

RESCUE OF CACAO GENETIC RESOURCES RELATED TO THE NACIONAL VARIETY: SURVEYS IN THE ECUADORIAN AMAZON (2010-2013)

RESCATE DE LOS RECURSOS GENÉTICOS DEL CACAO RELACIONADOS CON LA VARIEDAD NACIONAL: EXPLORACIÓN EN LA AMAZONÍA ECUATORIANA (2010-2013)

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ABSTRACT

Traditional cocoa plantations in Ecuador are mostly composed of a complex mix of highly variable hybrid progenies, which has greatly reduced the population of native trees of the “Nacional” variety, to such a point that they are considered today as heading for extinction, which is increasingly worrying the international chocolate industry. Some years ago, we used genetic molecular markers to identify trees considered to be relics of the ancient original population of the “Nacional” variety, and some wild cocoa trees in a particular region of the southern Ecuadorian Amazon were identified as highly related to the “Nacional” variety. This paper presents the results of two surveys carried out in the southern Ecuadorian Amazon, in the Zamora-Chinchipec Province, in 2010 and 2013. The objective of these surveys was to search for, identify and rescue cocoa trees that might be the wild ancestors of the “Nacional” variety. In 2010, 83 mother trees were collected (budwood, pods and leaves) and 48 in 2013. They were preserved at the Granja Domono experimental farm, near Macas (Morona-Santiago province) and at the Tropical Experimental station Pichilingue, near Quevedo. The trees collected are currently being characterized for their genetic diversity, using molecular markers, and for the biochemical diversity of their beans.

Keywords: Cocoa, genetic diversity, Nacional cocoa variety, Zamora Chinchipe, Ecuador.

RESUMEN

Las plantaciones tradicionales de cacao están compuestas en su mayoría de una mezcla compleja de progenies híbridas con un alto grado de variabilidad, lo que ha reducido las poblaciones de árboles nativos de la variedad “Nacional”, a tal magnitud que ahora se consideran en vía de extinción, lo que preocupa a la industria chocolatera mundial. Hace algunos años, se utilizaron marcadores genéticos moleculares para identificar árboles considerados como reliquias de la población original antigua de la variedad “Nacional”, y algunos árboles silvestres de cacao de una región particular del sur de la Amazonía Ecuatoriana fueron identificados como altamente relacionados con la variedad “Nacional”. Este artículo presenta los resultados de dos exploraciones realizadas en el sur de la Amazonia Ecuatoriana, en la provincia Zamora-Chinchipe, en los años 2010 y 2013. El objetivo de estas exploraciones fue buscar, identificar y rescatar los árboles de cacao que pudieran ser los ancestros silvestres de la variedad “Nacional”. En el 2010, 83 árboles madres fueron recolectados (yemas, mazorcas y hojas) y 48 en el 2013. Las muestras fueron preservadas en la granja experimental Granja Domono, cerca de Macas (Provincia de Morona Santiago) y en la Estación Experimental Tropical Pichilingue, cerca de Quevedo. En la actualidad, los árboles recolectados están siendo caracterizados en su diversidad genética mediante la utilización de marcadores moleculares y también están siendo caracterizados en cuanto a la diversidad bioquímica de sus granos.

Palabras clave: Cacao, diversidad genética, variedad de cacao Nacional, Zamora Chinchipe, Ecuador.



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INTRODUCTION

Ecuador produces more than half of the fine and flavour cocoa marketed annually in the world. The South American country draws this advantage from a local variety that was massively planted by settlers in the lowland of Ecuador (in the upper reaches of the Guayas river) from the beginning of the 17th century. This variety was later named “Nacional”.

Various hypotheses have been put forward as to the origins of the “Nacional” variety and its links with the indigenous peoples of Ecuador (Lerceteau *et al.* 1997), and all agree that the origins and uses of this variety date back much further than the arrival of the Spanish.

At the moment, traditional cocoa plantations in Ecuador are mostly composed of a complex mix of highly variable hybrid progenies, which has greatly reduced the population of native trees of the “Nacional” variety, to such a point that they are considered today as heading for extinction (Loor *et al.* 2009), which is increasingly worrying the international chocolate industry.

In this context, genetic molecular markers have been used to identify trees considered to be relics of the ancient original population of the “Nacional” variety (Loor *et al.* 2009). After analysis of wild material collected in Amazonia by Allen and Lass (1983), some wild trees were identified as highly related to the “Nacional” variety in a particular region of the southern Ecuadorian Amazon, considered now as its potential region of origin and domestication (Loor *et al.* 2012).

These discoveries are of paramount importance, be it scientifically, or economically and socially. Indeed, first and foremost, they enable a more systematic study of the variability of this variety in its natural environment, thereby making it possible to gain better knowledge of its origins and evolution. Then, by conserving and using these wild trees in breeding programmes, it will be possible to develop some new improved “Nacional” cocoa cultivars that can then be recommended for new plantations.

Given the situation and these prospects, the Ecuadorian Amazon holds a privileged position, as it is one of the regions in the world that shelters the greatest genetic diversity of the *T. cacao* species (Bartley, 2005), be it in its wild or domesticated state, which is reflected in the fact that

representatives of all commercial types of cocoa can be encountered in the same zone, along with others which, although potentially rich in sensory terms, are still unknown or little used at local level. On the other hand, the galloping degradation of its primary forest is a cause for global concern, as is the obvious loss of many wild cocoa trees of great interest.

The aim of this paper is to present the results of two surveys carried out in the southern Ecuadorian Amazon following the work by Loor *et al.* (2012), in order to search for, identify and rescue some cocoa trees that might be the wild ancestors of the “Nacional” variety. By setting up living collections of those trees, it will be possible to make use, in the breeding programs, of the genetic variability encountered in the Amazonian region, to increase fine cocoa production and maintain or increase its flavour quality.

MATERIALS AND METHODS

The survey zones

The region surveyed was a vast, heterogeneous territory identified as being the most likely centre of origin for the “Nacional” variety (Loor *et al.* 2012), comprising the eastern slopes of the Andes Cordillera and southern Ecuadorian Amazonia in Zamora Chinchipe province (see figure 1). Rainforest, range from 724 to 1188 meters above sea level.

In this region, there has been substantial environmental degradation, which has been transformed from an immense primary tropical rainforest into a region of large deforested zones, mainly due to cattle farming and the wood and mineral industries, where some small woodlands survive as the final and precarious remains of what was once a large virgin forest. It is precisely inside these forest remnants that some large and old cocoa trees can be found. Two surveys were successively undertaken in 2010 and 2013:

In 2010: The surveys were carried out in August and involved six cantons of Zamora-Chinchipe province: El Pangui, Yantzaza, Yacuambi, Centinela del Condor, Zamora and Nangaritzza. The Shuar communities in the zone were informed in detail about the purpose of the surveys and involved in the plant collections.

Methodologies followed during the collections

During the two surveys, for each mother tree of interest, we collected:

- Young budsticks of a size suitable for side cleft grafting on rootstocks around 3 months old, a technique adopted by INIAP (Molina 2008; Anon. 2012), with paraffin applied to the tips and protected in isothermal bags (Lachenaud and Sallée 1993)
- Pods, where available, for sowing (as a priority) and, if sufficient, for micro fermentations. The fermented (in “Rohan trays”; Jiménez *et al.* 2011), dried cocoa was sent to CIRAD in Montpellier (France) for biochemical analysis of the aromatic compounds and, where appropriate, sensory analyses. The micro fermentations were carried out at Granja Domono and Pichilingue in 2010, and at Pichilingue in 2013.
- A few leaves, for further microsatellite molecular marker analysis of the diversity collected (Pugh *et al.* 2004), and the genetic kinship of the collected material with the ‘Nacional’ variety. The leaves were vacuum packed (in 2013) or dried (in 2010) and sent to CIRAD in Montpellier.

- For each tree encountered, the location was recorded using a GPS, along with some morphological data used as descriptors (architecture, pods, flowers), as well as sanitary and environmental data (habitat, stand size, topology and pedology).

Rescuing the planting material

As indicated, in 2010 the collected material was preserved, i.e. grafted and sown, at Granja Domono and Pichilingue, and then planted in plots; in 2013, it was all preserved at Pichilingue.

RESULTS AND DISCUSSION

2010 surveys

Pods could not be collected from all the mother trees as the climate in 2010 was particularly dry and the main harvest peak was earlier than expected. The exceptionally low level of the Nangaritza river also disrupted the scheduled survey plan, by preventing travel by water.

Table 1 presents the 83 mother trees collected, of which only 39 bore pods. The surveyed zones (Figure 1) were as follows:

Table 1. Identification, location (coordinates in degrees, minutes and seconds), elevation, habitat, morphology of the 83 mother-trees (accessions) collected in August 2010, and the stand to which they belonged. FT = a few trunks, ST = single trunk, T = tuft, BiT = bi-trunk, WB = witches’ broom, FPR = frosty pod rot. In the “Observation” column, “W-B” means “White and purple beans”. (N/A = not available)

| Accession | Lat. S | Long. W | Elevation | Habitat | Stand | Architecture | Height | Pods harvested | Shape | Diseases | Observations |
|-----------|----------|----------|-----------|--------------|-------|--------------|--------|----------------|----------|--------------|--------------|
| ZAMO 001 | 03.51.37 | 78.45.00 | 829 | grazing land | 3 | FT | 10 | 1 | Nacional | WB, FPR | White beans |
| ZAMO 002 | 03.51.37 | 78.45.00 | 829 | grazing land | 3 | FT | 8 | 1 | Nacional | WB, FPR | Purple beans |
| ZAMO 003 | 03.51.36 | 78.44.39 | 1003 | Forest | Alone | T | 9 | 3 | Nacional | 0 | W-P beans |
| ZAMO 004 | 03.51.35 | 78.44.38 | 1033 | Forest | Alone | T | 8 | 1 | Nacional | 0 | Purple beans |
| ZAMO 005 | 03.51.33 | 78.44.40 | 1033 | Forest | Alone | FT | 10 | 4 | Nacional | 0 | Purple beans |
| ZAMO 006 | 03.51.33 | 78.44.40 | 1033 | Forest | Alone | T | 8 | 0 | N/A | WB, FPR | |
| ZAMO 007 | 03.51.32 | 78.44.42 | 1015 | Forest | Alone | FT | 10 | 4 | Nacional | 0 | White beans |
| ZAMO 008 | 03.51.31 | 78.44.43 | 1010 | Forest | Alone | FT | 13 | 0 | Nacional | 0 | |
| ZAMO 009 | 03.51.28 | 78.44.45 | 1005 | Forest | Alone | FT | 15 | 1 | Nacional | 0 | White beans |
| ZAMO 010 | 03.51.28 | 78.44.47 | 989 | Forest | Alone | FT | 16 | 1 | Nacional | FPR | |
| ZAMO 011 | 03.51.25 | 78.44.49 | 985 | Forest | 4 | ST | 8 | 3 | Nacional | WB | Purple beans |
| ZAMO 012 | 03.51.24 | 78.44.49 | 953 | Forest | Alone | T | 15 | 3 | Nacional | WB | |
| ZAMO 013 | 03.51.22 | 78.44.47 | 949 | Forest | Alone | FT | 9 | 1 | Nacional | Phytophthora | W-P beans |
| ZAMO 014 | 03.51.20 | 78.44.49 | 892 | Forest | 2 | FT | 12 | 5 | Nacional | 0 | W-P beans |
| ZAMO 015 | 03.51.20 | 78.44.49 | 892 | Forest | 2 | FT | 9 | 3 | Nacional | 0 | W-P beans |
| ZAMO 016 | 03.51.14 | 78.44.54 | 858 | Forest | Alone | ST | 8 | 4 | Nacional | 0 | W-P beans |

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|----------|----------|----------|------|--------------|-------|-----|------|----|----------|------------------|--------------|
| PANG 001 | 03.32.56 | 78.36.38 | 858 | grazing land | Alone | FT | 8 | 1 | Nacional | 0 | W-P beans |
| PANG 002 | 03.33.01 | 78.36.38 | 878 | grazing land | Alone | BiT | N/A | 3 | Nacional | Phytophthora | W-P beans |
| PANG 003 | 03.33.03 | 78.36.42 | 888 | grazing land | 2 | ST | 10 | 0 | N/A | N/A | |
| PANG 004 | 03.33.04 | 78.36.41 | 935 | grazing land | 2 | T | 15 | 1 | Nacional | N/A | |
| PANG 005 | 03.33.04 | 78.36.42 | 942 | grazing land | Alone | T | 9 | 0 | N/A | N/A | |
| PANG 006 | 03.33.05 | 78.36.44 | 947 | grazing land | Alone | T | 18 | 0 | N/A | N/A | |
| PANG 007 | 03.33.05 | 78.36.45 | 945 | Forest | Alone | T | 8 | 1 | Nacional | Phytophthora, WB | |
| PANG 008 | 03.33.05 | 78.36.47 | 956 | Forest | 3 | T | 8 | 1 | Nacional | WB | W-P beans |
| PANG 009 | 03.33.05 | 78.36.46 | 956 | Forest | 3 | FT | 8 | 0 | N/A | WB | |
| PANG 010 | 03.33.05 | 78.36.47 | 956 | Forest | 3 | BiT | 15 | 0 | N/A | N/A | |
| PANG 011 | 03.33.05 | 78.36.47 | 956 | Forest | Alone | T | 18 | 0 | N/A | N/A | |
| PANG 012 | 03.33.04 | 78.36.45 | 960 | Forest | Alone | T | 20 | 0 | N/A | N/A | |
| PANG 013 | 03.33.03 | 78.36.47 | 992 | Forest | Alone | T | 12 | 0 | N/A | N/A | |
| PANG 014 | 03.33.03 | 78.36.48 | 997 | Forest | Alone | T | > 20 | 0 | N/A | WB | |
| PANG 015 | 03.33.02 | 78.36.49 | 1024 | Forest | 10 | FT | 10 | 0 | N/A | N/A | |
| PANG 016 | 03.33.03 | 78.36.49 | 1010 | Forest | 10 | FT | 10 | 1 | Nacional | N/A | |
| PANG 017 | 03.33.03 | 78.36.49 | 1010 | Forest | 10 | FT | 8 | 2 | Nacional | N/A | W-P beans |
| PANG 018 | 03.33.03 | 78.36.50 | 1011 | Forest | 10 | BiT | > 20 | 0 | N/A | N/A | |
| PANG 019 | 03.33.00 | 78.36.51 | 1015 | Forest | Alone | FT | 15 | 0 | N/A | N/A | |
| PANG 020 | 03.32.58 | 78.36.51 | 1015 | Forest | Alone | ST | 15 | 0 | N/A | WB | |
| PANG 021 | 03.32.58 | 78.36.51 | 1015 | Forest | Alone | T | 15 | 1 | Nacional | WB | |
| PANG 022 | 03.32.53 | 78.36.50 | 972 | Forest | Alone | T | 15 | 1 | Nacional | N/A | W-P beans |
| PANG 023 | 03.32.52 | 78.36.50 | 967 | Forest | Alone | FT | 10 | 18 | Nacional | N/A | W-P beans |
| PANG 024 | 03.32.46 | 78.36.48 | 935 | Forest | Alone | ST | 8 | 5 | Nacional | 0 | W-P beans |
| YACU 001 | 03.54.50 | 78.50.55 | 837 | Friche | Alone | BiT | 4 | 3 | Nacional | Phytophthora | W-P beans |
| YACU 002 | 03.41.52 | 78.54.14 | 1064 | grazing land | 10 | T | 6 | 0 | N/A | WB | |
| YACU 003 | 03.41.52 | 78.54.14 | 1064 | grazing land | 10 | T | 8 | 0 | Nacional | WB | |
| YACU 004 | 03.42.03 | 78.54.17 | 1056 | grazing land | Alone | ST | 6 | 0 | N/A | 0 | |
| YACU 005 | 03.42.03 | 78.54.17 | 1056 | Ravine | 2 | T | 8 | 0 | N/A | 0 | |
| YACU 006 | 03.42.04 | 78.54.11 | 1085 | Ravine | 2 | T | 8 | 0 | N/A | 0 | |
| YACU 007 | 03.42.02 | 78.54.10 | 1054 | grazing land | Alone | FT | 9 | 0 | N/A | 0 | |
| YACU 008 | 03.42.02 | 78.54.08 | 1055 | grazing land | Alone | ST | 5 | 0 | N/A | 0 | |
| YACU 009 | 03.42.03 | 78.54.08 | 1044 | grazing land | Alone | ST | 5 | 0 | N/A | N/A | |
| YACU 010 | 03.42.03 | 78.54.04 | 1006 | grazing land | Alone | T | 18 | 0 | N/A | N/A | |
| YACU 011 | 03.46.27 | 78.53.39 | 825 | grazing land | Alone | FT | 10 | 0 | N/A | WB | |
| YACU 012 | 03.47.10 | 78.54.20 | 882 | grazing land | 3 | ST | 8 | 1 | Nacional | WB | Purple beans |
| YACU 013 | 03.47.10 | 78.54.20 | 882 | grazing land | 3 | ST | 8 | 0 | N/A | WB | |
| YACU 014 | 03.47.10 | 78.54.20 | 882 | grazing land | 3 | ST | 10 | 0 | N/A | WB | |
| YACU 015 | 03.46.57 | 78.54.17 | 895 | grazing land | 3 | FT | 8 | 0 | N/A | WB | |
| YACU 016 | 03.46.56 | 78.54.16 | 902 | grazing land | 3 | FT | 7 | 0 | N/A | WB | |
| YACU 017 | 03.46.56 | 78.54.16 | 902 | grazing land | 3 | ST | 5 | 0 | N/A | WB | |
| YACU 018 | 03.47.00 | 78.54.10 | 894 | grazing land | Alone | FT | 12 | 2 | Nacional | N/A | W-P beans |
| YACU 019 | 03.47.00 | 78.54.09 | 887 | grazing land | Alone | FT | 10 | 0 | N/A | N/A | |
| YACU 020 | 03.54.47 | 78.51.00 | 955 | Fallow | Alone | BiT | 8 | 0 | N/A | WB | |
| YACU 021 | 03.59.50 | 78.51.00 | 873 | Fallow | Alone | T | 6 | 1 | Nacional | 0 | W-P beans |
| BEVI 001 | 03.52.09 | 78.40.35 | 844 | Forest | 4 | FT | 9 | 3 | Nacional | WB | Purple beans |
| BEVI 002 | 03.52.09 | 78.40.35 | 844 | Fallow | 4 | N/A | 8 | 3 | Nacional | N/A | Purple beans |
| NANK 001 | 03.46.30 | 78.39.27 | 842 | grazing land | 8 | BiT | 15 | 3 | Nacional | WB, Phytophthora | Purple beans |
| NANK 002 | 03.46.30 | 78.39.27 | 842 | grazing land | 8 | FT | 12 | 3 | Nacional | N/A | Purple beans |
| NANK 003 | 03.46.30 | 78.39.27 | 842 | grazing land | 8 | BiT | 12 | 3 | Nacional | 0 | Purple beans |
| NANK 004 | 03.46.37 | 78.39.29 | 854 | grazing land | Alone | FT | 15 | 3 | Nacional | WB | White beans |
| NANK 005 | 03.47.06 | 78.40.27 | 842 | grazing land | Alone | FT | 12 | 5 | Nacional | WB | W-P beans |
| NANK 006 | 03.48.39 | 78.41.07 | 838 | Crop | Alone | N/A | 12 | 3 | Nacional | 0 | Purple beans |

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|----------|----------|----------|-----|--------------|-------|----|-----|---|----------|----|----------------------|
| PAQU 001 | 03.55.48 | 78.40.54 | 815 | Fallow | Alone | FT | 12 | 0 | N/A | 0 | Pink flower peduncle |
| SHAI 001 | 04.20.24 | 78.39.03 | 924 | Cleared zone | 3 | T | 1.5 | 0 | N/A | 0 | |
| SHAI 002 | 04.20.25 | 78.39.03 | 962 | Cleared zone | 3 | ST | 5 | 0 | N/A | 0 | |
| SHAI 003 | 04.20.25 | 78.39.03 | 962 | Cleared zone | 3 | ST | 1.5 | 0 | N/A | 0 | |
| SHAI 004 | 04.20.19 | 78.39.22 | 925 | Forest | Alone | ST | 5 | 0 | N/A | 0 | |
| SHAI 005 | 04.19.52 | 78.39.52 | 802 | Forest | Alone | FT | 7 | 1 | Nacional | 0 | |
| NUPA 001 | 04.22.21 | 78.39.47 | 914 | Crop | 4 | FT | 10 | 4 | Nacional | WB | |
| NUPA 002 | 04.22.21 | 78.39.47 | 914 | Crop | 5 | T | 8 | 0 | N/A | WB | |
| SHAM 001 | 04.19.57 | 78.41.19 | 925 | Fallow | 3 | T | 3 | 0 | N/A | 0 | |
| SHAM 002 | 04.19.57 | 78.41.19 | 925 | Fallow | 3 | T | 12 | 0 | N/A | 0 | |
| SHAM 003 | 04.19.57 | 78.41.19 | 925 | Fallow | 3 | T | 10 | 0 | N/A | 0 | |
| SHAM 004 | 04.19.57 | 78.41.19 | 925 | Fallow | Alone | ST | Nc | 0 | N/A | 0 | Red pods |
| GUAY 001 | 04.05.05 | 78.40.00 | 893 | grazing land | 3 | T | 12 | 0 | N/A | WB | |
| GUAY 002 | 04.05.03 | 78.40.01 | 887 | grazing land | 3 | FT | 10 | 0 | N/A | WB | |

- Towards Zumbi, between the Zamora and Nangaritza rivers: accessions Zamo-001 to Zamo-016
- Towards El Pangui: accessions Pang-001 to Pang-024
- On the Yacuambi river, towards La Paz (around the Shuar village of Kiim): accessions Yacu-002 to 019, and towards La Saquea: accessions Yacu-001 and Yacu-020, 021
- On the Nangaritza river, towards Guazimi (accessions Guay-001 and 002), towards Paquisha (accession Paqu-001), towards Bella Vista (accessions Bevi-001 and 002) and towards Nankais (accessions Nank-001 to 004, along the Tses-entse river; Nank 005 and 006, on the roadside)
- Further upstream on the Nangaritza river, towards Shaime (accessions Shai-001 to 005)
- On the Numpatakaima river, towards Nuevo Paraiso (accessions Nupa-001 and 002)
- On the Shamatak river, not far from Shaime (accessions Sham-001 to 004).

The surveys and collections were fruitful, despite the end of the main harvest, apart from in the Bella Vista zone and especially the Nuevo Paraiso zone, where we only found a few cocoa trees.

The main traits of the places and collected trees were as follows:

- Often found on grazing land, sometimes in more or less degraded forests, on generally steep slopes and at a high average elevation (from 800 to 1,100 m). Some of the cocoa trees had recently been cut back by the farmers and were therefore greatly threatened; this situation could be explain by the land use change and

expansion of intensive agriculture (Sala *et al.* 2006). For other hand, these changes of land could lead to habitat loss for more species and can even drive species decline and extinction (Vebrova *et al.* 2014).

- Even though many of the collected cocoa trees were not cultivated, it is impossible to reach any conclusion, at this stage, as to their wild nature. The elevations of our collections (up to 1,188 m) corresponded to the cultivation limits cited by Wood & Lass (1985).
- Age generally (according to appearance) old and height sometimes exceeding 20 m.
- Pods mostly of the “Nacional” shape, or similar follows. Almost all pods were green, ripening to yellow, but the color of seeds per pod was more variable than other character. This offers insight into relationships among the collected materials, and provide direct evidence of important commercial traits (Bartley 2005).
- Seeds of variable size and colour showed a great diversity inside and among fruits collected, pods with a mixture of white and purple seeds, pods with only white seeds follows, as reported by Allen and Lass (1983), or pods with seeds sometimes small or very flattened.
- Many diseases (witches’ broom, frosty pod rot, black pod rot) and an often highly degraded physiological condition, with many deficiency symptoms and often numerous parasites.

The collected budsticks and pods (from 83 and 39 mother trees, respectively) enabled 1,370 grafts to be carried out and 1,106 open progenies were sown at Granja Domono and Pichilingue. Some bean micro-fermentations were carried out for 15 origins.

Photo 1. Cocoa fruits (pods and seeds) showing the diversity among some collected materials.



2013 surveys

Table 2 presents the 48 mother trees collected in 2013, of which 33 bore pod.

The surveyed zones (Figure. 1) were as follows:

- In the vicinity of Palanda:
 - Towards the La Florida archaeological site, along the Valladolid river: accessions PAL 1 to 7
 - At “La Mina”: accessions PAL 8 and 9
 - At “La Cuesta de Santa Ana”: accessions PAL 15 to 19
 - Near the town, along the Palanda river, accessions PAL 20 to 24.
- Towards San Francisco del Vergel: accessions PAL 10 (at Santa Clara del Vergel) and PAL 11 to 14
- In the vicinity of Zumba:
 - At “La Hoya del cacao”: accessions PAL 25 to 28, and nearby, PAL 29 to 32

- At Huamchunangui: accessions PAL 33 to 37

- At La Gayusa: accessions PAL 38 to 40

- At Isimanchi: accessions PAL 44 to 48

- Towards Chito: accessions PAL 41 to 43 (at La Fortuna, along the San Francisco river, on the border with Peru).

Unlike the collections in 2010, those in 2013 all involved cultivated trees, included in plots, or very close to dwellings. Some trees of the Trinitario type (red) were even found (but not collected). The pod shape was “Nacional” in 13 cases out of 35 (i.e. 37%), and Angoleta, Amelonado or “Other” in 22 cases follows. The general condition of the trees was better than those surveyed in 2010 (fewer diseases and deficiencies).

The collected budsticks and pods (from 48 and 33 mother trees, respectively) enabled 643 grafts to be carried out and 2,150 open progenies were sown at Pichilingue. Some bean micro-fermentations were carried out for 27 origins.

Table 2. Identification, location (UTM-UPS coordinates, in the 17M square of the World Geodetic System), elevation, habitat, morphology of the 48 mother trees (accessions) collected in May 2013. FT = a few trunks, ST = single trunk, T = tuft, BiT = bi-trunk, WB = witches' broom. The size of the stands and tree heights are evaluations. The elevations, provided by a GPS, are approximations.

| Accession | GPS East | GPS North | Elevation | Habitat | Stand | Architecture | Height | Pods harvested | Shape | Diseases | Observations |
|-----------|----------|-----------|-----------|--------------|-------|--------------|--------|----------------|-----------|--------------|-----------------|
| PAL 1 | 707385 | 9487090 | 979 | Crop | 5 | T | 10 | 9 | Nacional | Wb | |
| PAL 2 | 707385 | 9487090 | 979 | Crop | 5 | T | 8 | 3 | Nacional | Wb | |
| PAL 3 | 707392 | 9487092 | 979 | Crop | 5 | T | 7 | 10 | Other | Wb | |
| PAL 4 | 707392 | 9487092 | 979 | Crop | 5 | FT | 5 | 10 | Nacional | | |
| PAL 5 | 707472 | 9487355 | 985 | Crop | 10 | ST | 4 | 8 | Angoleta | Wb | |
| PAL 6 | 707497 | 9487374 | 993 | Crop | 30 | FT | 8 | 5 | Angoleta | | |
| PAL 7 | 707497 | 9487374 | 993 | Crop | 30 | FT | 5 | 5 | Amelonado | | |
| PAL 8 | 707791 | 9488780 | 1015 | Grazing land | 2 | FT | 7 | 9 | Other | | |
| PAL 9 | 707769 | 9488812 | 1019 | Grazing land | 2 | T | 4 | 4 | Other | | |
| PAL 10 | 718399 | 9494627 | 1133 | Crop | Nb | FT | 10 | 4 | Nacional | | |
| PAL 11 | 716849 | 9482131 | 1181 | Crop | 30 | FT | 10 | 6 | Nacional | | |
| PAL 12 | 716850 | 9482109 | 1187 | Crop | 30 | ST | 6 | 7 | Other | | |
| PAL 13 | 716850 | 9482118 | 1188 | Crop | 30 | FT | 10 | 8 | Other | 0 | Pale beans |
| PAL 14 | 716850 | 9482118 | 1188 | Crop | 30 | FT | 8 | 8 | Nacional | | |
| PAL 15 | 707384 | 9487961 | 1080 | Crop | 5 | T | 10 | 0 | | 0 | |
| PAL 16 | 707243 | 9488112 | 1132 | Forest | 3 | T | 12 | 0 | Amelonado | 0 | |
| PAL 17 | 707273 | 9488134 | 1129 | Crop | 10 | T | 12 | 0 | | Wb | |
| PAL 18 | 707273 | 9488134 | 1129 | Crop | 10 | T | 12 | 0 | | Wb | |
| PAL 19 | 707259 | 9488113 | 1130 | Crop | 15 | T | | 0 | | Wb | |
| PAL 20 | 707617 | 9486443 | 975 | Crop | 20 | T | 6 | 0 | | Wb | Pale flushes |
| PAL 21 | 707626 | 9486419 | 977 | Fallow | 20 | T | 15 | 3 | Other | Wb | |
| PAL 22 | 707621 | 9486463 | 978 | Fallow | 20 | T | | 0 | Other | Wb | |
| PAL 23 | 707422 | 9485906 | 1063 | Crop | 3 | T | 7 | 0 | Other | Wb | Red staminodes |
| PAL 24 | 707422 | 9485906 | 1063 | Crop | 3 | T | 5 | 1 | Other | Wb | |
| PAL 25 | 708103 | 9460158 | 1150 | Crop | 4 | T | 8 | 7 | Nacional | Phytophthora | |
| PAL 26 | 708105 | 9460145 | 1150 | Crop | 4 | T | 5 | 10 | Nacional | 0 | |
| PAL 27 | 708092 | 9460177 | 1087 | Crop | 4 | FT | 6 | 1 | Nacional | 0 | |
| PAL 28 | 708092 | 9460177 | 1079 | Crop | 4 | T | 7 | 0 | | 0 | |
| PAL 29 | 707586 | 9460322 | 1129 | Crop | 1 | FT | 6 | 2 | Other | Wb | |
| PAL 30 | 707323 | 9460554 | 1105 | Grazing land | 3 | FT | 5 | 3 | Angoleta | 0 | Deficiencies |
| PAL 31 | 707323 | 9460554 | 1105 | Grazing land | 3 | FT | 7 | 2 | Other | | |
| PAL 32 | 707323 | 9460554 | 1105 | Grazing land | 3 | FT | 8 | 0 | Other | | Deficiencies |
| PAL 33 | 703610 | 9454260 | 1044 | Crop | 45 | T | 6 | 1 | Other | Wb | Almost dead |
| PAL 34 | 703610 | 9454260 | 1044 | Crop | 45 | FT | 8 | 2 | Nacional | Wb | Pale flushes |
| PAL 35 | 703633 | 9454282 | 1044 | Crop | 45 | FT | 12 | 3 | Nacional | Wb | |
| PAL 36 | 703564 | 9454042 | 1180 | Crop | 300 | ST | 8 | 7 | Amelonado | | |
| PAL 37 | 703572 | 9454029 | 1086 | Crop | 300 | BiT | 15 | 7 | Angoleta | | Pale flushes |
| PAL 38 | 704134 | 9454592 | 1028 | Crop | 30 | T | 12 | 4 | Other | Wb | |
| PAL 39 | 704134 | 9454592 | 1028 | Crop | 30 | T | 10 | 5 | Amelonado | Wb | Watery pod rot? |
| PAL 40 | 704122 | 9454641 | 1048 | Crop | > 20 | FT | 6 | 5 | | Wb | Pink flushes |
| PAL 41 | 722464 | 9451467 | 865 | Forest | 10 | FT | 10 | 0 | | | Steep slope |
| PAL 42 | 722464 | 9451467 | 865 | Forest | 10 | ST | 6 | 0 | | | Pale flushes |
| PAL 43 | 722467 | 9451539 | 864 | Forest | 3 | FT | | 0 | | | |
| PAL 44 | 708584 | 9465793 | 880 | Crop | 3 | FT | 10 | 9 | Nacional | | |
| PAL 45 | 708584 | 9465793 | 880 | Crop | 3 | FT | 10 | 8 | Nacional | | |
| PAL 46 | 711490 | 9463787 | 724 | Forest | 10 | T | | 1 | Amelonado | | |
| PAL 47 | 711473 | 9463789 | 731 | Forest | 10 | FT | 6 | 0 | | Wb | |
| PAL 48 | 709743 | 9454418 | 837 | Crop | 6 | FT | 10 | 0 | | | |

CONCLUSION

The outcome of these two survey and collection campaigns in Zamora-Chinchi province proved positive, as it allowed rescue very old cacao trees that show morphological characteristics of the Nacional variety.

A total of 131 mother trees were collected and rescued in living collections at two strategic locations, under INIAP control: the experimental farm “Granja Domo-

no” and the tropical experimental station “Pichilingue”. More than 35% of the collected trees were of the typically ‘Nacional’ phenotype (pods and flowers), especially trees Zamo-014, 015, Pang-008, 024, Yacu-021, Nank-005, Pal 1, 2, 4, 10, 11, 14, 25, 26, 27, 34, 35, 44 and 45.

17% of the accessions collected had pale, sometimes white and even sometimes totally white seeds, like trees Zamo-001, Zamo-007, Zamo-009 and Nank-004.

The living collection at Pichilingue and Domono are cu-

currently being characterized for its genetic diversity, using molecular markers, and for the biochemical diversity of the beans.

These surveys and collections prove essential for safeguarding the province's cocoa heritage, which is under

serious threat from agriculture (mainly livestock farming) and diseases, and also for identifying possible ancestors of the 'Nacional' cocoa tree. Studies on the genetic variability of the collected material and its closeness to the 'Nacional' type may possibly reveal the need for further surveys in targeted sectors.

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