Cacao Germplasm Collection in the Middle Amazon

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Abstract

Over the period from 6 to 12 of May, 2007, a botanical expedition was conducted in the Middle Amazon, Brazil. The objective was to sample the cacao populations of that region in order to support the cacao genetic improvement program of CEPLAC by enriching the germplasm collection. The region was chosen for representing important areas traditionally cultivated with cacao varieties domesticated by local producers (landraces), in the high varzeas of the Amazon River. These varieties always presented a better field performance than the hybrids. The cacao plantings are quite small, about three hectares in average, and maintained with very little technology. During the expedition, budwood sticks and seeds were collected from 87 mother trees on 19 small farms, representing five sub-populations of large stands of cacao. The biometrical analysis of some characters related to pods and seeds revealed a high phenotypic variability among the populations. The occurrence of certain similarities among them may have been caused by genetic flow resulting from the transport of plant material along the rivers and also due to the habit of the local producers to select genetic material based on phenotypic traits for the establishment of new plantings. The local farmers always chose planting materials from plants with pods of average size and thin husk, with a larger number and weight of seeds, and with sweet pulp. The results lead to the inference that these explored populations should belong to the same genetic complex due to the morphological and biometrical similarities for pod characteristics and to the genetic flow. Other aspects such as those related to local adaptation, cacao diseases, genetic erosion, the species domestication process, and multiplication of botanical materials are also discussed.

Introduction

Collection of cacao (*Theobroma cacao* L.) from Brazilian Amazonian populations has kindled the interest of the scientific community since the 1940s. The collecting expeditions undertaken in 1942, 1947, 1949 and in 1955 by the former Agronomic Institute of the North (IAN), Belém, Pará, Brazil, aimed at the use of new accessions in the development of new improved varieties. This activity increased in scale, particularly from the 1960s, with the creation of the Cacao Research Center of CEPLAC, in Ilhéus, Bahia. It gave support, initially, to the cacao genetic improvement programme in South-east Bahia and, later, to the expansion of the cacao crop in the Amazon region.

The botanical expeditions accomplished from 1960 to 1990, in the different hydrographic basins that constitute the area of dispersion of cacao, generated significant scientific knowledge, especially on the ecology of the Brazilian Amazon cacao populations, either in the wild or semi-cultivated. Their potential for use in genetic improvement was assessed by Vello and Medeiros 1965; Vello and Rocha 1967; Vello and Silva 1968; Bartley 1977; Machado 1981, Almeida 1982, 1983; Barriga 1982, 1988; Barriga *et al*. 1985; Almeida and Almeida 1987.

The fact that the cacao plantings in the Amazon River basin constitute a repository of genes for important agronomic traits is a further reason for interest in these populations. Riverside producers have always been inclined to cultivate certain types of cacao plants based on defined phenotypic characteristics such as pod shape, seed size, among others (Almeida *et al*.1995; Almeida 1996), contributing, therefore, to the fixation of such traits. That situation is quite common in the Middle Amazon, a region of very ancient cultivation and a reference centre for cacao exploration since the 19th century (Nery 1979). The cacao plantings in the Middle Amazon have only been partially explored so far for breeding purpose (Barriga 1988).

The objective of this article is to present the main results of the 2007 botanical expedition for the collection of cacao germplasm in the Middle Amazon describing the characteristics of the populations, the variability, the occurrence of diseases, the genetic erosion, the process of domestication of the species, among others, in order to contribute towards improvement achieved in cacao breeding programmes.

Material and methods

The expedition to collect cacao germplasm was aimed at semi-cultivated plantings on the banks of the Amazon River at locations sited between the municipal districts of Urucurituba (2° 47'S; 57° 20' W) and Itacoatiara (3° 8' S; 58° 25' W), and was mounted between May 6th and 12th, 2007. These plantings are distributed in a quite homogeneous micro-region known as the Middle Amazon (Figure 1), representing one of the traditional regions of cacao cultivation, where a great concentration of plantings of common varieties were established, having been domesticated by local producers over decades. The latter employed the process of phenotypic selection solely. These populations have been barely surveyed and represent a great potential for breeding.

The area in focus belongs to the sub-area of the Alluvial Plain of the Amazon River, which is characterized by the dense tropical forest and periodically flooded hydromorphic soils originating from the deposits of the recent Quaternary. These soils, known as high "*varzeas*", are annually enriched with sediments from the waters of the river flowing from the Cordillera of the Andes. During the collection mission, the water level of the Amazon River was about 50 to 80 cm below the soil level of the high *varzeas*, while in the previous year the flood reached a height of 80 cm in the cacao plantings.

The vegetation of the floodplain (*varzea*) forest is characterized by a small variety of medium-sized species and a great percentage of soft wood (Projeto Radambrazil 1976). The climate is rainy tropical, characterized as *Ami type*, with a well-defined dry season. The average, maximum and minimum temperatures in the region are approximately 27, 32 and 22°C, respectively. The number of daylight hours exceeds 1600 per year, and the relative humidity reaches an annual average between 71 and 82%, resulting in a constantly humid atmosphere. The annual average rainfall is more than 2000 mm, of which 75% is concentrated in the months December to May. Monthly rainfall less than 80 mm occurs from August to October. The average altitude of the region varies from 10 to 30 m above sea level.

In the region earmarked for prospecting, the Amazon River constitutes the main hydrographical network, which is also fed by the Madeira River, as well as several tributaries, "*igarapes*" and "*furos*" (channels) of lesser water flows. It also represents the only means of access to the cacao plantings for visitors from other regions.

The collection mission team consisted of seven employees of CEPLAC: two cacao breeders (Caio Márcio Vasconcellos Cordeiro de Almeida and Wilson Reis Monteiro); a plant pathologist (Paulo Sérgio Bevilaqua de Albuquerque); two technicians (Admilson Mota de Brito and Afonso Rabelo Costa); a field worker (Wálter da Silva Barbosa) and a driver responsible for support in the transport by land of materials from Itacoatiara to Manaus and vice-versa. Additionally, the services of a boat pilot and a cook were also procured.

The collection strategy consisted of sampling the cacao populations of the region. Only the more extensive cacao areas in terms of population size were considered, because these supposedly best represent the genetic variability locally. This decision was supported by information from the local people, who know the region and the cacao crop system in Middle Amazon better. It was decided to use a large sample of plants for each collection area to ensure better genetic representation. Cacao areas with small population sizes were not visited.

In this way, five large cacao concentrations were chosen at the following sites: Igarape of Jurupari, Costa das Garças, District of Augusto Montenegro, Costa do Moura and Costa do Conceição. Ripe pods were collected initially, or in their absence, almost ripe pods, to represent the observed phenotypic variation. In addition, whenever it was of interest to preserve the mother tree characteristics, budwood was collected for vegetative propagation. The cacao types were described according to the terminology proposed by Engels *et al*. (1980) with some adaptations.

The collected botanical materials were assigned an alphanumeric code using the letters AM - acronym for the Amazon State, followed by a number, starting from 1064, in sequence to the series of collections already accomplished in the Amazon State.

Average values were obtained for pod and seed traits such as weight of the pods (WP), fresh seed weight per pod (FSW/P), number of seeds per pod (NS/P) and fresh weight of a single seed (FWSS). These data were submitted to an analysis of variance, using the hierarchical classification scheme (Steel and Torrie 1960) and the comparison of means was made with Tukey's test.

The extremities of the budwood sticks of each mother tree were protected with paraffin wax and the sticks were then wrapped in moistened newspaper. The seeds were conditioned in moistened powdered charcoal, after the removal of the mucilage with sawdust. Finally, all the propagules were stored in styrofoam boxes and sent to four locations: Experimental Station of Rio Negro (ESTEX-RN), in Manaus, Amazon State, Experimental Station of Ouro Preto (ESTEX-OP), in Ouro Preto d'Oeste, Rondonia State, Jose Haroldo Cacao Genetic Resource Station (ERJOH), in Marituba, Pará State, and also to the Service of Plant Introduction - SIPLA, in Salvador, Bahia State, for subsequent transference to the Cacao Research Center (CEPEC) located in Itabuna, Bahia.

Results and discussion

Characteristics of the cacao populations cultivated in the Middle Amazon

The cacao plantings visited in the Middle Amazon belong to a great cacao complex that has its origin in much earlier times. Thus, currently it is impossible to establish a chronological mark as reference. However, the ancestors of the riverside cacao producers established most of the plantings more than a hundred years ago in many cases. Only a minority was planted more recently, *i.e*., in the last ten years. Initially, the cacao plantings were established on the margins of the Amazon River and then spread towards the interior of the *varzeas* at a variable distance, predominantly between 100 and 150 m and, in some cases, up to 300 m.

The local cocoa producers, believing that clumps with a larger number of trunks are more productive, traditionally do not prune orthotropic branches (chupons). Consequently, the clumps are formed by many trunks of various ages overlapping over generations. In some cases, the number of adult trunks can be greater than ten. The formation of the cacao plantings in the sub-forest of *varzeas*, under conditions of excessive shading, causes great competition for light, water and nutrients leading to an exaggerated growth with plants reaching heights of over 20m. With the aging of the clumps and bending of the higher trunks, new clumps arise near to the original ones through the growth of orthotropic branches from the bended or fallen trunks, as already reported by Almeida (1996). Thus, in these plantations, the cacao architecture is completely different from that observed in other producing areas where the management practices are used to maintain the individuality of the plants.

The handling of these plantings is carried out in quite a precarious way, limited to cultural treatments such as weeding, pod harvesting, seed fermentation and seed drying in the sun (Brito *et al*. 2002).

There are also some cacao plantings technically established under the guidance of the Rural Extension Services of CEPLAC. In these, the cacao tree is intercropped with other regional species of economic value, especially Açaí (*Euterpe oleracea*) and Taperebá (*Spondia lutea*). Under these circumstances, the producer was guided to use as planting material, the seeds from one to three selected cacao clumps from his own old planting or even from the neighbours by selecting the ones with a lower incidence of witches' broom disease, fruits of medium size with a thin husk and large seeds.

Cacao collections were undertaken on a total of 19 small farms including five larger cacao concentrations, located at the margins of the Amazon River or in the neighbourhood. These were grouped as following: i) Igarapé of Jurupari - 20 mother trees collected in five farms in the Communities of Igarapé do Jurupari and Nova Amazonas; ii) Costa das Garças - 16 mother trees in six farms in the Communities of Santa Cruz and Ponta Grossa; iii) Vila Augusto Montenegro - 11 mother trees in one farm in the Community of São José; iv) Costa of Moura - 18 mother trees in five farms in the Community of the Nossa Senhora do Perpétuo Socorro and v) Costa do Conceição - 22 mother trees in two farms in the Community of Nossa Senhora das Graças. The most western point of sampling was the Igarapé of Jurupari (2°31' 16.2 " S; 57° 34' 36.3 " W), in the municipal district of Urucurituba, upstream from the city of Parintins and the most eastern point was Igarapé of Canumanzinho (3°18' 38.4 " S; 58° 46' 28.8 " W), in the municipal district of Itacoatiara, about 40 km upstream of that city. The geographical distance between these extreme points is approximately 162 km in a straight line or about 185 km following the main course of the Amazon River.

The cacao population of Igarapé of Jurupari is 17 km far away from Costa das Garças; and the latter is about 20 km from that in the district of Augusto Montenegro. The distance from the latter population to the one of Costa do Moura is 80 km and a further 41 km to the cacao population in Costa da Conceição.

In Table 1, the mean squares, coefficients of variation, overall means and the range of variation for characters observed in the mentioned populations, such as pod weight (PW), fresh seed weigh per pod (FSWP), number of seeds per pod (NSP) and fresh weight of single seed (FWSS), are presented. A sample size of four pods was used for most of accessions (94.2%), and for only a few of them was a sample of two pods employed. For the character NSP, the mean square was not significant, suggesting that in the circumstances of the evaluation, the mentioned populations are quite similar for this trait. However, for other characters, the mean square values were significant at the levels of 5% and 1% probability, indicating the existence of phenotypic diversity among the explored populations.

The coefficients of variation ranged from 13.4% (for NSP) to 21.4% (for PW), indicating the presence of variability that can be exploited further with this germplasm. The overall means are quite high for semi-wild cacao material, especially for PW (535.5 g), FSWP (133.8 g) and FWSS (3.1 g), as are the maximum values. This may suggest that some type of phenotypic selection has been conducted for many generations by the riverside producer communities in the choice of planting materials for the establishment of the new plantings, as mentioned by Almeida *et al.* (1995) and Almeida (1996).

In Table 2, the number of cacao clumps observed in the populations and the results of the comparison of means, using the Tukey test, at the 5% level of probability are presented. For the characters showing significant differences (PW, FSWP and FWSS), the population of Augusto Montenegro was the one with lowest values, while that from Costa do Garças had the highest. In addition, it is evident that the values observed for the populations of Costa da Conceição and Igarapé do Jarupari, which represent the extreme points of the explored region, do not differ indicating phenotypic similarity although they are geographically separated by 162 km in a straight line.

In general, the morphological characteristics of pods and flowers that prevailed in the explored populations are:

- Flower – Average size, with an absence of anthocyanin or very little pigmentation in the filament, intensely pigmented staminodes, and guide lines with intermediate pigmentation.
- Fruit - Elliptic form, medium to large, little to moderate basal constriction, acuminate to obtuse apex, pod surface moderately rough, furrows lightly or moderately separate and of little or moderate depth. The colour of immature pods is light green with or without brightness, as shown in Figure 2, where samples of small <15 cm), medium (15 - 20 cm) and large (>20 cm) pods are illustrated.

Besides those characteristics, some less common characteristics in the *T. cacao* L. species were also observed, such as:

- Presence of anthocyanin pigment on the surface of the fruit in the accession, AM 1086, as much in the immature as in ripe pods, when exposed to the sunlight.
- Presence of round, green areas on the yellow surface of the ripe fruit as in the accession AM 1141;

Table 1: Estimates of mean squares between and within populations, coefficients of variation (CV), overall means and range of variation, in grammes, for characters measured in cacao trees of Middle Amazon, Amazon State, Brazil, 2007

Characters	Mean squares		CV	Overall means	Range of variation
	Between	Within	%		
Pod weight (g)	31,026.4*	12,194.8	21.4	535.5	280.0 – 915.0
Fresh seed weight per pod (g)	3,512.6**	545.8	19.6	133.8	82.8 – 237.5
Number of seeds per pod	26.1ns	34.0	13.4	43.1	27.0 - 61.0
Fresh weight of a single seed (g)	1.2**	0.2	17.0	3.1	2.0 - 5.0

[1]. * and ** = significance at the 5% and 1% probability levels, respectively, using the F test.
ns = not significant.

Table 2: Number of cacao clumps observed (NCC) and overall mean of populations for pod weight (PW, in grammes), fresh seed weight per pod (FSWP, in grammes), number of seeds per pod (NSP) and fresh weight of a single seed (FWSS, in grammes) obtained in cacao plantings of the Middle Amazon, Amazon State, Brazil, 2007

Cacao populations	NCC	PW	FSWP	NSP	FWSS
Augusto Montenegro	11	460.7a	111.6a	41.2a	2.7a
Costa da Conceição	22	508.3ab	127.3ab	43.3a	2.9ab
Igarapé do Jurupari	20	547.0b	131.9b	42.8a	3.2bc
Costa do Moura	18	568.7b	139.6bc	42.5a	3.3c
Costa das Garças	15	575.3b	155.4c	45.1a	3.5c

Means followed by the same letters do not differ amongst themselves according to Tukey's test at a 5% level of probability level.

- Fruits with erect peduncle, forming an angle of insertion of 90° in relation to the trunk, as in the accessions AM 1081 and AM 1132;
- Fruits with a hard husk and strong peduncle as in the accession AM 1102.

In some communities, the riverside producers used to employ local terms to characterize certain cacao types based on the pod shape as follows:

- "Cacau Jacaré", with big pods resembling an alligator, very rough pod surface, thick husk, as in accessions AM 1083, AM 1109 and AM 1123;
- "Cacau Bola", with small pods melon-shaped pods, thin husk, as in accessions AM 1066, AM 1081, AM 1130 and AM 1148;
- "Cacau Abiu", with a pod shape that resembles a native fruit tree known as "abiu". It is a type of cacao with that has a large, long pod, with an attenuate apex, as in AM 1067, AM 1075 and AM 1138.

The accessions, AM 1133 and AM 1134, are progenies of vigorous four-year-old mother trees, with a single trunk, that presented more than 30 fruits of medium size, in an advanced stage of development.

On some farms, a great uniformity for type of pods was observed, indicating that genes that control such characters are already fixed in the population, as was the case in the community of Nossa Senhora do Perpetuo Socorro where the amelonado pod shape prevailed, with a thinner pod husk and pod size varying between small and medium. This is attributed to a narrow genetic base that might be restricted to a few selected components. One farmer from the district of Augusto Montenegro provided an example of the practice of phenotypic selection when he revealed that in the past he used to establish cacao plantings with seeds obtained from those selected cacao clumps that were distinguished from the others for being more productive, free of witches´ broom symptoms, and produced large pods with thin and soft husks that easily break when pods fall on the ground during harvesting.

Considering certain similarities in morphological and biometrical characteristics of pods among the populations as well as the occurrence of the genetic flow among them, it is believed that these five explored populations might be closely related and, consequently, might belong to the same genetic complex. Genetic flow is thought to have occurred via the movement of botanical material subsequent to landslides on river banks caused by erosion, and also due to the phenotypic selection of planting materials among the riverside producers

Occurrence of diseases

The occurrence of witches' broom disease, caused by *Moniliophthora perniciosa,* was observed in all cacao plantings that were visited, as evident by the symptoms in the canopy, fruits and flower cushions. The incidence of disease varied among communities. However, it was a common manifestation expressed at different levels of severity in the same plantation. In general, high inoculum pressure of *M. perniciosa* was observed in plantings of the communities of Igarapé do Jurupari, Novo Amazonas, Santa Cruz and Ponta Grossa; low pressure in the district Augusto Montenegro and in the community of Nossa Senhora das Graças; and variable intensities in the community of Nossa Senhora do Perpetuo Socorro (low, medium and high). In 45 accessions out of 87 selected mother trees that supposedly represent the observed variability in that area, no symptom of the disease was observed.

Nascimento, Almeida and Alvim (1984) and Barriga (1988) observed a low incidence of witches' broom in the plantings when collecting cacao in the Middle Amazon. The authors attributed this fact to the variation in the level of the water of the Amazon River on *varzea* soils that could supply an efficient and natural control of that disease through an escape mechanism (absence of synchronization between the presence of spores of *M. perniciosa* and new tissues of the cacao tree). However, the observations made in the current expedition led to the assumption that the low incidence of witches' broom in these cacao plantings may be attributed not

only to the escape, but also to the fact that the phenotypic selection has been based on criteria related to production and resistance to *M. perniciosa.* This is probable since some riverside farmers selected certain types of pods based on productive and resistant cacao clumps, according to some reports.

The incidence of *Phytophthora* was observed in pods and in orthotropic branches, especially in two farms of the “Costa do Conceição”. These appeared to be related to very favourable environmental conditions for the disease, characterized by the excess of shade and the swampy soils.

Genetic erosion in the Middle Amazon cacao plantings

The cacao plantings of the Amazon floodlands (*varzeas*) are submitted to a process of genetic erosion due to the occurrence of floods and to the action of the local people when looking for more profitable and productive agri-businesses such as passion fruit planting (Brito *et al.* 2002). Reports from 50 years ago contained information that there was a great cacao concentration in the area between the "Parana do Ramos", in the municipal district of Urucurituba, and the "Parana do Albano", in the municipal district of Parintins, on the right margin of the Amazon River. This was a continuous area with an extension of approximately 95 km, only interrupted by the water outlets of *igarapes*, *paranas* and *furos* (channels), belonging to several producers. Currently, that cacao area is reduced to less than half as a consequence of facts already mentioned. The same problem has been observed with the cacao plantings of Costa da Conceição, between the *igarapes* of Canumazinho and Padre, which were reduced to 10% of the total area.

The domestication of cacao in the Middle Amazon

The term domestication is used to describe the evolutionary process under human selection, in which the wild state changes slowly and gradually to a cultivated condition. In this context, Almeida (1996) discussed some indicative aspects of the occurrence of domestication in *T. cacao* L.

The observation of the cacao cultivation in the *varzeas* of the Middle Amazon allows the analysis of the ongoing process of domestication of the species. The local farmers have contributed effectively to the adaptation of the species to that ecosystem through cultivation and phenotypic selection for some hundreds of years.

These cacao plantings were initially established by using seed from wild populations collected in the region or from under-storey seedlings within their farms. With either of these possibilities, natural selection might have acted on the cacao population, since the environmental factors had an influence not only on seed germination, but also on growth of the seedlings, thus establishing a reproductive differential. It was also confirmed that the local farmers always chose as planting materials those cacao plants with pods of average size and thin husk, larger number and weight of seeds, and with sweet pulp. This characterizes the practice of phenotypical selection. In agreement with their observations, large pods generally have a thick husk and a small number of seeds.

After the establishment of these plantings, individuals in the cacao population are naturally exposed to many abiotic stresses, having to compete for environmental factors, especially light and nutrients of the soil due to a great number of shade species in the area. Additionally, the annual floods may have exerted a strong selection pressure on the genotypes, favouring the natural selection of those that are more adapted to a habitat with periodic flooding. This survival capacity of the genotypes may be positively correlated to adaptability to the *varzea* conditions, as

well as with productivity, since the evolutionary process also influences the capacity of different genotypes to transmit their genes to future generations (Shorrocks 1980). This becomes evident when comparing the plantings in this region to those established with hybrid varieties developed during the decade of the 1970s in Bahia. The latter always had very poor vegetative and reproductive performance.

It was also observed that the high number of stems per cacao clump may possibly constitute a strategy for adaptation of the species to the environment, in the sense of assuring reproductive success and its own preservation, as mentioned by Almeida (1996). In the period of pod ripening (March to June), the *varzeas* are, in general, submitted to the floods of the Amazon river, a condition that is favourable for the transportation of fruits and of seeds, but unfavourable for the germination of seeds.

Thus, it is shown that the cacao plantings of the Middle Amazon are under continuous selective pressures for ecological adaptability of the species to the prevalent conditions in that ecosystem, and constitute a real local landrace variety.

Multiplication of the genetic materials

Seeds or budwood sticks were collected from 87 mother trees as follows: only budwood from one mother tree only; budwood and seed from 16 plants; and only seed from 70 plants. This resulted in 17 clones and 86 progenies. Such propagules were submitted to multiplication processes over periods that varied from two to four days after the collection, depending on the location of the three CEPLAC experimental stations. Fifty days later, the efficiency indexes were quite variable for the two multiplication processes: i) vegetative propagation - 17 mother trees were multiplied onto 733 rootstock seedlings, with 503 successful grafts (68.6%); ii) seed propagation - 86 progenies were multiplied through sowing of 11,559 seeds with 10,892 seedlings obtained (94.2%).

In general, multiplication through vegetative propagation always results in a lower efficiency due to the difficulty of obtaining budwood sticks in good physiological condition for that purpose, a consequence of the excessive shading. In addition, it is common to experience a total loss of the accessions due to the inefficiency of the process in cases where the budwood sticks are in an unsuitable condition. The multiplication through seminal propagation besides assuring better genetic representation of the population also has a better efficiency index due to the convenience inherent in sowing. Thus, if seed collection is the objective of a collecting mission, it becomes critical to accomplish the collection during the main periods of cacao pod ripening.

Conclusions

Some conclusions that can be drawn from this botanical expedition are:

- The observed sub-populations have been genetically improved for more than three centuries by the local communities, through phenotypic selection only;
- Within some of these sub-populations, characteristics related to shape and size of pod, number of seeds per pod and resistance to diseases, especially, witches' broom, were fixed;
- There is variability regarding pod shape, size and rugosity. The variation among sub-populations is larger and significant, while within sub-populations it is not;

- For the cacao sub-populations of Costa do Garça, Costa do Moura and Igarapé do Jupari, the average pod size was larger than that observed for the sub-populations of Costa do Conceição and Augusto Montenegro.
- The Augusto Montenegro's sub-population is more uniform, with pods and seeds being smaller in size.
- All sub-populations appear to present a high level of resistance to witches' broom disease in fruits, flower cushions and the canopy.
- The selection procedures employed by the Middle Amazon communities were efficient in obtaining highly productive and resistant cacao plants;
- The level of productivity of the sub-populations can be increased by the adoption of modern production technologies;
- A seed collection is more representative than a clonal collection. Five pods from each selected mother tree facilitated transport, shipment and packing of seeds, decreases the risk of losing any accession, and allows *in situ* evaluation of the sub-population;
- The 87 accessions collected are considered as a good representation of the sub-population of cacao in the Middle Amazon region.

Acknowledgements

Our acknowledgements go to the Common Fund for Commodities (CFC), the International Cacao Organization (ICCO), Bioversity International and also CEPLAC for giving the necessary support for this botanical expedition. They are also extended to Afonso Rabelo Costa (technician), Walter da Silva Barbosa (field assistant) and to the administrative body of CEPLAC in Manaus for the great collaboration rendered during this botanical expedition. Also, we would like to express our gratitude to the cacao producers of the Middle Amazon for being so kind, allowing us to visit their plantings for the collection of germplasm and to Raul R. M. Valle, João L. Pereira and Uilson V. Lopes for the revision of this article.

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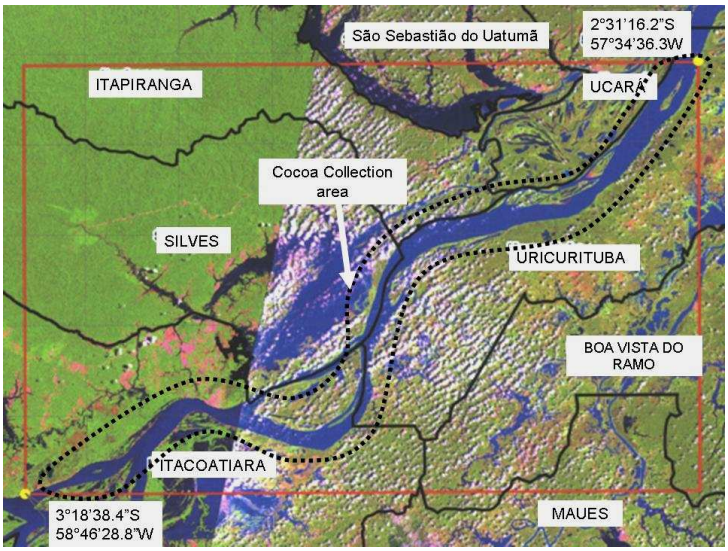

Figure 1: Map of the Middle Amazon, Amazon, Brazil, showing the area where the collecting action took place

Figure 2: Example of the variability in the cacao populations of Middle Amazon, Amazon, Brazil