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Description of Amazonian *Theobroma* L. collections, species identification, and characterization of interspecific hybrids

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RESUMO – (Descrição de coleções Amazônicas de *Theobroma* L., identificação de espécies e caracterização de híbridos inter-específicos). No Estado do Pará, Brasil, localizadas nas cidades de Belém e Marituba, existem duas coleções *in vivo* de espécies brasileiras do gênero *Theobroma* L. que abrigam, além das espécies naturais, híbridos inter-específicos obtidos natural ou artificialmente. Objetivando organizar uma base de dados sobre as espécies brasileiras do gênero *Theobroma*, descreve-se neste artigo as coleções existentes, com informação detalhada sobre os híbridos inter-específicos. Inclui-se chave artificial para identificação dos táxons verificados.

Palavras-chave: híbridos inter-específicos, *Theobroma*, germoplasma, *Herrania*

ABSTRACT – (Description of Amazonian *Theobroma* L. collections, species identification, and characterization of interspecific hybrids). There are two major *in vivo* collections of species of the genus *Theobroma* L. in Belém and Marituba, state of Pará, Brazil, and in both there are natural species, as well as natural and artificial interspecific hybrids. In order to organize a database of Brazilian *Theobroma* species, a description of the existing collections and detailed information about the interspecific hybrids, including an artificial key for their identification, are presented in this article.

Key words: interspecific hybrids, *Theobroma*, germplasm, *Herrania*

Introduction

Theobroma L. is the genus of the most relevance of the family Sterculiaceae, because of the economic importance of the cacao tree (*T. cacao* L.) (Purseglove 1968). *Theobroma* is of exclusive neotropical origin, with natural dispersion in tropical lowland rainforests extending from the Amazon basin through Southern Mexico (18ºN to 15ºS) (Cuatrecasas 1964). Recent phylogenetic studies, based on combined analysis of plastid *atpB* and *rbcL* DNA sequences, morphological, anatomical, palynological, and chemical characteristics have been used to propose the inclusion of the family Sterculiaceae into a broadly defined Malvaceae *sensu latu* (Judd & Manchester 1997; Alverson *et al.* 1999; Bayer *et al.* 1999).

The genus *Theobroma* contains 22 species classified into six sections: Andropetalum (*T. mammosum* Cuatr. & León); Glossopetalum (*T. angustifolium* Moçiño & Sessé, *T. canumanense* Pires & Fróes, *T. chocoense* Cuatr., *T. cirmolinae* Cuatr., *T. grandiflorum* (Willd. ex Spreng.) Schum., *T. hylaeum* Cuatr., *T. nemorale* Cuatr., *T. obovatum* Klotzsch ex Bernoulli, *T. simiarum* Donn. Smith., *T. sinuosum* Pavón ex Hubber, *T. stipulatum* Cuatr., *T. subincanum* Mart.); Oreanthes (*T. bernouillii* Pittier, *T. glaucum* Karst., *T. speciosum* Willd., *T. sylvestre* Mart., *T. velutinum* Benoist); Rhytidocarpus (*T. bicolor* Humb. & Bonpl.); Telmatocarpus (*T. gileri* Cuatr., *T. microcarpum* Mart.) and Theobroma (*T. cacao* L.). Representative species from all the sections can be found in Brazil, except for Andropetalum. The species that occur in Brazil are: *T. grandiflorum*, *T. obovatum*, *T. subincanum*, *T. speciosum*, *T. sylvestre*, *T. microcarpum*, *T. glaucum*, *T. canumanense*, *T. bicolor*, and *T. cacao*, all restricted to the Amazon basin (Cuatrecasas 1964). The existence of the species *T. canumanense* is based in the description of two exsiccates collected near the river Canumão (municipality of Borba, AM). Seeds of *Theobroma* are recalcitrant, restricting conventional *ex situ* preservation of genetic resources. Therefore, long-term preservation of *Theobroma* species requires the establishment of germplasm collection either *in situ* or *ex situ*.

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Interspecific hybridization between *Theobroma* species is a potential strategy for cacao (*T. cacao*) breeding, including the introduction of genes for resistance against major pathogens, such as the witches' broom disease caused by *Crinipellis perniciosa* (Stahel) Singer, and other traits, such as abscission of mature fruits and changes in tree canopy architecture. The development of new biotechnologies might facilitate the generation of new interspecific hybrids, increasing the importance of *Theobroma* germplasm collections for their potential use in *T. cacao* breeding programs.

There are two important collections of *Theobroma* species in the state of Pará. The "George O'Neill Addison" collection at "Empresa Brasileira de Pesquisas Agropecuária Amazônia Oriental/ EMBRAPA Amazônia Oriental" in Belém, and the "Basil George David Bartley " collection, established at the "Estação de Recursos Genéticos do Cacau (ERJOH)", at the "Comissão Executiva do Plano da Lavoura Cacaueira" (CEPLAC), in Marituba, that contains natural and artificial interspecific hybrids between Brazilian *Theobroma* species. Nevertheless, most of the information about these collections have not been neither organized nor published. Unfortunately, some of the information has been completely lost, such as the original field map and the notebook of the "George O'Neill Addison" collection. Recently, this collection has been reinventoried by Silva & Venturieri (1998).

Due to the importance of these two collections, they were subjected to the following procedures: a) redefinition of the collection boundaries, with a survey of existing specimens and localization in field maps; b) correct botanical identification of the species and their hybrids; and c) formulation of a descriptive synopsis about the species and their hybrids, based on morphological descriptors found in the literature, field observations, and comparison with herbarium samples from "EMBRAPA Amazônia Oriental" and from the "Museu Paraense Emílio Goeldi". A review about reported interspecific hybrids of *Theobroma* and *Herrania* Goudot, and a critical survey of available information were also presented to organize and establish a consulting source about *Theobroma*.

Material and methods

Survey of the *in vivo* collections - The Amazonian *Theobroma* collections were re-characterized, generating a list of specimens (species and interspecific hybrids) properly identified by botanical characters and by molecular and biochemical markers (Silva 2000; Silva *et al*. 2001). All specimens were identified and physically mapped in the areas.

"Basil George David Bartley" Collection - A field survey was conducted to correctly identify all species represented at ERJOH-CEPLAC, with special attention in identifying natural hybrids. Based on an existing map, the identification of all existing specimens was checked, and a field map was established. The identification of the species, with the establishment of a list of species and hybrids and location on a field map, followed the same methods used for the "George O'Neill Addison" collection.

"George O'Neill Addison" Collection - Initially, the original field map was searched in the library of "EMBRAPA Amazônia Oriental", followed by interviewing many researchers who might had been involved with the collection, without success. A new map was drawn based on the field survey of the collection, checking with existing information from reports and fragments of information from persons directly or indirectly involved on the establishment and/ or maintenance of the collection. The map was drawn dividing the area into lines and columns, naming and locating each plant into Cartesian coordinates.

Identification of species and interspecific hybrids - Botanical vouchers of *Theobroma* and *Herrania* species deposited at the herbarium of "EMBRAPA Amazônia Oriental" and from the "Museu Paraense Emílio Goeldi" were consulted to proceed morphological comparative studies of the *Theobroma*, the interspecific hybrids, and *Herrania* species. Descriptions available in the literature were also used during identifications.

Results

Survey of the *in vivo* collections

"Basil George David Bartley" collection

Location - This collection is located at the Eastern quarter, near the entrance of the "Estação de Recursos Genéticos do Cacau José Haroldo" (ERJOH), at the road BR 316, Km 15, in Marituba, Pará, Brazil (1º12'S; 49º13'W).

History - To increase the genetic basis of the species related to *T. cacao* for conservation and future use in

breeding programs, a field collection was started in January/1984 by Dr. Basil George David Bartley with representatives of all species of the genus *Theobroma* naturally occurring in the Brazilian Amazon, including some species of the genus *Herrania* [*H. mariae* (Mart.) Decaisne ex Goudot, *H. albiflora* Goudot and *Herrania* sp.]. A summary about this collection was published (Almeida *et al.* 1987; 1995). This collection, named "Basil George David Bartley Collection" contains natural hybrids between *T. grandiflorum* and *T. subincanum* (Silva & Venturieri 1998).

Inventory - The identification and location of the existing trees of the "Basil George David Bartley" collection can be verified in the field map presented by Silva & Venturieri (1998), and the species description can be consulted by the species identification key proposed by Cuatrecasas (1964).

There are 280 trees of the genus *Theobroma*: 65 of *T. grandiflorum*; 110 of *T. subincanum*; 12 of *T. bicolor*; 11 of *T. sylvestre*; 34 of *T. obovatum*; 7 of *T. obovatum* from Tefé (AM); 8 of *T. microcarpum*; 21 of *T. speciosum*; 7 of *Theobroma* sp. and 5 natural hybrids of *T. grandiflorum* × *T. subincanum*.

Observations - (1) There are many *Herrania mariae* in the area; (2) There are other *Herrania* in the area, but not *Herrania mariae* cf. *Herrania albiflora*; (3) In other areas of the station, there are many *T. cacao* accesses, forming the major cacao collection of Brazil, better described in Almeida *et al*. (1987; 1995).

Natural hybrids - Five natural hybrids between *T. grandiflorum* and *T. subincanum* were identified in the "Basil George David Bartley" collection, here reported as the first natural adult hybrids observed between these two species. Addison & Tavares (1951) obtained experimentally the same hybrid, and their observations were limited to seedling characteristics, since their hybrids were at the juvenile stage, and had not yet flowered. These hybrids were planted in a single row and introduced as half-sibs, identified as F11-1, F11-5, F11-15, F11-17 and F11-19. Two of them (F11-1 and F11-5) show an excellent hybrid vigor, in comparison to neighboring *T. grandiflorum* trees, but both were highly susceptible to witches' broom disease. The hybrid F11-19 is a considerable smaller plant than its half-sibs F11-1 and F11-5. The F11-5 tree blossoms intensively but does not bear pods. Up to now, only the F11-1 and F11-19 have produced pods. Not all of the trees from the collection had flowered when this manuscript was prepared, therefore it is possible that other natural hybrids between *T. grandiflorum* and *T. subincanum* might be occurring in the area, especially those assumed as *T. grandiflorum* in row F11.

"George O'Neill Addison" collection

Location - This collection belongs to the "Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA Amazônia Oriental", located in Belém, Pará, Brazil (1º20'S; 48º30'W).

History - In February/1945, George O'Neill Addison and Rosendo Tavares established a "Cacao Breeding Program" at the "Instituto Agronômico do Norte, IAN" (nowadays "EMBRAPA Amazônia Oriental") to obtain interspecific hybrids of *Theobroma* which could combine *T. cacao* yield with resistance to the "...major cacao diseases, such as witches' broom, and black pod". So, nine species of *Theobroma* were established: *T. cacao* (in two areas, one for rootstock formation to be grafted with varieties of interest, and another for seedlings of "Catongo", an albino-seed mutant variety from the state of Bahia); *T. mariae* (later reclassified as *Herrrania mariae*); *T. microcarpum*; *T. obovatum*; *T. subincanum*; *T. grandiflorum*; *T. spruceanum* (now considered as *T. sylvestre*); *T. speciosum*; and *T. bicolor* (Addison & Tavares 1951; IAN 1946).

From November/1945 to February/1946, studies about methods of controlled pollination and pod set among different trees of *Theobroma* species were conducted using trees from the "Museu Paraense Emílio Goeldi". From there, ten *T. cacao* plants were selected for crosses with other species of the genus. It was noticed that one of the plants (nº 5), despite the fact that it was self-sterile, it was the only one to set interspecific hybrid pods (Addison & Tavares 1951; IAN 1946).

In 1947, the species *T. cirmolinae* (from Colombia), *T. simiarum* (from Costa Rica), and varieties from different Amazonian regions of *T. cacao*, *T. speciosum*, *T. sylvestre*, *T. subincanum*, *T. grandiflorum*, *T. bicolor*, *T. microcarpum*, *Herrrania mariae*, and *T. obovatum* were introduced in the "George O'Neill Addison" collection. During the same year, attempts were conducted to develop polyploids to investigate their behavior in relation to economic important traits. From these experiments, two *T. cacao* polyploids, 8 plants from *T. speciosum* and 2 from *T. subincanum* (IAN 1947) were obtained, however there is no further report about these polyploids. In 1948, grafting experiments between the different Brazilian species of *Theobroma* were started,

to better understand the physiological affinity among these species (IAN 1948).

During 1949, the "Plant Breeding Unit" ("Secção de Melhoramento de Plantas") was dedicated to the conservation and multiplication of the introduced material; grafting and hybridization between *Theobroma* species; and to multiply the *T. cacao* clone "ICS-1", introduced from Trinidad, to provide budstick for cacao growers (IAN 1949). From the hybridizations conducted in that year, only hybrids between *T. grandiflorum* × *T. subincanum*, *T. grandiflorum* × *T. obovatum*, *T. subincanum* × *T. obovatum*, and *T. speciosum* × *T. sylvestre* developed normally. The hybrid seedlings derived from pollinations between *T. cacao* × *T. grandiflorum* and *T. speciosum* × *T. bicolor* died before they reached 15cm (IAN 1949).

In 1950, shade trees were planted, including "bracatinga" (*Mimosa schomburgkii* Benth.), "visgueiro" (*Parkia pendula* Bent.), "pau-de-balsa" (*Ochroma lagopus* Swartz) and "ingazeiro" (*Inga dulcis* Mart.), which some still remains in the area, to establish a 10ha field for plant propagation. Part of this area was used as experimental field trials, and for introductions of new varieties. This area nowadays is the "George O'Neill Addison" collection of *Theobroma* species (IAN 1952).

During 1951, the interspecific *Theobroma* hybridizations continued, including backcrossing of the hybrids, but without success. In 1951, a manuscript was published in Portuguese containing the results from the interspecific hybridization work conducted since 1945, including a detailed illustration of the hybrids (Addison & Tavares 1951; IAN 1951). In the following year, an English version of the paper was published (Addison & Tavares 1952).

The original hybrids between *T. grandiflorum* × *T. subincanum*, *T. grandiflorum* × *T. obovatum*, *T. subincanum* × *T. obovatum* and *T. speciosum* × *T. sylvestre* developed by Addison & Tavares (1952) still exist at "EMBRAPA Amazônia Oriental" in Belém. However, during all these years, the collection was not properly maintained. In the same area, a fertilizer trial with *T. grandiflorum*, followed by studies in plant pathology were established (Hans Müller, pers. communication). A loss of around 1/3 of the original plants is estimated. Most of the collection (around 2/3) was left up the year 2000 under scrubs. The original field map and plant identification were lost.

Inventory - The identification and location of the existing trees of the "George O'Neill Addison" collection can be checked in Silva & Venturieri (1998), and the species description can be consulted by the species identification key proposed by Cuatrecasas (1964). A key for the identification of the interspecific hybrids is here proposed. In the area, there are 1,488 trees of the genus *Theobroma*, identified as follows: 840 trees of *T. grandiflorum*, of which only 121 are from the original collection; 2 of *T. subincanum*; 18 of *T. obovatum*; 3 of *T. bicolor*; 4 of *T. sylvestre*; 200 of *T. speciosum*, 2 of *T. glaucum*; 3 *T. grandiflorum* × *T. subincanum* hybrids; 8 *T. grandiflorum* × *T. obovatum* hybrids; 8 *T. subincanum* × *T. obovatum* hybrids; 285 *T. sylvestre* × *T. speciosum* hybrids; 115 trees of an unidentified *Theobroma* species from Oreanthes section. There were a few individuals of *Herrania* sp. at the collection site.

Occurrence of Artificial Interspecific Hybrids - Representatives of all *Theobroma* interspecific hybrids reported by Addison & Tavares (1951) were identified: *T. grandiflorum* × *T. subincanum*, *T. grandiflorum* × *T. obovatum*, *T. obovatum* × *T. subincanum* and *T. speciosum* × *T. sylvestre*. A brief description of the main characteristics of these hybrids is presented below, emphasizing those that differentiate from the parental species and their ability to produce offspring.

Identification of interspecific hybrids - Based on the identification key for the Brazilian *Theobroma* species proposed by Ducke (1953), an artificial key for the identification of interspecific hybrids was developed.

Artificial Key to the Species and Hybrids *Teobroma*

1. Multibranched tree, erect trunk, jorquette with tree branches, simple leaves. Petal-lamina smaller than three times the length of the petal-hood
   2. Petal-lamina broadly elliptic. Staminodes subulated. Pods with five weak ribs, not reticulated
      3. Stamens with three antheriferous ..... ***T. speciosum***
      3. Stamens with two antheriferous
         4. Petal-lamina rose or brownish red, with 2×2.2-2.5mm. Flowers mainly on small branches, some in the trunk, in small fascicles, scentless or with a weak vanilla scent ..... ***T. sylvestre***

4. Petal-lamina red, with 5-6×7-8mm. Flowers on branches and trunk, in small fascicles, with lemon scent ..... ***T. sylvestre* × *T. speciosum***

2. Petal-lamina triangular in keel form, tenuous hoofed at base. Staminodes with petal form. Pods are neither ribbed nor reticulated
   5. Pods ellipsoid-obovoid or oblong-ellipsoid
      6. Pods with granular epicarp ..... ***T. obovatum***
      6. Pod without granular epicarp
         7. Pods measuring 10×6cm. Leaves frequently beyond 30×15cm ..... ***T. subincanum***
         7. Pods measuring up to 7×4cm. Presence of dimorphism on leaves: large leaves (30×15cm) as in *T. subincanum* coexisting with small leaves (20×7cm) as in *T. obovatum* ..... ***T. subincanum* × *T. obovatum***
   5. Pods ellipsoid, subglobose or subcylindrical
      8. Staminodes lanceolate, abruptly acuminated on the apex ..... ***T. grandiflorum***
      8. Staminodes oblong, inflexed, with acute apex
         9. Epicarp with soft tomentous unevenly distributed. Staminodes measuring 11-13×3mm ..... ***T. grandiflorum* × *T. obovatum***
         9. Epicarp with soft tomentous evenly distributed. Staminodes measuring 10-11×3mm ..... ***T. grandiflorum* × *T. subincanum***

1. Unbranched trunk, slender, with apical growth and compound palmate leaves, 5-9 digitate, in top. Petal-lamina exceeding many times the length of the petal-hood ..... ***Herrania***

Hybrid description

T. grandiflorum × *T. subincanum* hybrids - These hybrids were more vigorous than the larger parental species, *T. grandiflorum*, suggesting the occurrence of hybrid vigor, but they were highly susceptible to witches' broom disease. The pods from these hybrids were slightly larger than those from *T. subincanum*, but the format and color of its tomentous pericarp were alike to *T. grandiflorum* pods. In relation to the organoleptic characteristics, the seed-pulp scent resembled *T. grandiflorum*, but it was less acidic and its taste remembered banana flavor. These hybrids had a remarkable characteristic of abscission of mature pods, apparently a dominant character inherited from *T. grandiflorum*, because pods from *T. subincanum* do not abscise. However, it was common to observe dry pods hanging from the trunk of these hybrids (as observed in *T. subincanum*), but it was not clear if this was a characteristic of the hybrids or it was due to the attack of witches' broom disease. Their seeds had shape and size more similar to *T. subincanum*, and when sown, germinated and produced seedlings. The general aspect of the tree was similar to *T. grandiflorum*, but some of the leaves were remarkably larger than in the parentals (length and width). The flowers were very similar to *T. grandiflorum* (shape and color), but it was possible to distinguish the flowers from the hybrid tree by the smaller size of the petals (petal-lamina and petal-hood) and also by the staminodes that did not show an abruptly acuminated apex as in *T. grandiflorum*. The flowers from the hybrids presented stamens with 3 antheriferous (Silva & Venturieri 1998).

T. grandiflorum × *T. obovatum* hybrids - The general aspect of these hybrid trees resembled *T. grandiflorum*, and the best way to identify was based on flower characteristics, mainly the size and shape of the staminodes or by the intermediate characteristics of their pods (Fig. 1A). It was possible to observe a few seedlings under the hybrid tree canopy, from fallen cracked pods. Some seeds from these hybrids did not germinate, while some germinated, producing a radicule and small shoots, but the development was arrested when the seedlings reached around 10cm, without expanding the first leaves. Nevertheless, some exhibited a normal, or almost normal development, while some presented "leaf burning", similar as described for the F_1 hybrids by Addison & Tavares (1952). One hybrid tree produced a large amount of pods, and it was apparently resistant to witches' broom disease, and it can be indicated for further tests for yield (Silva & Venturieri 1998).

T. subincanum × *T. obovatum* hybrids - These hybrids had flowers with intermediate characteristics between the parental species. The hybrid could be identified by its flowers, that resembled more *T. obovatum* (petal-

lamina with an invagination) and by the general aspect of the canopy, that showed leaf dimorphism, with large leaves, such as in *T. subincanum*, co-existing with small leaves, as in *T. obovatum* or also by the presence of pods with intermediate characteristics between the parental species (Fig. 1B). The pods from the hybrids were more similar in shape to *T. obovatum* pods, but they were a bit longer, smoother and browner, when ripen, than *T. subincanum*. These pods, when not harvested, dried on the trees. These hybrids presented tomentum at the abaxial face of the young leaves, which remained on senescent leaves (Silva & Venturieri 1998).

T. sylvestre × *T. speciosum* hybrids - The trees were very similar to the parental species, which are very much alike. The hybrid trees produced flowers on the trunk and branches (Fig. 1C). These flowers were similar in shape and petal-lamina color (red) to those of *T. speciosum*, but were small as in *T. sylvestre*, because they contained stamens with 2-antheriferous, and *T. speciosum* shows 3-antheriferous. The best criteria to discriminate the hybrids from *T. sylvestre* were the larger size of their flowers (mainly the petal-lamina); their flower shape was more similar to *T. speciosum*; the lime scent similar to *T. speciosum* flowers, but less intense. Some hybrid trees produced flowers with 5, 6 or 7 (rarely) staminodes and 5 or 6 petals. In one case, a hybrid tree (R22/23-56) showed around 50% of the flowers with 6 staminodes, plus flowers containing all possible combinations of the number of staminodes and petals mentioned above. All the hybrids are currently producing pods, which are similar to those from *T. sylvestre*. These pods, even ripe, remain on the trees and dry. A few young *T. sylvestre* × *T. speciosum* hybrid seedlings were found at variable distances from adult hybrid trees suggesting to be resulting from natural dispersion (Silva & Venturieri 1998).

Discussion

Natural hybrids between species of *Theobroma* are extremely rare. According to Reksodihardjo (1964), there was only one report by Baker *et al*. (1953) about a tree, found in the Caquetá river, Remolino forest

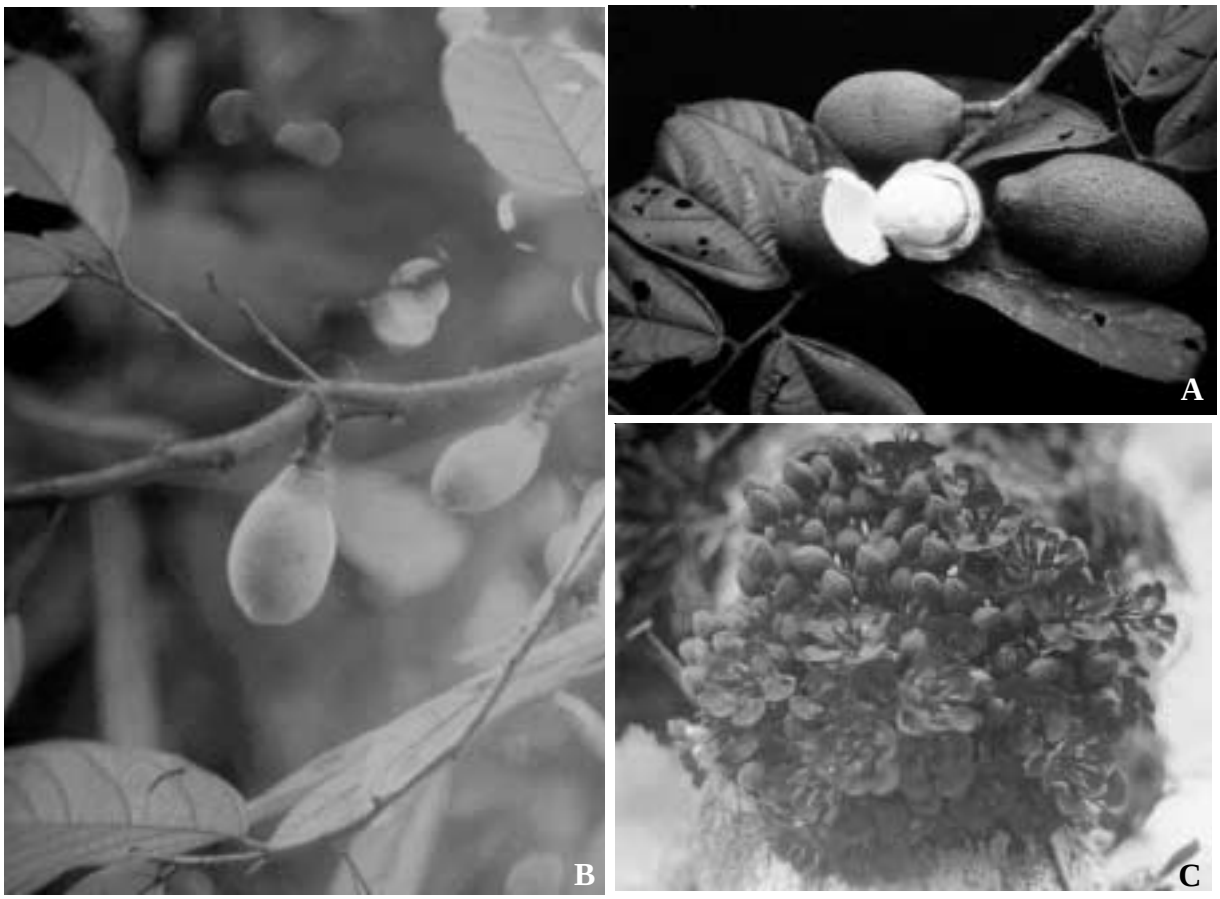

Figure 1. A. Pod from *Theobroma grandiflorum* × *Theobroma obovatum* hybrid. B. Pod from *Theobroma subincanum* × *Theobroma obovatum* hybrid. C. Flower cushion of *Theobroma sylvestre* × *Theobroma speciosum* hybrid.

(southwestern Colombia) with intermediate characteristics between *T. obovatum* and *T. subincanum* (called as *Theobroma guianense* in Reksodihardjo 1964), that could possibly be of a hybrid origin. This tree is the same mentioned in the last revision of the genus *Theobroma* conducted by Cuatrecasas (1964), who described the finding of this natural hybrid tree, of about 11-12 meters, by Cope and Holliday. This tree had some characters of *T. obovatum* and others of *T. subincanum*, described as having almost smooth pods, but beared floccose, and exhibiting a woolly pubescence on the young shoots and leaves [characteristics of exsiccate T122, deposited in the Imperial College of Tropical Agriculture (St. Augustine, Trinidad and Tobago); Instituto de Ciencias Naturales (Bogotá, Colombia); and the U.S. National Herbarium (Washington, USA) herbaria].

The examination of specimens at herbaria suggested the existence of others natural hybrids. Reksodihardjo (1964) also referred to others exsiccates of hybrids between *T. obovatum* and *T. subincanum*, one collected in Colombia (Cope & Holliday T117) and deposited at the Imperial College of Tropical Agriculture (Trinidad and Tobago) and at the "Instituto de Ciencias Naturales" (Bogotá, Colombia) herbaria, and two collected in Brazil (Fróes 20684 and 33783), both deposited at the IAN (now "EMBRAPA Amazônia Oriental") herbarium, plus two other exsiccates of a presumable natural hybrid between *T. glaucum* and *T. sylvestre* (*T. sylvestre* was treated in the paper under its synonym name *T. martianum*), both (exsiccates Fróes 22114 and 25554) deposited at the IAN ("EMBRAPA Amazônia Oriental") herbarium.

The scarcity of natural hybrids between *Theobroma* species might be due to three factors: (1) an efficient reproductive isolation that genetically avoid or turn difficult the formation of hybrids with asynchrony of reproduction; different pollinators; and alloincompatibility system (Reksodihardjo 1964; Rodrigues & Venturieri 1997; Venturieri 1994); (2) rare sympatry; (3) rare specimens collected because hybrids were unnoticed during sampling (Reksodihardjo 1964).

The experimental interspecific hybridization between species of the genus *Theobroma* was first attempted in 1937 in Trinidad, but only a few preliminary results were reported (Posnette 1945). A series of hybridization experiments between Brazilian species of *Theobroma* were performed between 1945 and 1951, by George O'Neill Addison and Rosendo Tavares at the "Instituto Agronômico do Norte", in Belém, Pará, Brazil, and up to now it is the most comprehensive work in the area, and includes a detailed illustration of those hybrids (Addison & Tavares 1951; 1952). The crosses involved all the Brazilian species of the genus *Theobroma* and from now considered the related genus *Herrania*. Other researchers also tried to develop artificial hybrids, such as Dr. Basil George David Bartley from the Imperial College of Tropical Agriculture (Trinidad) and Dr. Jorge Soria from the Interamerican Institute of Agricultural Sciences in Turrialba (Costa Rica), but there are no published reports about the results (Reksodihardjo 1964). A few decades ago, it was thought that the hybridization between *Theobroma* species from distinct sections was extremely difficult, if not impossible (Reksodihardjo 1964), however this hypothesis was discarded by the development of hybrids between species of sections Glossopetalum and Andropetalum [hybrids between *T. angustifolium* and *T. mammosum*; and between *T. simiarum* and *T. mammosum* obtained at CATIE, Costa Rica] (Cuatrecasas 1964) and in 1966 between *T. grandiflorum* (section Glossopetalum) and *T. cacao* (section Theobroma) [Martinson 1966]. A summary of reported interspecific *Theobroma* hybrids is shown at Tab. 1.

The seeds of *Theobroma* are recalcitrant, and do not tolerate low temperatures or desiccation, turning the conventional *ex situ* conservation system for genetic resources based on seeds unviable. The conservation of germplasm must be done with *ex situ* and/or *in situ* active germplasm collection, that properly managed, can preserve the evolutionary potential of the species (Debouck 1993). However, the exact distribution and origin of the species of the genera are not known and are restricted to reports of specimen collection in herbarium, and the risks of losses of certain populations is not recognized. According to Almeida *et al*. (1995), despite the huge efforts made by CEPLAC to exploit areas under high risk of genetic erosion, it is known that many natural areas of *T. cacao* were destroyed by deforestation of the Amazonian rain forest. The annual rate of deforestation between 1998 and 1999 was estimated to be 17,259km^2, and 19,836km^2 between 1999 to 2000 (FAO 2001). Under these rates of deforestation, probably no program to rescue Amazonian autochthonous species could compete.

Collected specimens of *Theobroma*, except by *T. cacao*, represent only a very small sample of their biodiversity. Therefore, the evaluation of the genetic diversity of the various species of the genus is still

Table 1. Interspecific hybridizations in the genera *Theobroma* and *Herrania*. (Based on field observations and on the literature) (Posnette 1945; Addison & Tavares 1951; Addison & Tavares 1952; Backer *et al*. 1953; Cuatrecasas 1964; Reksodihardjo 1964; Martinson 1966).

Species × Species	*T. angustifolium*	*T. cacao*	*T. obovatum*	*T. simiarum*	*T. speciosum*	*T. subincanum*	*T. sylvestre*
T. mammosum	■■■	■		■■■			
T. angustifolium		■					
T. grandiflorum		■■■	■■■■■			■■■■■	
T. obovatum						■■■■	
T. microcarpum		■■					
T. bicolour					■■		■
T. glaucum							■■■
T. sylvestre					■■■■■		
T. cacao	■■		■	■	■		
H. balaensis		■■					
H. mariae		■					

Status: ■ Hybrid fruit; ■■ Seedling hybrid; ■■■ Adult hybrid; ■■■■ Adult hybrid with production of fruits; ■■■■■ F2.

underestimated. The establishment of *in situ* germplasm collections and the organization of new botanical expeditions are necessary to enlarge current collections.

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References

Addison, G.O. & Tavares, R.M. 1951. Observações sobre as espécies do gênero *Theobroma* que ocorrem na Amazônia. **Boletim Técnico do Instituto Agronômico do Norte 25**: 3-20.

Addison, G.O. & Tavares, R.M. 1952. Hybridation and grafting in species of *Theobroma* which occur in Amazonia. **Evolution 6**: 380-386.

Almeida, C.M.V.C. de; Barriga, J.B.; Machado, P.F.R. & Bartley, G.B. 1987. Evolução do programa de conservação dos recursos genéticos de cacau na amazônia brasileira. **Comissão Executiva do Plano da Lavoura Cacaueira/ Departamento Especial da Amazônia - Boletim Técnico 5**: 1-108.

Almeida, C.M.V. de; Barriga, J.P.; Machado, P.F.R. & Silva, F.C.O. da 1995. **Coleta de cacau (*Theobroma cacao* L.) da amazônia brasileira: uma abordagem histórica e analítica**. CEPLAC/SUPOC, Porto Velho.

Alverson, W.S.; Whitlock, B.A.; Nyffler, R.; Bayer, C. & Baum, D.A. 1999. Phylogeny of the core Malvales: evidence from *ndhF* sequence data. **American Journal of Botany 86**: 1474-1486.

Baker, R.E.D.; Cope, F.W.; Holliday, P.C.; Bartley, B.G.D. & Taylor, J. 1953. **The Anglo Colombian cacao collecting expedition**. Rep. cacao Research. St. Augustine, Trinidad. Reprint Arch. Cacao Research, v. 1. H. Toxopeus (ed.), Am. Cocoa Res. Inst.

Bayer, C.; Fay, M.F.; De Bruijn, P.Y.; Savolainen, V.; Morton, C.M.; Kubitzki, K.; Alverson, W.S.; Chase, M.W. 1999. Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: a combined analysis of plastid *atpB* and *rbcL* DNA sequences. **Botanical Journal of The Linnean Society 129**(4): 267-303.

Cuatrecasas, J. 1964. Cacao and its allies: a taxonomic revision of the genus *Theobroma*. **Contributions From The United States National Herbarium 35**: 379-614.

Debouck, D.G. 1993. Biodiversity of forest species and overview of conservation needs for cocoa germplasm (*Theobroma* spp., Sterculiaceae). In: **Proceedings International Workshop on Conservation, Characterisation, and Utilisation of Cocoa Genetic Resources in the 21st Century**. Port of Spain, 1992. The Cocoa Res. Unit, Trinidad.

Ducke, A. 1953. As espécies brasileiras do gênero *Theobroma*. **Boletim Técnico do Instituto Agronômico do Norte 28**: 1-89.

FAO - Food and Agriculture Organization of the United Nations. 2001. **The State of the World's Forests**. FAO, Rome.

IAN - Instituto Agronômico do Norte. 1946. **Relatório Anual/Secção de Melhoramento de Plantas**. Ministério da Agricultura/Serviço Nacional de Pesquisa Agronômicas, Belém, p. 101-104.

IAN - Instituto Agronômico do Norte. 1947. **Relatório anual/Secção de Melhoramento de Plantas**. Ministério da Agricultura/Serviço Nacional de Pesquisa Agronômicas, Belém, p. 54-56.

IAN - Instituto Agronômico do Norte. 1948. **Relatório anual/Secção de Melhoramento de Plantas**. Ministério da Agricultura/Serviço Nacional de Pesquisa Agronômicas, Belém, p. 11-16

IAN - Instituto Agronômico do Norte. 1949. **Relatório anual/Secção de Melhoramento de Plantas**. Ministério da Agricultura/Serviço Nacional de Pesquisa Agronômicas, Belém, p. 14-19.

IAN - Instituto Agronômico do Norte. 1951. **Relatório anual/Secção de Melhoramento de Plantas**. Ministério da Agricultura/Serviço Nacional de Pesquisa Agronômicas, Belém, p. 27-28.

IAN - Instituto Agronômico do Norte. 1952. **Relatório anual/Secção de Melhoramento de Plantas**. Ministério da Agricultura/Serviço Nacional de Pesquisa Agronômicas, Belém, 2 p.

Judd, W.S. & Manchester, S.R. 1997. Circumscription of Malvaceae (Malvales) as determined by a preliminary cladistic analysis of morphological, anatomical, palynological, and chemical characters. **Brittonia 49**: 384-405.

Martinson, V.A. 1966. Hybridisation of cacao and *Theobroma grandiflora*. **Journal of Heredity 57**: 134-136.

Posnette, A.F. 1945. Interspecific pollination in *Theobroma*. **Tropical Agriculture 22**: 188-190.

Purseglove, J.W. 1968. **Tropical Crops-Dicotyledons**. Longman Green London, London.

Reksodihardjo, W.S. 1964. **The Species of the genus Theobroma**. Ph.D. thesis. Harvard University, Cambridge, Massachusetts.

Rodrigues, A.A. & Venturieri, G.A. 1997. Floral biology of "cupui" (*Theobroma subincanum*) - Sterculiaceae. In: **Anais do I Encontro de Botânica Econômica de Espécies Nativas da América Latina**, CATI, Campinas.

Silva, C.R.S. 2000. **Filogenia do gênero *Theobroma* utilizando marcadores moleculares e bioquímicos**. Dissertação de Mestrado. Centro de Energia Nuclear na Agricultura. Universidade de São Paulo, Piracicaba, SP.

Silva, C.R.S.; Figueira, A.V.O. & Souza, C.A.S. 2001. Diversidade no gênero *Theobroma*. Pp. 49-80. In: L.A.S. Dias (ed.). **Melhoramento genético do cacaueiro**. FUNAPE, UFG, Goiás.

Silva, C.R.S. & Venturieri, G.A. 1998. Híbridos do gênero *Theobroma*: levantamento, histórico e perspectivas. In: **Relatório do projeto biologia aplicada à domesticação de fruteiras amazônicas do gênero *Theobroma***. Universidade Federal do Pará. PTU/CNPq/MCT. Belém, PA.

Venturieri, G.A. 1994. **Floral Biology of Cupuassu (*Theobroma grandiflorum* (Willdenow ex Sprengel) Schumann)**. Ph.D. thesis. University of Reading, UK.