ASSESING GENETIC DIVERSITY OF CACAO (*Theobroma cacao* L.) NATIVO CHUNCHO IN LA CONVENCIÓN, CUSCO-PERÚ.

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Abstract

Perú is a megadiverse country with high biological, cultural, ethnic and historic richness, recognized as world center of genetic resources, being as a strategic for food security of its population MINAM, (2010). The Ministry of Agriculture declared; "Natural Patrimony of the Nation to the Peruvian Cocoa (*Theobroma cacao* L.)" and created the National Registry of Peruvian Cocoa Cultivars. Peru is one of the main origin centers of cocoa with high diversity and genetic variability; Native "Chuncho cocoa", "Porcelain of Piura", Nacional of Perú".

The Peruvian Amazon has a great genetic diversity of cocoa, one of them is "Chuncho cocoa", it is native to the province of La Convención in Cusco-Perú, where around 20 thousand hectares are cultivated, 60% are "Chuncho". The name comes from the place, since the area is inhabited by indigenous ethnic groups "Matsiguenga Chunchos", who probably domesticated this Cutivar (Bioversity, 2009).

To asses genetic diversity of Chuncho cocoa it was necessary to do bioprospection among the farmers' fields and native lands, located in the province of La Convención-Cusco 250 km), 51 morphological and agronomical descriptors were used to characterize 260 trees. 72 plants were selected by its superior agronomic characteristics to be the first genebank of Chuncho Cocoa, in fact there are several trees in this area waiting to be rediscovered.

The variability is in shape, size of seeds and fruits (all yellow), color of seeds (from whitish to almost black), tolerant to resistant against adverse factors.

35 sensorial profiles were encountered, Chuncho Cocoa has fine aroma and exceptional flavor; floral and fruity, sweet with low acidity, very low bitterness and astringency, herbal and nutty.

To do molecular prospection were used; 260 leaves samples of Native Chuncho Cocoa, 96 SNPs molecular markers, DNA Stool kit extraction and purification, DNA quantification by NanoDrop 2000 and genotyping by EP1™ System FLUIDIGM

60% of the samples are pure Chunchos, different population from the classification presented by Motamayor C. (2008), Chuncho is not Contamana. The result of Structure analysis confirmed that Chuncho Cocoa is indigenous to southern Peru, I can say the valley of Urubamba river in la Convención, Cusco-Perú is the center that originates cocoa.

To assess genetic variability and to know the characteristics of the Chuncho Cocoa will give us the basis for breeding, which will contribute to enhanced agricultural productivity, economic development, and food security for farmers involved in the cocoa value chain.

1. **Introduction**

The Peruvian Amazon has a great genetic diversity of cacao, one of them is "Chuncho" cocoa, it is native to the province of La Convención, Cusco-Perú, where more than 20 thousand hectares of cacao are cultivated, of which, 80% are "Chuncho". The name comes from the place, since the area is inhabited by indigenous ethnic groups "Matsiguengas Chunchos", who probably domesticated this Cultivar (Bioversity, 2009). Chuncho cocoa, is at risk of genetic erosion and in danger of becoming extinct and be replaced.

The study of the genetic variability of cocoa based on **SNP**s **molecular markers** are more accurate alternative for genotyping, identification and further studies, since these techniques allow the discrimination of individuals depending on the genotype, regardless of the environmental effect or interactions between genes.

Chuncho cacao is one of the most ancient and unique for this valley, with very high qualities, mainly in aroma and flavor, we have identified 35 sensorial profiles among the tested Chuncho genotypes.

Chocolate manufactures prefer it to make finest chocolates. When Chuncho cacao is properly cultivated, it can produce high yield and some Chuncho cacao trees are also tolerates and resist to the major diseases of cocoa, hence Chuncho cacao is an invaluable group of genetic resource for cocoa production and for breeding programs.

2. **Materials and methods**

2.1. **Sampling location**

- Latitude: 12 ° 50 '31.31 "S - 12 ° 29' 29.29" S.
- Longitude: 72 ° 43 '11.30 "W - 73 ° 56' 39.50" W.
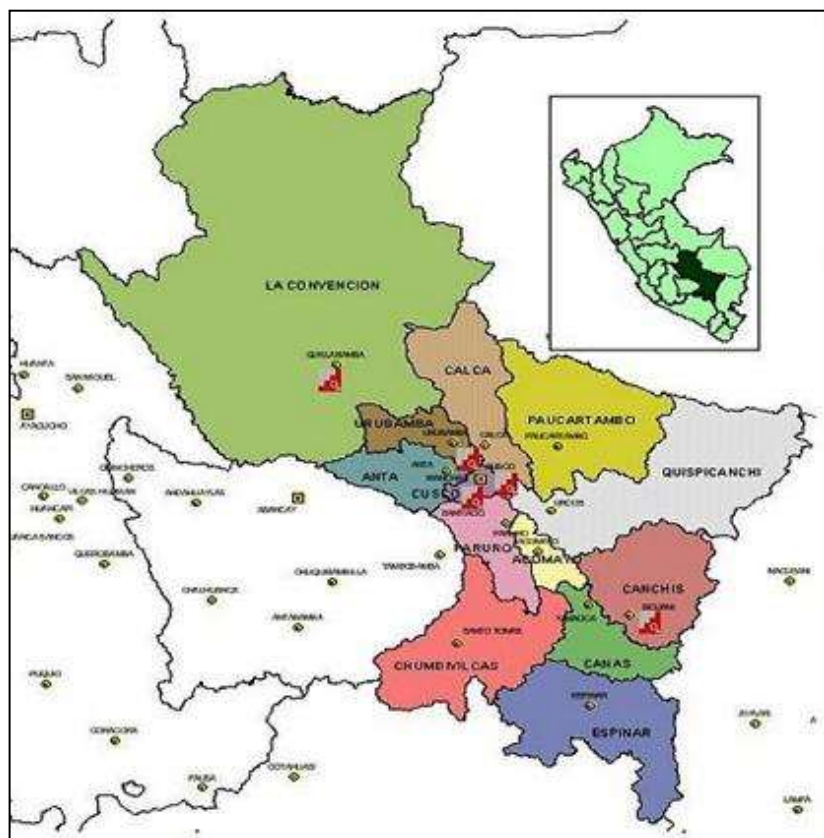- Maximum altitude: 1600 m.a.s.l.   Minimum altitude: 726 m.a.s.l.

Figure 1:   Map location of La Convención-Cusco Perú.

The collected material was 250 Chuncho cacao samples from selected trees along 300 km of La Convención Valley- Cusco, Perú (fig.2).
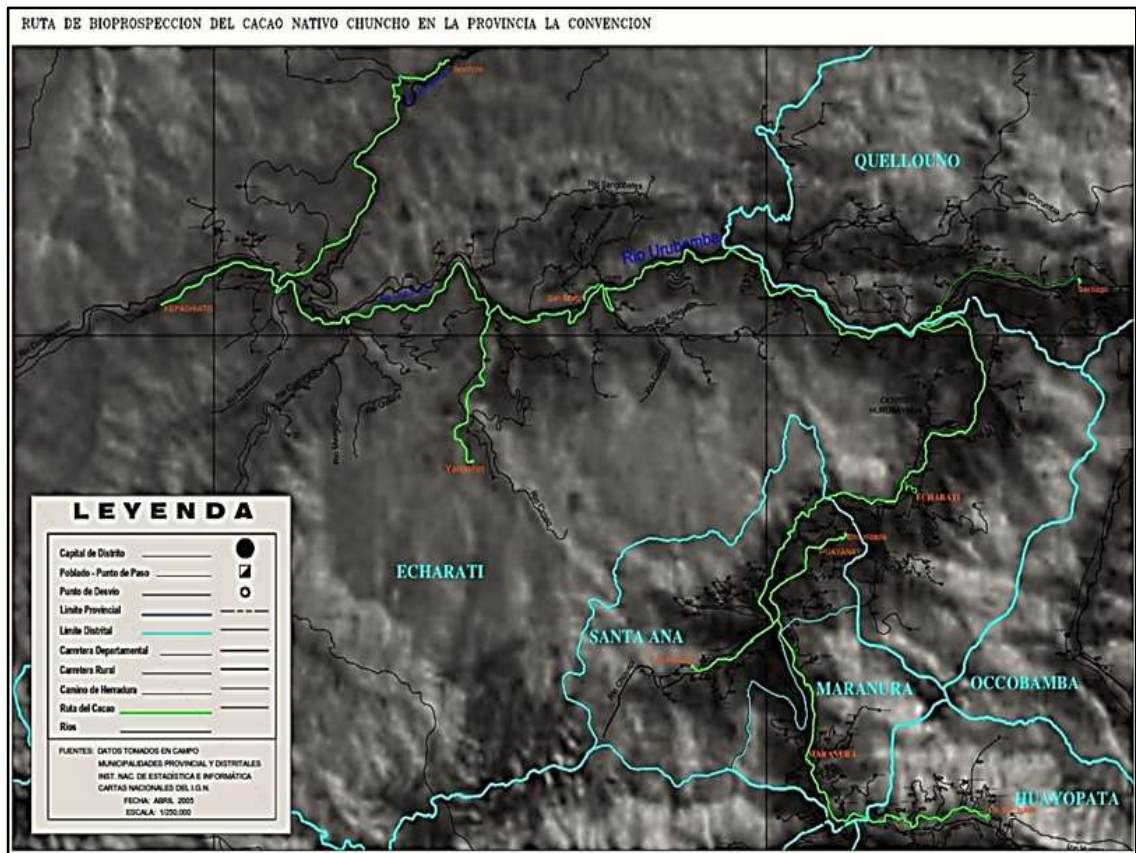

Figure 2:   Map of bioprospecting Chuncho cocoa (along 250 Km).

Figure 3:   Chuncho Cacao Genbank, Quillabamba, Cusco-Perú.

3. Methods

a. Extraction and purification of DNA: Utilizing QIAamp DNA Stool kit, QIAquick PCR Purification Kit, was successfully for all cocoa leaf samples (250).

b. DNA quantification: For this purpose, we used NanoDrop 2000, UV-Vis Spectrophotometer, and the ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA. A ratio of ~1.8 is generally accepted as "pure" for DNA. More than 95% of the Chuncho cacao samples were in the range of quality for SNP genotyping (fig.3).

c. SNP Genotyping: all 250 DNA Chuncho cacao Samples were genotyped by EP1™ System FLUIDIGM, The EP1, along with Fluidigm® integrated fluidic circuits (IFCs), streamlines the entire workflow from the setup of Dynamic Array™ IFCs and Digital Array™ IFCs to PCR thermal cycling, endpoint detection and data analysis, with 96 selected SNPs markers settled in a Digital Array.
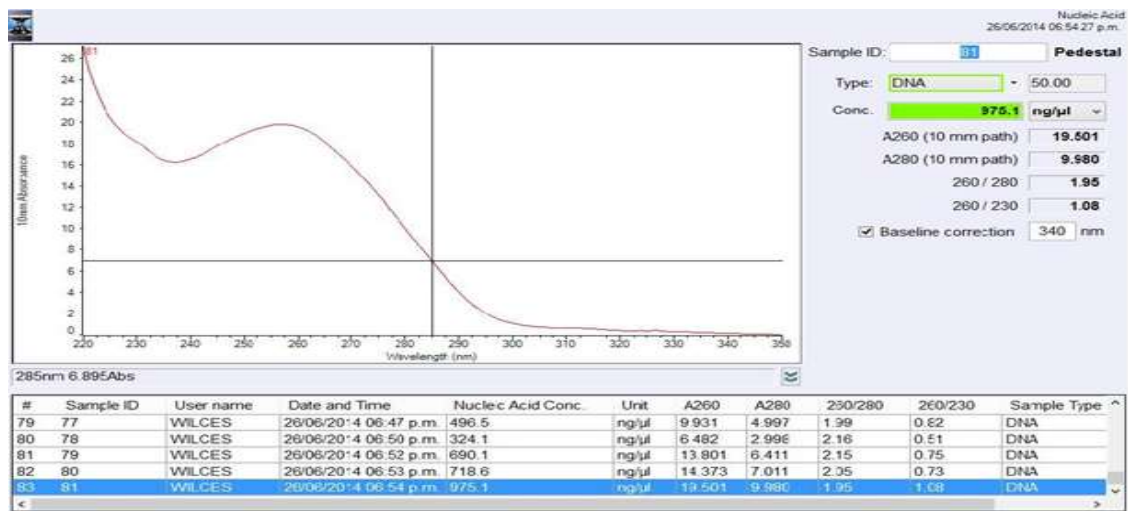
4. Results

Figure 4: DNA quantification by NanoDrop 2000.

Table 1: Summary statistics 0f 94 SNP markers across 240 Chuncho Cacao samples and 13 reference cacao germplasm groups.

Germplasm groups		Information index	Observed heterozygosity	Expected heterozygosity	Inbreeding coefficient
Nacional	Mean	0.192	0.142	0.125	-0.111
	SE	0.026	0.023	0.018	0.033
LCT EEN	Mean	0.358	0.236	0.238	0.018
	SE	0.028	0.026	0.020	0.045
IMC	Mean	0.274	0.251	0.183	-0.284
	SE	0.029	0.031	0.021	0.035
Nanay	Mean	0.314	0.229	0.197	-0.061
	SE	0.024	0.027	0.018	0.040
Parinari	Mean	0.349	0.292	0.233	-0.173
	SE	0.029	0.030	0.021	0.032
Ucayali	Mean	0.298	0.158	0.196	0.140
	SE	0.028	0.017	0.020	0.025
Beni	Mean	0.194	0.136	0.121	-0.003
	SE	0.024	0.027	0.017	0.048
Purus	Mean	0.271	0.186	0.176	-0.021
	SE	0.028	0.026	0.020	0.037
Morona	Mean	0.259	0.196	0.170	-0.040
	SE	0.028	0.029	0.020	0.048
Madre Dios	Mean	0.264	0.193	0.176	-0.052
	SE	0.029	0.026	0.021	0.034
Amelonado	Mean	0.234	0.124	0.142	0.128
	SE	0.024	0.018	0.017	0.039
Criollo	Mean	0.157	0.125	0.102	-0.166
	SE	0.025	0.023	0.017	0.037
F. Guiana	Mean	0.171	0.115	0.111	-0.034
	SE	0.026	0.019	0.017	0.027
Chuncho	**Mean**	**0.372**	**0.207**	**0.230**	**0.111**
	SE	**0.020**	**0.017**	**0.016**	**0.019**

From the initial 96 SNPs markers chosen to study genetic diversity, 94 generated high call rates in 240 Chuncho cocoa Samples.

Summary statistics for the combined Chuncho cocoa samples and references are shown on table 1. For Chuncho cocoa the Mean Shannon's mutual information index is 0.372 that means moderate diversity for these samples, the mean Observed heterozygosity 0.209 the mean Expected heterozygosity 0.230 express intermediate gene diversity and the mean of Inbreeding coefficient 0.111 that confirms most of the Chuncho cacao trees are cross fertilized. The percentage of Polymorphic Loci is 100%.

Table 2: Pairwise Population Matrix of Nei Genetic Distance.

	Nac	LCT	IMC	NA	PA	Uca	Beni	Pur	MOR	MAD	Ame	Crio	F.G	Chun
Nacional	0.00													
LCT EEN	0.18	0.00												
IMC	0.34	0.28	0.00											
NA	0.45	0.34	0.08	0.00										
PA	0.48	0.31	0.17	0.12	0.00									
Ucayali	0.20	0.19	0.30	0.41	0.33	0.00								
Beni	0.32	0.23	0.39	0.45	0.38	0.11	0.00							
Purus	0.32	0.20	0.23	0.24	0.20	0.24	0.27	0.00						
MOR	0.06	0.12	0.27	0.38	0.38	0.15	0.24	0.26	0.00					
MAD	0.30	0.20	0.32	0.37	0.34	0.12	0.07	0.17	0.23	0.00				
Amelonado	0.80	0.55	0.27	0.21	0.15	0.67	0.71	0.40	0.69	0.60	0.00			
Criollo	0.55	0.28	0.66	0.68	0.64	0.53	0.47	0.53	0.47	0.47	0.86	0.00		
F_Guiana	0.50	0.36	0.25	0.22	0.14	0.39	0.41	0.29	0.44	0.38	0.23	0.74	0.00	
Chuncho	0.26	0.19	0.28	0.34	0.31	**0.06**	**0.06**	0.21	0.18	**0.07**	0.58	0.47	0.36	0.00

The genetic distance by the Pairwise Population Matrix of Nei Genetic Distance gives to Ucayali 0.06, Beni 0.06 and Madre de Dios 0.07 populations, those values compared to Chuncho population are the closest, hence they may belong to a same metapopulation, which is significantly different from the other Peruvian populations listed in table 2.
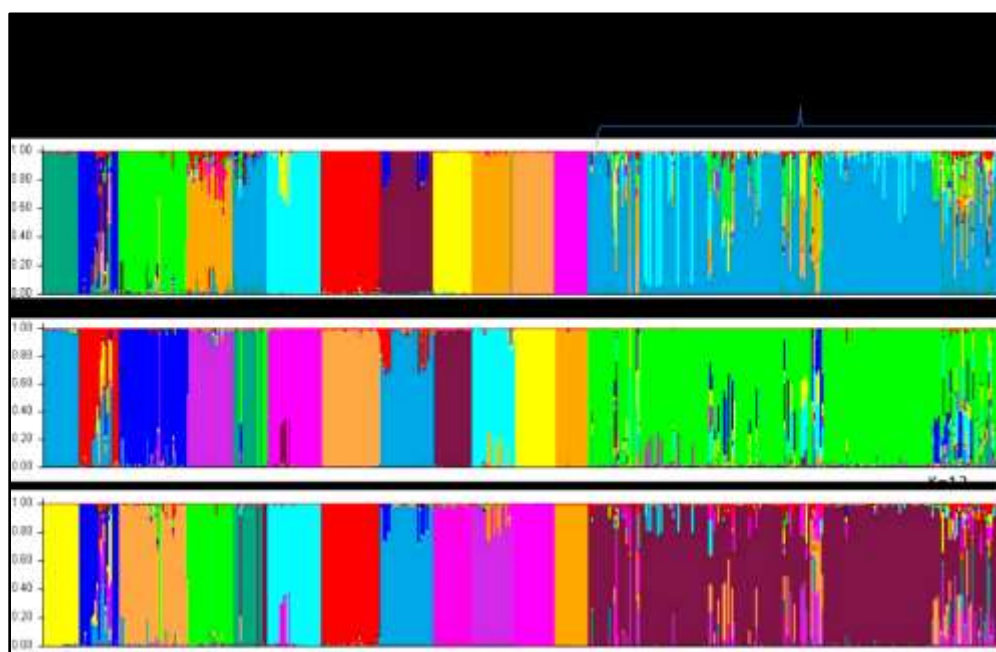

Figure 5:   Population assignment tests for Chuncho cocoa and references (13), using STRUCTURE software.

Analysis of population structure separated all the reference groups in different clusters. When K=11, Chuncho cacao was assigned in the same cluster with Ucayali group. This is because the Ucayali group included a number of clones from Rio Urubamba, where Chuncho cacao was originated. However, when K value increased to 12 and 13, Chuncho cacao was separated from Ucayali and became an independent cluster. This result demonstrated that Chuncho has its unique genetic identity may not be treated as a member of Ucayali population. Majority of the Chuncho cocoa samples were assigned as a full membership (Q value > 0.9). Nonetheless, substantial number of Chuncho samples was revealed having different percentage of hybrid origin, with the closest relatives as: Ucayali, Beni, and Madre de Dios populations. The result of Structure analysis confirmed that Chuncho Cacao is indigenous to southern Peru.
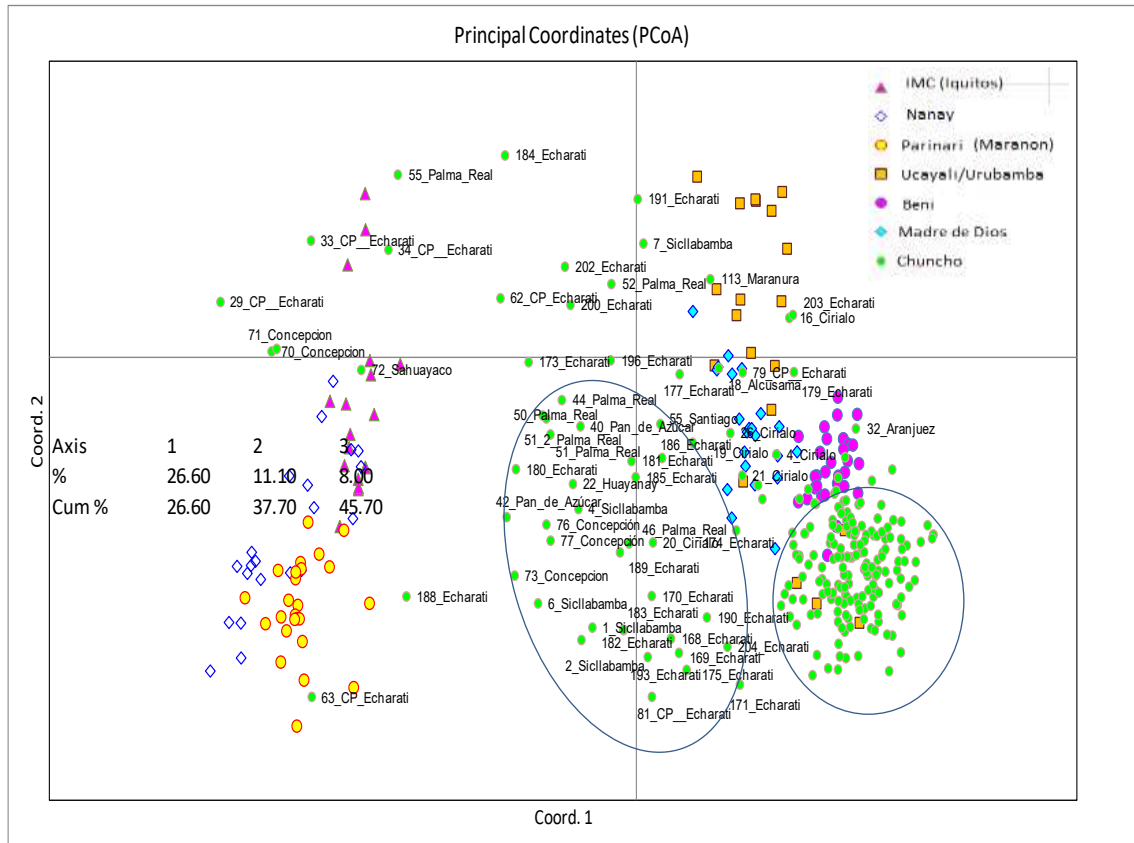

Figure 6:　　　Principal coordinate analysis (PCoA) for Chuncho Cacao and reference populations.

The Principal coordinate analysis (PCoA) for Chuncho Cacao and references (13), Figure 5 shows clustering results for "Core" set of Chuncho, hybrids between Chuncho and other germplasm groups and the reference clusters. The green dots concentrated in the right blue circle represent the core Chuncho samples and the others are spread around showing their affiliation with other germplasm clusters.

Therefore it will be necessary to continue a second stage that is to select a subset of Chuncho samples and genotype them using Next Generation Sequencing, this will allow us to fully understand the genetic relationship between germplasm in Chuncho, Ucayali, Madre de Dios and Beni, thus provide scientific baseline information for ex situ and on-farm conservation.

Based on the SNP analysis and phenotypic data, we will select the clones with high yielding, disease resistance and finest flavor and arrange formal field trials. After field evaluation, the promising clones will be recommended to local farmers. Breeding effort will be launched to improve yield and disease resistance under the background of fine flavor.

I can say now, Perú is know not just to have one of the wonders of the world "Machupicchu", but also for having the finest food of gods "Chuncho Cocoa", the information acquired in this research will strengthen the sustainability of cocoa production chain.

5. References

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