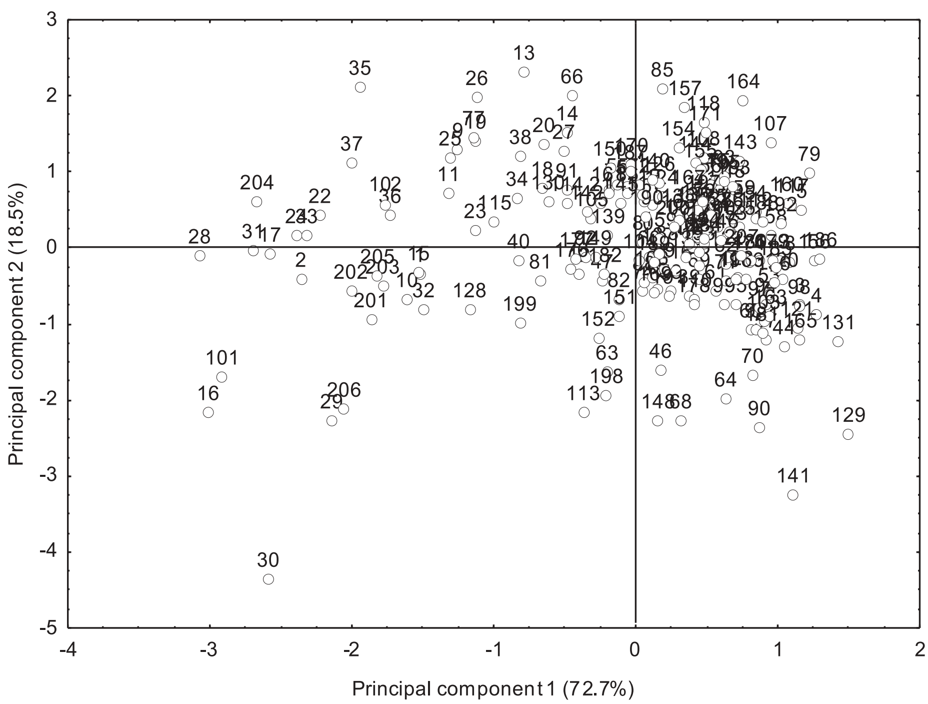

Figure 1. Biplot of principal components of 207 cacao accessions (Table 1) evaluated for five traits listed in Table 2

10, 15, 17, 22, 24, and 28), together with clones of the series STM (clones 201, 202, 203, 204 and 205), BE (clones 1 and 2), MA (clones 32 and 33) and also IMC 67 make up another subgroup; clone EEOP 17 (clone 16), in turn, has a more distant position and is close to RO 263 (clone 101), selected in Rondônia in 1983, on a plantation of hybrid varieties, suggesting a different subgroup. This information seems to evidence the participation of different parents in the formation of clones of the EEOP series, a relevant fact in the search for durable resistance to witches' broom by genetic improvement.

Furthermore, the relative proximity of position of clones of the series STM, MA and BE seems to suggest a common origin, which is an acceptable hypothesis since they are originated from one and the same watershed, a fact that makes the long-distance migration of genes viable, as postulated by Almeida (2001). Also, clones of the series POUND (clones 36 and 37), coherently have close positions and among them RO 265 (clone 102), with the same origin as RO 263. One could therefore speculate that clone RO 265 is a descent from POUND 7 or POUND 12, which have participated as parents in the formation of the hybrid varieties distributed so far.

The accessions of the series RO (except for RO 263 and RO 265) and CAM constitute, predominantly, a distinct subgroup. They all represent diverse wild subpopulations that existed in the past in upland areas in the state of Rondônia, comprising, however, only the watersheds of the rivers Jamari and Ji-Paraná. In this macroregion there are no natural barriers that could constitute a factor of geographic or ecological isolation for these subpopulations. The area comprises a rectangular shape of approximately 27000 km^2 (270 x 100 km) along the highway Marechal Cândido Rondon (BR 364), between the cities Ariquemes and Cacoal. Almeida et al. (1995) speculated on the possibility that such subpopulations would belong to a same gene complex. The reason for the observed similarity of morphological nature and presence or absence of anthocyanin pigments may have been the gene exchange throughout the history of the species' evolution. The biplot (Figure 1) also allows conclusions on the existence of genetic similarities in these subpopulations, corroborating the observations of Almeida et al. (1995).

A look at the biplot (Figure 1) further shows that clone ICS 9 (clone 30) is more divergent than the others. This distinction is relevant for the planning of crossings aiming at transgressive hybridization, especially when crossed with clone MA 15 (clone 35) or clones EEOP 14, EEOP 7 and RO 168 (clones 13, 26 and 66, respectively), which are more distant to ICS 9. Additionally, the subgroups formed by clones EEOP 17 and RO 263 (clones 16 and 101), ICS 100 and STM/CASA (clones 29 and 206) and RO 44, RO 236 and RO 507 (clones 129, 90 and 141), are also more distant from those suggested for crossings with ICS 9, which allows the exploitation of the heterotic hybrid generation. Summing up, our study shows the existence of multivariate divergence in the germplasm that is available for the realization of more promising crossings.

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Variabilidade em recursos genéticos do cacaueiro em Rondônia, Brasil

RESUMO - *A limitada variação genética explorada no melhoramento leva ao estreitamento da base genética e a vulnerabilidade do cultivo. Por isso, deve-se prover o melhorista com informações sobre a variabilidade nos bancos de germoplasma. Nesse estudo, 207 acessos clonais do banco de germoplasma de cacau foram avaliados por cinco épocas, com base no peso médio do fruto, peso médio das sementes úmidas fruto^{-1}, número médio de sementes normais fruto^{-1}, peso médio individual da semente úmida e peso médio individual da semente seca. Os dados médios foram submetidos à análises uni- e multivariadas. Os acessos apresentaram diferenças altamente significativas para os cinco caracteres, indicando expressiva variabilidade. Identificaram-se genótipos de interesse para o programa de melhoramento do cacaueiro em Rondônia, especialmente para incremento de produção. Acessos como EEOP 3, EEOP 5, MA 12, RO 263, RO 265 e STM 66/04 se destacaram para a maioria dos caracteres.*

Palavras-chave: *Theobroma cacao*, recursos genéticos, caracterização, variabilidade.

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