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Karyotype variation in cultivars and spontaneous cocoa mutants (*Theobroma cacao* L.)

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ABSTRACT. Four mutant cocoa accessions with morphological changes and a cultivar sample were karyomorphologically characterized. Slides were prepared by enzymatic digestion of the root meristem and squashed in 45% acetic acid, followed by 2% Giemsa staining. The chromosome number of $2n = 20$ was seen in all accessions. The karyotype formula for Cacau Comum and Cacau Rui was $2n = 20$m. Submetacentric chromosomes were observed in Cacau Pucala and Cacau Jaca, both with $2n = 18$m + 2sm, but the karyotype formula for Cacau Sem Vidro was $2n = 16$m + 4sm. Satellites were located on the long arm of the 1st and 2nd chromosome pairs of Cacau Comum, whereas Cacau Pucala had satellites on the 6th chromosome pair. Greater karyotypic variation in Cacau Sem Vidro was found, whose 1st and 2nd chromosome pairs had satellites on the long arm and 6th and 10th pairs had satellites on the short arm. Analysis revealed a lower average chromosome length in Cacau Comum (1.53 ± 0.026 μm) and a higher length in Cacau Sem Vidro (2.26 ± 0.038 μm). ANOVA revealed significant difference ($P < 0.01$) for the average chromosome length and the length of chromosome pairs within and between accessions. The average chromosome lengths of mutants of Cacau Rui and Cacau Jaca were not statistically different by the Tukey test at 5% probability. The karyotypic diversity observed in this study is not necessarily associated with the changing character of the accessions analyzed, but may reflect the genetic variation observed in *Theobroma cacao*.

Key words: *Theobroma*; "Cacau" mutants; Karyomorphology; Karyotypic diversity

INTRODUCTION

The cocoa plant (*Theobroma cacao* L.*)* belongs to the family Malvaceae and is distributed in Neotropical regions. This crop is of worldwide significance, providing raw materials for the cosmetics, pharmaceutical, and food industries, especially for the manufacture of chocolate (Leung, 1980; Whitlock et al., 2001; Streinberg, 2002; Zuidema et al., 2005). In Brazil and in other countries in South America, cocoa production was considerably reduced with the spread of Witches' broom disease, which is caused by the fungal pathogen *Moniliophthora perniciosa* (Pereira et al., 1989; Bartley, 2005). Nonetheless, natural sources of resistance to cocoa diseases are needed for the genetic improvement of the crop, as the control methods are little effective and very expensive (Surujdeo-Maharaj et al., 2004; Oliveira and Luz, 2005).

The great genetic variability of *T. cacao* is observed in the major morphological descriptors, such as flowers, leaves, and fruit, and in the natural sources of environmental stress tolerance and disease resistance (Vello et al., 1967; Bartley, 2005). The observed genetic variation of qualitative and quantitative parameters in *T. cacao* can be attributed to the large number of varieties and to the existence of spontaneous mutants (Bartley, 2005; Eremas, 2008). Amongst the already reported spontaneous mutants of concern, in this study we can list Cacau Rui, Cacau Jaca, Cacau Pucala, and Cacau Sem Vidro, which have morphological variants and resistance factors to biotic and abiotic agents, in addition to being promising for the genetic improvement of *T. cacao* (Bartley, 2005).

The chromosomal number of some species of the genus *Theobroma* was initially reported by Davie (1935) and Carletto (1946), where $2n = 20$ was observed for Cacau Catongo, Cacau Criolo, and Cacau Tigre (Carletto, 1946). An asymmetrical karyotype composed of metacentric, submetacentric, telocentric, and acrocentric chromosomes and a satellite chromosome pair have been described for the "Cupuaçu tree" (*Theobroma grandiflorum* Schum) (Santos, 2002). As regards the chromosomal morphology, there was polymorphism in *T. cacao*; the chromosomes were grouped into distinct groups according to their length (Muñoz, 1948). Given the age of the publication and the fact that the nomenclature for the centromeric position of Levan et al. (1964) had not yet been proposed, the chromosome classification was described as the "J" and "L" morphologies. A comparative study between the species *T. cacao* and *T. grandiflorum* has revealed the presence of symmetrical karyotypes in both species, with small metacentric chromosomes (Dantas and Guerra, 2010).

The presence of a satellite chromosome pair has, until this moment, been considered as a cytological marker for the genus *Theobroma* and has also been reported in meiotic chromosomes and in association with nucleoli (Muñoz, 1948; Glicenstein and Fritz, 1989; Dantas and Guerra, 2010). The chromosomal banding held on commercial accessions of *T. cacao* and *T. grandiflorum* with base-specific fluorochrome staining with chromomycin A_3 (CMA_3) and 4′,6′-diamidino-2-phenylindole (DAPI) has revealed the presence of a chromosome pair with a CMA_3^+/$DAPI^-$ band (Dantas and Guerra, 2010). Fluorescent *in situ* hybridization with a 45S rDNA probe has revealed a hybridization site on a chromosome pair that coincided with the CMA_3^+/$DAPI^-$ band. Using 5S rDNA probes, only one interstitial hybridization site was found to be located in one of the three largest chromosome pairs of *T. cacao* (Dantas and Guerra, 2010).

The karyomorphology of *T. cacao* has been little studied, whereas analyses performed so far are limited to commercial accessions, disregarding the large number of cultivated varieties and mutant genotypes. Accordingly, there is no substantial information on the intraspecific karyotype diversity of *T. cacao* in the literature. Given the lack of karyotypic studies for *T. cacao* and the existence of mutant accessions, this study has assessed the karyomorphology of five accessions, of which one was a cultivar and four were spontaneous mutants, in order to verify the intraspecific karyotypic variability of *T. cacao*.

MATERIAL AND METHODS

Biological material

One accession of the Cacau Comum cultivar and four spontaneous mutant accessions of *T. cacao* characterized according to Bartley (2005) (Table 1, Figure 1) have been used. The mutant accessions are kept in the active germplasm bank of the Centro de Pesquisas do Cacau (CEPEC) located in Ihéus, BA, Brazil. The mutant accessions Cacau Jaca, Cacau Rui, and Cacau Sem Vidro originated in the Cocoa Region of Bahia, whereas the mutant Cacau Pucala originated in Peru (Table 1). The Cacau Comum variety native to the Lower Amazon region was identified and collected from a Cabruca Cocoa Agroforestry system located on the campus of Universidade Estadual de Santa Cruz (UESC), in Ilhéus, Bahia, Brazil.

Table 1. Identification of *Theobroma cacao* accessions, origin, and morphological features of the accessions analyzed.

Descriptive name	Accession	Origin	Varying morphological characteristics
Cacau Comum	Cultivar	Low Amazonian Region, Brazil	Normal morphological characteristics and big-size plants
Cacau Rui	640	Bahia, Brazil	Gradation of leaves and narrow leaves
Cacau Pucala	642	Peru	Corrugated leaves
Cacau Jaca	643	Bahia, Brazil	Rounded leaves similar to the jackfruit's (*Artocarpus heterophyllus*)
Cacau Sem Vidro	647	Bahia, Brazil	Thin skin fruits and seed adhesion; vitreous fluid is almost non-existent

Preparation of samples for cytogenetic study

In spontaneous mutants, the cytological preparations were made using young radicles sourced from cuttings obtained from plagiotropic rooted shoots of mother plants (Sodré, 2007). For the propagation of cuttings, the shoots were collected and their bases were immersed into 0.05% Derosal. After that, the shoots were cleaned and cut into 15-cm long stem cuttings and the leaves were reduced to 1/3 of their original size; thus, each cutting had five leaves. The bases of the cuttings were immersed for 5 s in a butyric acid solution at 6000 ppm. The cuttings were maintained in PVC rings containing a moistened substrate of well-matured sawdust compound and washed sand (4:1, w/w), and held in a greenhouse at CEPEC. Rootlets from the Common Cocoa cultivar were obtained from pulped and germinated seeds in a humid chamber, containing a filter paper substrate moistened with distilled water, at room temperature.

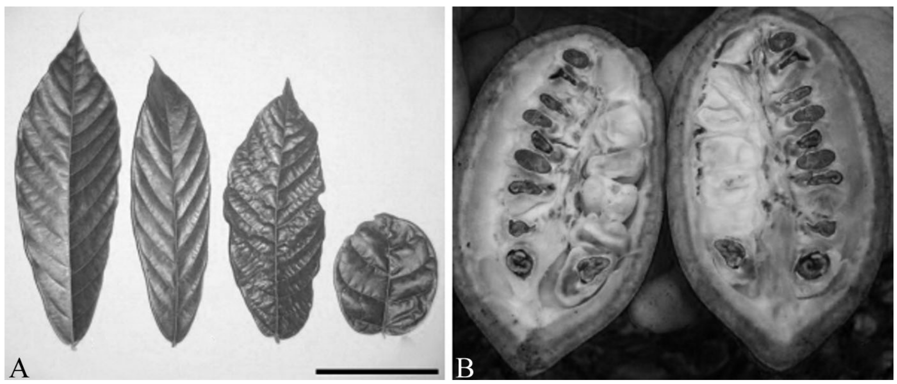

Figure 1. Varying morphological characteristics in *Theobroma cacao*. **A.** Leaves of Cacau Comum, Cacau Rui, Cacau Pucala, and Cacau Jaca. **B.** Cacau Sem Vidro fruit. Bar = 11 cm.

Rootlets were collected and immersed in an antioxidant solution of 1 M ascorbic acid in distilled water. Then, the rootlets were pretreated with 0.002 M 8-hydroxyquinoline for 4.5 h in a container stored on ice. The small roots were washed and fixed with Carnoy's I solution (3:1 ethanol-acetic acid) (Johansen, 1940) for 12 h, and maintained at -20°C until analysis. Slides were prepared as proposed by Guerra and Souza (2002), with a modification that consisted of hydrolysis with 1 N HCl at 37°C for 20 min. The slides were washed and subjected to enzymatic digestion with a 2:20 cellulase-pectinase solution (%, v/v), for 60 min at 37°C, and subsequent maceration and crushing of the meristem in a drop of 45% acetic acid. After freezing the slide-coverslip assembly in liquid nitrogen, the coverslip was withdrawn. Cytological preparations were stained in 2% Giemsa (v/v) for 15 to 20 min and then mounted in a Neo-mount medium (Merck, Darmstadt, Germany).

Cytogenetic data analysis

Chromosome measurements were carried out to obtain the short arm (SA), long arm (LA), and satellite (SAT) lengths. The ratio between chromosome arms ($r = L/S$), total chromosome length ($TCL = SA + LA + SAT$), average chromosome length ($\chi = \Sigma CT$/number of chromosomes), haploid lot length (HLL), and asymmetry index (TF%) (Huziwara, 1962) were calculated. Chromosome classification was done using the nomenclature proposed by Guerra (1986): metacentric ($r = 1\text{-}1.49$), submetacentric ($r = 1.50\text{-}2.99$), acrocentric ($r = 3\text{-}\infty$), and telocentric ($r = \infty$). Satellites were classified as microsatellite and macrosatellite, according to Battaglia (1955). Cytogenetic analysis consisted of five metaphases that were photo-documented using an Olympus CX41 light microscope coupled to an Olympus 7.1 digital camera, and chromosome measurements were carried out using the software Image Tool version 3.0.

Statistical analysis

A completely randomized design with five replicates was used. Statistical analyses were performed using the software Sisvar (Ferreira, 2003) on the Windows Vista® platform. Analysis of variance (ANOVA) was performed to evaluate expressive differences in the average chromosome length as a function of the accessions, the chromosome length from the 1st to the 10th pair within each accession, and amongst all accessions. Moreover, the Tukey test at 5% probability was carried out for comparing the average chromosome length.

RESULTS

Cytogenetic analyses revealed that all of the observed *T. cacao* accessions had the $2n = 20$ chromosome number (Figure 2). The chromosome length of the analyzed accessions (Table 2) had a 53% variation in the Cacau Comum cultivar, 59% in Cacau Pucala, 53% in Cacau Rui, 68% in Cacau Jaca, and 52% in Cacau Sem Vidro. The HLL exhibited a 68% variation in the Cacau Comum cultivar compared with the HLL of Cacau Sem Vidro. Cacau Comum and Cacau Rui contained exclusively metacentric chromosomes ($2n = 20$m); however, Cacau Jaca and Cacau Pucala had one submetacentric chromosome pair each, pairs 5 and 7, respectively. The karyotypes were symmetrical, with a higher asymmetry index for the mutants Cacau Rui and Cacau Jaca, both with a 45% TF, and a lower index for the mutants Cacau Sem Vidro, with a 40% TF (Table 3); therefore, there was a 12% variation between the analyzed accessions.

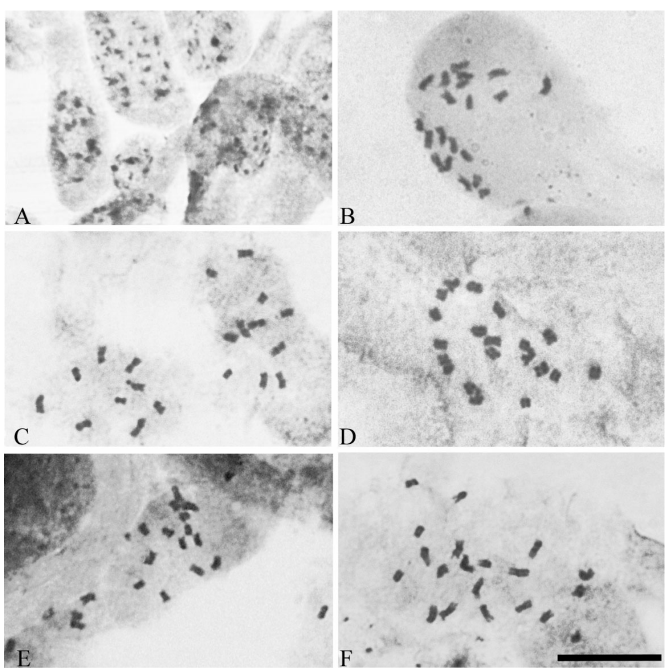

Figure 2. Conventional staining in nuclei and metaphase chromosomes of *Theobroma cacao*. **A.** Interphase nucleus of Cacau Sem Vidro; (**B-F**). Metaphase chromosomes of cocoa. **B.** Cacau Comum; **C.** Cacau Pucala; **D.** Cacau Rui; **E.** Cacau Jaca; **F.** Cacau Sem Vidro. Bar = 10 μm.

Table 2. Karyomorphological data in metaphase chromosomes of *Theobroma cacao*.

Accession	Data	Chromosome pair (µm)									
		1	2	3	4	5	6	7	8	9	10
Cacau Comun	SA	0.71	0.69	0.71	0.82	0.70	0.63	0.55	0.55	0.56	0.52
	LA	0.75	0.70	1.03	0.86	0.79	0.72	0.74	0.74	0.65	0.62
	SAT	0.67	0.55	-	-	-	-	-	-	-	-
	TCL	2.13	1.94	1.74	1.68	1.49	1.35	1.29	1.29	1.21	1.14
	L/S	1.06	1.01	1.45	1.05	1.13	1.14	1.34	1.35	1.16	1.19
	CLASS	m	m	m	m	m	m	m	m	m	m
Cacau Pucala	SA	1.00	0.86	0.87	0.86	0.55	0.73	0.73	0.69	0.58	0.56
	LA	1.10	1.08	1.06	0.95	0.85	0.86	0.77	0.72	0.80	0.68
	SAT	-	-	-	-	0.40	-	-	-	-	-
	TCL	2.10	1.94	1.93	1.88	1.80	1.59	1.50	1.41	1.38	1.24
	L/S	1.10	1.26	1.27	1.11	1.54	1.18	1.05	1.04	1.38	1.21
	CLASS	m	m	m	m	sm	m	m	m	m	m
Cacau Rui	SA	1.05	0.97	0.94	0.93	0.88	0.75	0.7	0.69	0.62	0.59
	LA	1.30	1.29	1.16	1.15	0.93	0.84	0.85	0.83	0.7	0.67
	SAT	-	-	-	-	-	-	-	-	-	-
	TCL	2.35	2.26	2.10	2.08	1.81	1.59	1.55	1.52	1.32	1.26
	L/S	1.24	1.33	1.23	1.24	1.06	1.12	1.21	1.20	1.13	1.14
	CLASS	m	m	m	m	m	m	m	m	m	m
Cacau Jaca	SA	0.98	0.87	1.00	0.88	0.84	0.78	0.60	0.77	0.68	0.72
	LA	1.17	1.22	1.07	1.15	1.04	0.88	0.97	0.78	0.79	0.75
	SAT	-	-	-	-	-	-	-	-	-	-
	TCL	2.15	2.09	2.07	2.03	1.88	1.66	1.57	1.55	1.47	1.47
	L/S	1.19	1.40	1.07	1.31	1.24	1.13	1.62	1.01	1.16	1.04
	CLASS	m	m	m	m	m	m	sm	m	m	m
Cacau Sem Vidro	SA	1.27	1.00	1.12	1.09	1.08	0.75	0.67	0.81	0.80	0.63
	LA	1.43	1.35	1.42	1.32	1.25	1.13	1.16	0.95	0.92	0.71
	SAT	0.53	0.52	-	-	-	0.41	-	-	-	0.36
	TCL	3.23	2.87	2.54	2.41	2.33	2.29	1.83	1.76	1.72	1.70
	L/S	1.13	1.35	1.27	1.21	1.16	1.51	1.73	1.17	1.15	1.11
	CLASS	m	m	m	m	m	sm	sm	m	m	m

SA = mean value of the short arm; LA = mean value of the long arm; SAT = satellite; TCL = total chromosome length; L/S = ratio between arms; CLASS = classification regarding chromosome morphology; m = metacentric; sm = submetacentric.

Table 3. Karyotypic parameters in *Theobroma cacao* accessions.

Acession	HLL (µm)	Means ± SD	TF%	KF
Cacau Comum	15.26	1.53 ± 0.026^{a}	42	20m
Cacau Pucala	16.77	1.67 ± 0.010^{b}	44	18m + 2sm
Cacau Rui	17.84	1.78 ± 0.043^{c}	45	20m
Cacau Jaca	17.93	1.79 ± 0.032^{c}	45	18m + 2sm
Cacau Sem Vidro	22.68	2.26 ± 0.038^{d}	40	16m + 4sm

HLL = haploid lot length; Means ± SD = average chromosome lengths and standard derivation; TF% = asymmetry index; KF = karyotype formula; m = metacentric; sm = submetacentric. Values followed by the same letter in rows do not statistically differ by the Tukey test at 5% probability.

There were variations in the location, position, and classification of satellites amongst the analyzed accessions (Figures 3 and 4; Table 2). The Cacau Comum cultivar had two chromosome pairs with macrosatellites, both located on the long arm of the 1st and 2nd chromosome pairs. In Cacau Pucala, only the 3rd chromosome pair had microsatellites on the short arm. Greater differences were observed in Cacau Sem Vidro, whose 1st and 2nd chromosome pairs carried macrosatellites on the long arm, and the 6th and 10th chromosome pairs carried microsatellites on the short arm. It was not possible to view satellites through the conventional staining of Cacau Rui and Cacau Jaca accessions.

Figure 3. Karyograms in *Theobroma cacao* accessions. **A.** Cacau Comum; **B.** Cacau Pucala; **C.** Cacau Rui; **D.** Cacau Jaca; **E.** Cacau Sem Vidro. Bar = 5 µm.

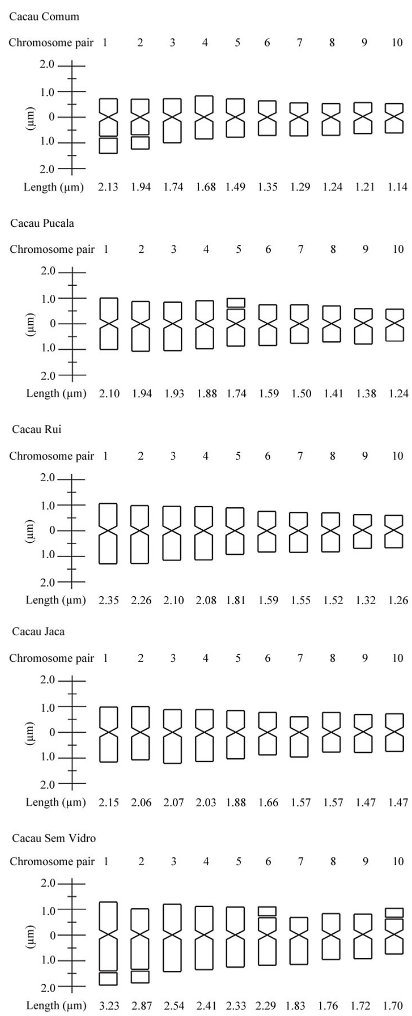

Figure 4. Ideograms of *Theobroma cacao* accessions.

ANOVA revealed an expressive effect (P < 0.01) with regard to the average chromosome length of the accessions (Table 4) and the chromosome length from the 1st to the 10th pairs within each accession (Table 5) and among all accessions (Table 6). The Tukey test at 5% probability revealed that only Cacau Rui and Cacau Jaca had no statistical differences with regards the average chromosome size.

Table 4. Summary of analysis of variance for the average chromosome length of the *Theobroma cacao* accessions analyzed.

Source of variation	d.f.	MS
Accession	4	0.385*
Error	20	0.001
CV(%)	1.78	-

d.f. = degrees of freedom; MS = mean square; CV(%) = coefficient of variation; *significant (P < 0.01).

Table 5. Summary of analysis of variance for the chromosome length from the fist to the 10th chromosome pair within all the accessions analyzed.

Source of variation	d.f.	MS				
		Cacau Comum	Cacau Pucala	Cacau Rui	Cacau Jaca	Cacau Sem Vidro
TCL	9	0.544*	0.464*	0.779*	0.383*	1.339*
Error	40	0.002	0.006	0.003	0.001	0.002
CV%		2.85	4.50	2.97	2.09	2.13

d.f. = degrees of freedom; MS = mean square; TCL = total chromosome length; CV% = coefficient of variation; *significant (P < 0.01).

Table 6. Summary of analysis of variance for the chromosome length from the 1st to the 10th chromosome pair among all the accessions analyzed.

SV	d.f.	Chromosome pair MS									
		1	2	3	4	5	6	7	8	9	10
TCL	4	1.057*	0.749*	0.431*	0.371*	0.463*	0.616*	0.193*	0.160*	0.188*	0.253*
Erro	24	0.013	0.003	0.002	0.001	0.002	0.001	0.001	0.001	0.000	0.000
CV%		4.86	2.66	2.43	1.51	2.24	2.27	2.44	2.40	1.93	1.63

SV = source of variation; d.f. = degrees of freedom; MS = mean square; TCL = total chromosome length; CV% = coefficient of variation; *significant (P < 0.01).

DISCUSSION

The diploid chromosome number of $2n = 20$ was observed in all accessions used in this study, confirming previous cytological studies conducted in *T. cacao* and related species in the genus *Theobroma* (Davie, 1935; Carletto, 1946; Dantas and Guerra, 2010). There is a known relationship between karyotype stability and chromosome number. However, disploidy species in the genus *Theobroma* have been reported in previous studies of *T. grandiflorum* (Santos, 2002) and *T. cacao*, in which disploidy was observed in a mutant genotype (M253) with $2n = 19$ chromosomes (Muñoz, 1948).

Karyomorphological inferences in the genus *Theobroma* are scarce in the literature, and most existing studies were conducted prior to the 80's and related to conventional staining mitotic and classical meiotic analyses. Cytogenetic studies performed on the genus *Theo-*

broma have revealed karyotypes with small chromosomes, ranging from 9.1 to 1.69 µm in *T. speciosum* (Yoshitome et al., 2008), 1.19 to 2.0 µm in *T. cacao*, and 1.15 to 2.21 µm in *T. grandiflorum* (Dantas and Guerra, 2010). Among the accessions analyzed, there was greater variation between chromosomes lengths (1.14 to 3.23 µm).

The assessment of the commercial and mutant accessions revealed a trend towards genetic material gains, with an expressive difference in the average chromosome length and the chromosome length from the 1st to the 10th pairs within each accession and amongst all accessions. The average comparison test (Tukey test at 5% probability) revealed that the mutants Cacau Rui and Cacau Jaca, which share common ancestry, do not differ statistically with respect to their average chromosome length. On the other hand, the average chromosome lengths of the Cacau Comum (low Amazonian region, Brazil) and Cacau Pucala (Peru) cultivars are statistically distinct.

In general, variations in chromosome length are associated with rearrangements and abnormalities such as deletions and duplications (Stebbins, 1971), the latter being less deleterious than the deletions; besides this, duplications can be regularly transmitted through the female gametophyte (Auger and Sheridan, 2012). Chromosomal rearrangements may result in abnormal meiosis (Souza and Pereira, 2011). The presence of univalents and other meiotic abnormalities has been reported in some *T. cacao* cultivars (Carletto, 1946; Opeke and Jacob, 1967). Despite the greater tolerance of plants to structural and numerical chromosomal changes such as aneuploidies (Matzke et al., 2003), aberrant morphological features and reproductive changes or losses may be related to karyotypic changes (Karsburg et al., 2009). Nevertheless, the morphological features observed in *T. cacao* are mostly associated with recessive genes. This means that only individuals homozygous for the mutant gene have the features (Bartley, 2005).

The position of the centromere has been found to vary in different *Theobroma* species. In *T. speciosum*, for example, the presence of submetacentric chromosomes was verified on the 4th and 9th chromosome pairs (Yoshitome et al., 2008). A comparative study addressing *T. cacao* and *T. grandiflorum* has revealed the presence of symmetrical karyotypes in both species, with a higher ratio between the chromosome arms on the 5th chromosome pair of *T. grandiflorum* (1.30) and the 7th chromosome pair of *T. cacao* (1.32) (Dantas and Guerra, 2010). By examining the commercial accession and *T. cacao* mutants, differences were observed in the position of the centromere, causing different karyotypic formulas to occur in the mutant accessions of Cacau Pucala, Cacau Jaca, and Cacau Sem Vidro when compared with Cacau Comum. Such variations in the chromosome morphology have reflected differences between the short and the long chromosome arms, indicating uneven genetic material gain (Stebbins, 1971). Gain or loss of genetic material is associated with chromosomal alterations such as deletions and insertions. On the other hand, translocations may be associated with changes in chromosome morphology; they do not imply loss or gain of genetic material, but may alter the chromosome morphology (Levin, 2002). If, on the one hand, changes in the chromosome structure may contribute to the diversification of the genus *Theobroma*, on the other hand, its intraspecific observation in *T. cacao* accessions indicate the involvement of different accessions in the germplasm analyzed in this work, as reinforced by the different origin of some accessions.

The asymmetry index (TF%) has shown that more symmetrical karyotypes are present in Cacau Pucala, Cacau Rui, and Cacau Jaca, which are more primitive, whereas Cacau

Comum and Cacau Sem Vidro exhibit karyotypes with a smaller karyotypic symmetry and are more derivative (Stebbins, 1971). Derivative karyotypes indicate recent chromosomal modifications during the establishment of a cultivated species or variety (Levin, 2002). Increased chromosomal asymmetry is associated with the accumulation of chromosomal differences as a function of the relative chromosome size amongst chromosomes of the complement of a given species. Such differences may result from chromosome modifications involving the chromosomal centromere, such as centric inversions, which can change its position from being median to subterminal or terminal (Stebbins, 1971).

The existence of a single satellite-bearing chromosome pair in the Cacau Pucala accession corroborates previous studies involving *T. cacao* (Glicenstein and Fritz, 1989; Dantas and Guerra, 2010). There were, however, variations in the number, location, and classification of satellites. Cacau Comum and Cacau Sem Vidro were found to have the greatest variation in the number and location of satellites, which were located on the long arm of chromosome pairs 1 and 2. Cacau Sem Vidro exhibited satellites on the short arm of the 6th and 10th chromosome pairs. In general, the polymorphism with respect to the number of secondary constrictions is assigned to post-hybridization events like nucleolar dominance and epigenetic factors (Pikaard, 2000).

The variation in the number and location of satellites, along with the different TF% and HLL values observed for Cacau Comum and Cacau Sem Vidro, indicate the distinct origin of such accessions in relation to others. One may assume that there has been genetic material loss in the Cacau Comum cultivar, and genetic gain in the Cacau Sem Vidro cultivar. This may be reinforced by the comparison between the HLL and the karyotypic character derived in both accessions, since Cacau Comum showed lower HLL as compared with Cacau Sem Vidro, and both accessions have shown high TF% in comparison with the other cultivars, hence indicating the presence of karyotypic modifications.

The extensive track history of domestication and genetic improvement of cocoa with the presence of contrasting varieties, accessions, and aberrant mutants may reveal particular peculiar cytogenetic and evolutionary parameters of *T. cacao*. Our results report the existence of an intraspecific karyotypic diversity in *T. cacao*, with stability in the chromosome number and variations touching the chromosome length and morphology, and the number and location of satellites.

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