PUBLISHABLE FINAL ACTIVITY REPORT

Project no. 015100

CHERLA

Promotion of Sustainable Cherimoya Production Systems in Latin America through the Characterisation, Conservation and Use of Local Germplasm Diversity

Period covered: January 1st 2006 – December 31st 2008

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EXECUTIVE SUMMARY

Cherimoya (*Annona cherimola* Mill.) is a perennial fruit tree species of Andean origin of excellent organoleptic qualities, nutritious value and market potential. Despite the presence of an enormous wealth of local diversity, limited and often little coordinated activities to use this potential have been carried out in the Andean region.

CHERLA (Promotion of sustainable cherimoya production systems in Latin America trough the characterisation, conservation and use of local germplasm diversity) aimed at the promotion of sustainable cultivation of cherimoya in Bolivia, Ecuador and Peru, based on the characterisation, conservation and use of local genetic resources combining the available European expertise (both at the scientific and at the production levels) with local ongoing activities. Innovative research activities (using molecular tools and Geographic Information Systems) have provided new scientific insight into the existing cherimoya diversity gathering the necessary information to optimise current germplasm conservation activities. This has allowed unlocking the present, but so far untapped, genetic potential of cherimoya and canalise the obtained results to tackle the essential constraints (pests, lack of adequate cultural practices and limited commercialisation) for optimal cherimoya cultivation for the end-users, the poor resource Andean farmers. Training of local scientists has helped to fortify the scientific capacity in the region ensuring a long-term impact of the project. The CHERLA consortium was built based on existing expertise and experiences and comprised nine partners in six countries, including NGOs and one international organization.

PROJECT OBJECTIVES

The main objective of CHERLA was to develop sustainable cherimoya production systems in three Andean countries (Bolivia, Ecuador and Peru) based on the characterisation, conservation and use of local genetic resources. This main objective was divided into three groups of interdependent activities:

1. **Assessment of local diversity**
A first step in CHERLA was to assess local cherimoya diversity using existing *ex situ* germplasm collections and studying *in situ* cultivated, semi-cultivated and wild materials. Assessment has been carried out through classic phenotypic descriptors in combination with state-of-the-art molecular tools and Geographic Information Systems.
Verification criteria: WP1, WP2, WP3 and deliverables D1-D6.

2. **Conservation of local diversity**
Local diversity has been conserved *ex situ* and *in situ* using protocols that have been developed as a function of local situation parameters in the three Latin American countries participating in CHERLA. Small living core collections have been established both at the local (managed by local farmer associations) and national (managed by national agricultural research institutes) levels, together with seed collections of wild materials. Plant materials have been fully documented and characterised and made available for subsequent improvement work in the intervention area object of this project and elsewhere.
Verification criteria: WP4, WP5 and deliverables D7-D11.

3. **Use of local diversity**
The conservation of local cherimoya diversity should have a direct impact on the end-users. Thus, the main problems faced by local farmers for sustainable cherimoya production have been addressed and guidelines to optimize cropping, processing and commercialization using a participative approach have been developed.
Verification criteria: WP6, WP7, WP8, WP9 and deliverables D12-D26.

SUMMARY OF THE MAIN RESULTS OBTAINED

1. Assessment of local diversity

- Evaluation of phenological and pomological commercially interesting traits have been performed in the different *ex situ* germplasm collections in Spain, Ecuador and Peru.
- A set of 20 polymorphic SSRs useful for fingerprinting and diversity studies in cherimoya has been developed, published and distributed among the members of the CHERLA consortium. The set has been used to fingerprint *ex situ* collections and to study the genetic diversity in cultivated, semicultivated and wild cherimoya material.
- A selection of the most informative traits has been performed
- A CHERLA database with three modules (*in situ* characterization, molecular characterization and *ex situ* characterization) has been developed and distributed among the project partners. This database allows to efficiently share the results gathered by the different project partners.
- Descriptors for cherimoya have been finished and they have been published

2. Conservation of local diversity

- A complete inventory of cherimoya genetic resources conserved *ex situ* in the different countries has been performed.
- A method to develop core collections in cherimoya has been described.
- Additional cherimoya accessions have been collected and planted in germplasm collections in order to increase the number of representativeness of the current *ex situ* collections in the different countries involved in CHERLA.
- A strategy for cherimoya *in situ* conservation measures has been elaborated.
- Spatial analyses have been performed providing an overview of cherimoya diversity in its centre of origin, including the definition of hotspots of diversity.

3. Use of local diversity

- Detailed studies on reproductive biology of cherimoya and its main bottlenecks have been performed.
- The main pests that affect cherimoya fruits in the different countries have been identified and the most important are fruit flies species of the genera *Ceratitis* and *Anastrepha*.
- Optimization of fruit production has been performed in different pilot areas in Ecuador, Peru and Bolivia establishing the main cropping limitations and applying different local strategies through workshops, fairs and direct interactions with local farmers.

- Support materials for the different workshops with farmers, technicians and professionals have been elaborated.
- The evaluation of the financial/economic damage caused by pests has been performed.
- Improved techniques for nurseries have been elaborated and released
- Detailed surveys have been carried out involving the main actors of cherimoya production, marketing and consumption in the different countries of the CHERLA consortium.

ACTIVITIES PERFORMED AND RESULTS OBTAINED

Workpackage number: WP1

Workpackage number: WP1. Phenotypic characterization and diversity in germplasm collections

Objectives

General objective: To characterize the phenotypic traits of germplasm conserved in *ex situ* germplasm collections.

Specific objectives: i) To study in the different germplasm collections a set of most discriminant traits. ii) To compare the results obtained in the same genotypes in different locations (multilocational comparison). iii) To establish common criteria to develop descriptors for cherimoya.

Description of work

- **Task 1.1**. **Study of phenological and pomological commercially interesting traits**. These studies were performed in the cherimoya accessions conserved in Spain, Ecuador, Peru and Bolivia. Based on previous experience by different partners participating in the project, a set of interesting traits including tree, leaf, flower and fruit characteristics were available and they were used to characterize the collections maintained in the different institutions with the goal of developing a set of most discriminant, stable traits. These traits formed the base for the study of the morphological diversity in the region. In order to perform this task common genotypes present in the different collections as a results of previous germplasm exchanges were analysed first.

- **Task 1.2**. **Development of descriptors for cherimoya**. Once the traits described in Task 1.1. were studied in the different locations and genetic materials, descriptors for cherimoya were developed in a similar way to the descriptors available for other crops.

Deliverables

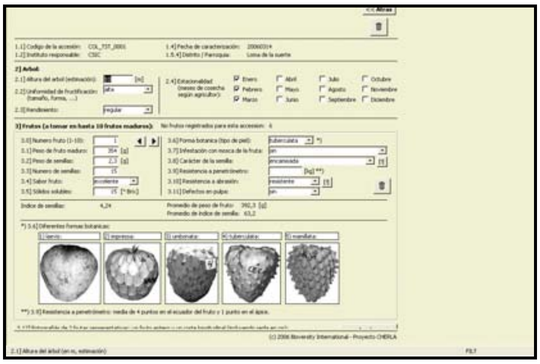
D1: Selection of the most informative traits.
D2: Development of descriptors for cherimoya.

Results

Task 1.1. Study of phenological and pomological commercially interesting traits.

In the germplasm collection of E.E. la Mayora (Spain) a total of 309 accessions are conserved by Partner 1 (CSIC). In Ecuador, the germplasm collection maintained by Partner 5 (INIAP) is conserved in the Granja Experimental Tumbaco of the INIAP in Tumbaco (Quito) in the province of Pichincha. This collection is composed of 126 trees of 42 accessions. In Peru (Huanchacc, Ayacucho) 340 accessions are conserved. In Bolivia 97 accessions are conserved.

In order to standardize the results obtained by each of the Partner, the CHERLA consortium decided to develop a database that could be shared among the partners. This database has three main modules (*in situ* characterization, molecular characterization and *ex situ* characterization).



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Deliverable 1 “Selection of the most informative traits” (D1) was completed and released.

Task 1.2. Development of descriptors for cherimoya

Based on previous works by members of the consortium and based on existing Bioversity descriptors for other tropical fruit species, the final version of the descriptors (D2) was published in both English and Spanish and it is available by request to any of the partners of CHERLA or in the Bioversity webpage Deliverable 1 “Selection of the most informative traits” (D1) was completed and released during the previous period and Deliverable 2 “Development of descriptors for cherimoya”.

Workpackage number: WP2

Genotypic characterization and diversity *ex situ* and *in situ*

Objectives

General objective: To characterize and study diversity in *ex situ* collections, local cultivated, semicultivated and wild germplasm using molecular tools.

Specific objectives: i) Development of a set of informative microsatellite markers to fingerprint genotypes and characterize diversity. ii) Fingerprinting genotypes and study of the genetic diversity conserved at the different germplasm collections. iii) Characterization and study of genetic diversity in cultivated and semi-cultivated materials. iv) Genetic diversity in selected wild cherimoya stands.

Description of work

- Task 2.1. Development of a set of informative microsatellite markers. The SSR loci developed were screened in a selected number of cherimoya genotypes. A set of 20 highly polymorphic and easily scorable loci were selected. Screening was performed both with the use of an automatic capillary electrophoresis apparatus and high resolution agarose gel electrophoresis. In order to develop this set of markers, the discrimination results in agarose gels was determinant since this methodology is easier to transfer.

- Task 2.2. **Fingerprinting and diversity studies in *ex situ* conserved germplasm**. DNA was extracted from fresh leaves. These studies were performed in the cherimoya accessions conserved in each country. Periodic replications of the analyses was performed in at least two laboratories to ensure the standardization of the protocols and the reproducibility of the results.

- Task 2.3. **Characterization and study of genetic diversity in cultivated, semi-cultivated and wild material**. DNA was extracted from fresh leaves. These studies were performed in particular areas of the departments of Ancash, Apurimac, Ayacucho, Cajamarca, Cusco, Huancavelica, Huanuco, Junin, Piura, La Libertad and Lima in Peru, the provinces of Guayllabamba-Pichincha, Loja and Azuay in Ecuador and the departments of Santa Cruz, Cochabamba, La Paz, Tarija and Chuquisaca in Bolivia. For wild material, the studies were performed in areas with important extensions of wild cherimoyas such as the departments of Ayacucho, Ancash, Cajamarca, La Libertad, Lambayeque and Piura in Peru or the province of Loja/Vilcabamba valley in Ecuador. For assessment of genetic diversity within and between populations, leaf samples were collected randomly from up to 40 individuals per population and DNA was extracted. Genetic data were analysed by standard statistical approaches. The free GIS tool DIVA-GIS, which has been specifically designed to carry out detailed spatial diversity analyses, was also used in this task together with the ESRI desktop programs ArcView/ArcGIS.

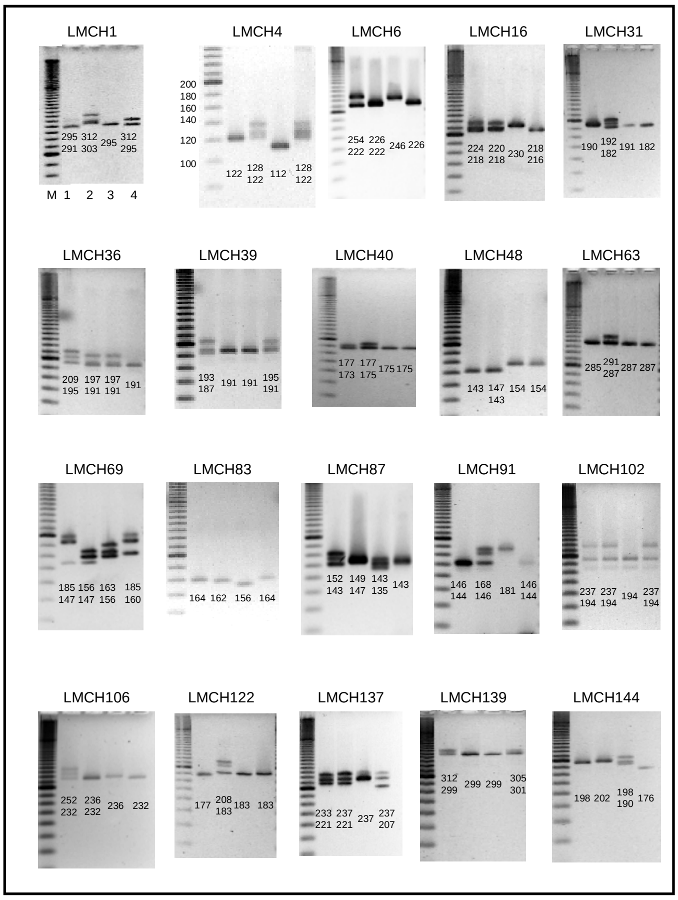
Deliverables

D3: Development of a set of informative microsatellite markers for fingerprinting and diversity studies
D4: Fingerprinting and diversity conserved *ex situ*
D5: Fingerprinting and diversity in cultivated, semi-cultivated and wild material

Results

Task 2.1. Development of a set of informative microsatellite markers.

A set of informative SSR loci (D3) was developed and distributed among the partners. This set is based on an initial study of 98 polymorphic cherimoya microsatellites and it has been optimized to allow a good resolution in acrylamide and agarose gels as well as in automatic analyzing fragment systems. The set of 20 selected microsatellites is represented below with the image in agarose gel electrophoresis after the amplification of each of them in four selected cherimoya genotypes. The sequences of the primers together with DNA of the 4 accessions of reference have been distributed to the partners involved in molecular analyses with the objective that all the groups use the same DNA as controls.



Similarly, the DNA extraction protocol has been optimized and distributed among the different groups involved in CHERLA with the objective of standardizing all the protocols allowing and easier exchange of data among laboratories.

The set of informative SSR loci (D3) was released during the first year of the project and the results on the development of the set of SSR markers have been published in 2008:

Escribano, P., M.A. Viruel, J.I. Hormaza. Development of 52 new polymorphic SSR markers from cherimoya (*Annona cherimola* Mill.). Transferability to related taxa and selection of a reduced set for DNA fingerprinting and diversity studies. *Molecular Ecology Resources* 8: 317 – 321.

Task 2.2. **Fingerprinting and diversity studies in *ex situ* conserved germplasm**

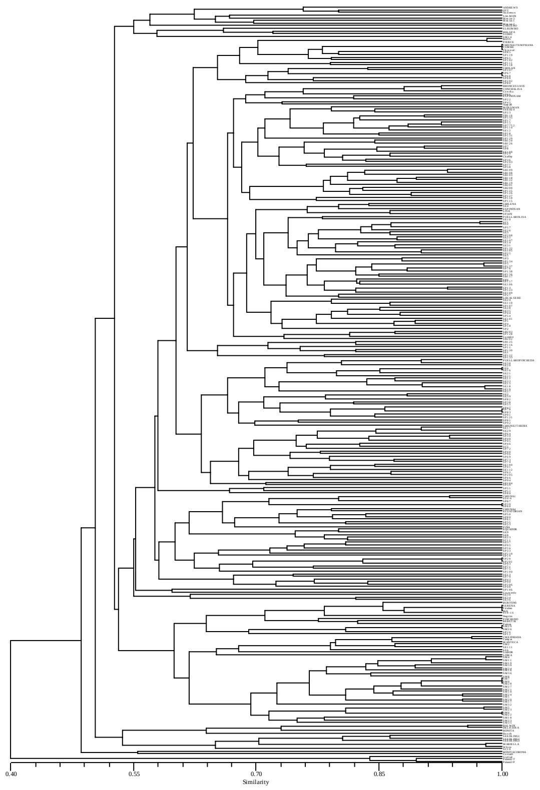
The different groups performed the analysis of the accessions conserved in the different collections using the set of selected SSRs developed in Task 2.1.

In Spain (Partner 1, CSIC), 269 accessions have been completely analyzed with SSR markers and their genetic similarity established. This analysis was performed with some of the

SSR loci of the consensus kit and with additional SSRs. The results have been published in 2007:

Escribano, P., M.A. Viruel, J.I. Hormaza (2007) Molecular analysis of genetic diversity and geographic origin within an ex situ germplasm collection of cherimoya by using SSRs. Journal of the American Society for Horticultural Science 132: 357-367.

As an example, the following figure represents the similarity relationships among the genotypes conserved using UPGMA analysis.



In Peru INIEA has characterized 290 accessions of the national germplasm collection with 20 SSR loci and the size of the amplification fragments have been optimized through a comparison with analyses of some of the genotypes with an automatic fragment analyzer by Partner 1 (CSIC) in Spain. In Ecuador, INIAP has been working on DNA extraction from 126 samples of a total of 42 different accessions of the INIAP collection conserved in Tumbaco-Pichincha.

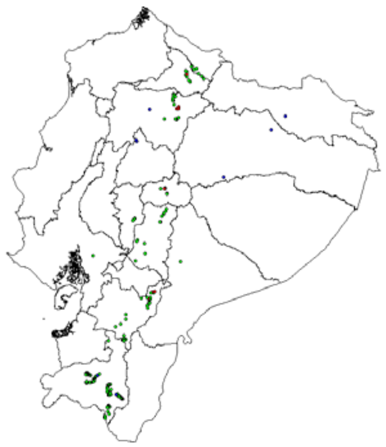
Task 2.3. **Characterization and study of genetic diversity in cultivated, semi-cultivated and wild material**

During the kick-off meeting the CHERLA consortium decided how to carry out these two tasks in the different countries comparing the diversity at three different levels:

- Comparison of the diversity among groups (cultivated/semicultivated/wild). A total of 100 genotypes will be collected randomly in each country for each of the three types of materials: 100 cultivated, 100 semicultivated and 100 wild (except in Bolivia where no wild cherimoya stands are known).
- Study of the diversity within each group (semicultivated and wild). A minimum of 50 genotypes per local populations will be collected and a minimum of 4 populations per group. Each country will decide how many populations will be analyzed.

- Diversity in wild material: 100 samples per wild forest: 1 forest in Ecuador and 1 in Peru.

In Ecuador, the collection of samples has been performed in collaboration between Partner 9 (NCI) and Partner 5 (INIAP). So far a total of 375 accessions have been collected and 267 of them have been analyzed with 8 SSRs of the consensus kit. The following map describes the location of the accessions sampled:

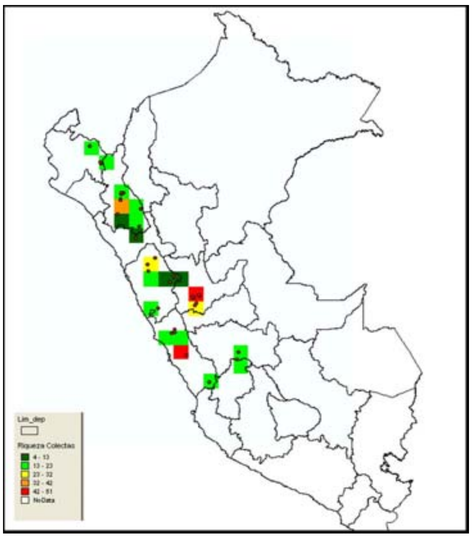


Cultivated: ●
Semicultivated ●
Wild ●

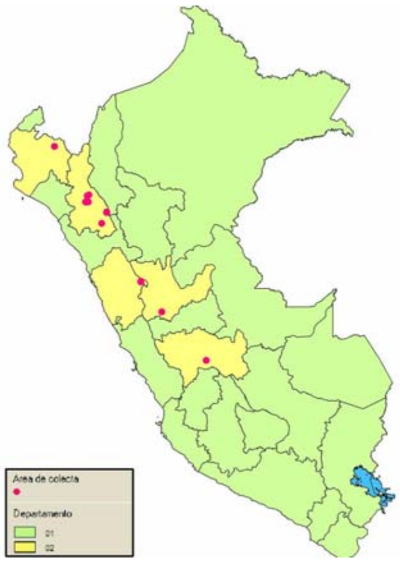
Moreover, 4 commercial orchards have been identified in Ecuador where a random sampling of 10% of the trees were carried out:

1. Guayllabamba, Pichincha: 1 ha with approximately 60 trees.
2. Tumbabiro, Imbabura: 1,5 ha with approximately 150 trees.
3. Paute, Azuay: 3 ha with approximately 300 trees.
4. Guachapala, Azuay: 0,3 ha with approximately 30 trees.

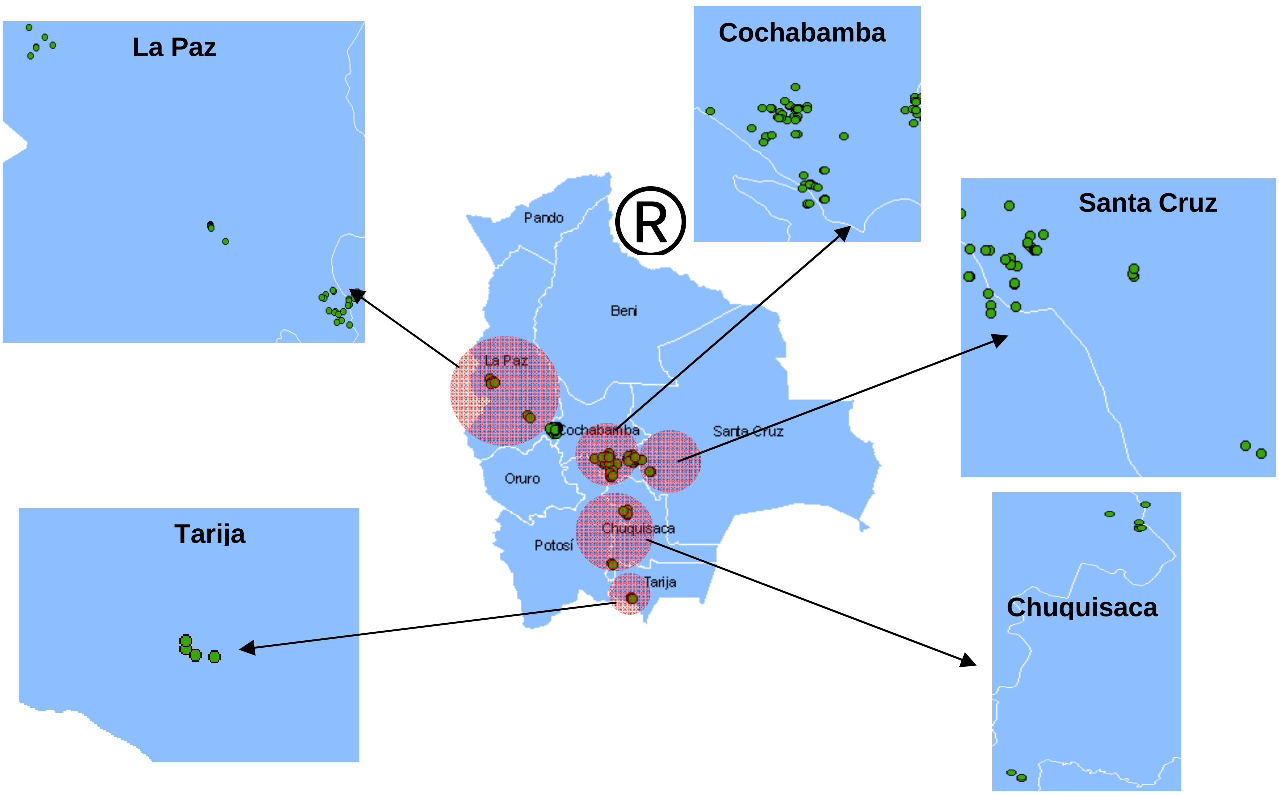
In Peru a total of 41 cultivated genotypes in Cajamarca (San Pablo) and La Libertad (Ascope) and 316 semi-cultivated genotypes in Cajamarca (Cajabamba, Cutervo, Chota, San Pablo, Celendín), Piura (Ayabaca and Huancabamba), Ancash (Chiquian, Huaylas, Sihuas and Huari) and Lima (Huaral, Huaruchiri and Yauyos) have been collected by Partner 6 (INIEA). Moreover, in this country, a total of 330 wild genotypes have been collected in Cajamarca (Cutervo, Chota, San Pablo and Celendín), Piura (Ayabaca) and Ancash (Huari). Those samples represent 7 regions in the country.



Regarding wild materials the following map indicates the areas where putative wild genotypes have been collected. They represent a total of 245 samples from 5 different regions: Cajamarca, Piura, Ancash, Junín and Huánuco.



In Bolivia, a total of 400 samples have been collected by Partner 7 (PROINPA) from cultivated and semicultivated genotypes in 5 departments: La Paz, Cochabamba, Santa Cruz, Chuquisaca and Tarija..



According to the distribution of the cherimoya producing areas in the countries, cherimoyas are found in an altitudinal range of 2000 to 2600 masl, that correspond to bioclimatic mesotermic interandean valleys. In this country, although initially no wild cherimoya populations had been reported, as a complement to the activities envisaged in this WP, prospection trips were carried out to identify wild populations of related species in the

Tropic and Chaco regions. Different species of semicultivated (*A. squamosa*, *A. reticulata* y *Rollinia* sp.) and wild (*A. nutans* and *A. montana*) materials were characterized in collaboration with the Martín Cárdenas herbarium of Cochabamba. The results indicate a low genetic structure of the different populations analyzed.

Partner 3 (UNIVIE) has investigated the phylogenetic position of *Annona cherimola* within the Annonaceae. It could be shown that the closest relatives are native elements originating from Mesoamerica. In this sense, the results obtained in CHERLA are currently being compared with results obtained through other projects in Costa Rica and Guatemala thanks to the access to additional material from Mesoamerican populations of *Annona cherimola* and related taxa. This is of great importance to finally ascertain the origin *A. cherimola* and related species.

Workpackage number: WP3

Localization of hotspots of diversity

Objectives

General objective: To establish the hotspots of diversity from the molecular data obtained from genotypes already conserved *ex situ* and of genotypes studied *in situ*

Specific objectives: i) Determination of the areas of highest diversity from material conserved *ex situ* in germplasm collections. ii) Determination of the areas of highest diversity from material studied *in situ.*

Description of work

- Task 3.1. **Identification of hotspots of diversity based on characterization results**. The results obtained in WP2 served to establish the main hotspots of cherimoya diversity both in wild and cultivated material. This information is highly relevant to establish the centre of origin of cherimoya. In order to accomplish this goal, observation of related species in the Annonaceae in the different areas studied was complement molecular data obtained in cherimoya.

- Task 3.2. **Decision on the location and number of wild trees from which seeds will be collected**. Whereas elite cultivated germplasm can be conserved in *ex situ* living collections, in order to guarantee that the diversity present in interesting wild cherimoya spots is not lost, seeds will be collected and conserved at low temperature.

Deliverables

D6. Establishment of hotspots of diversity.

Results

Task 3.1. **Identification of hotspots of diversity based on characterization results**.

Molecular analysis was based on the analysis of 9 microsatellites (SSRs) (cf Deliverable D03). Molecular analyses were carried out using GenAlEx 6.1 (http://www.anu.edu.au/BoZo/GenAlEx) and Structure (http://pritch.bsd.uchicago.edu/structure.html), while spatial analysis was carried in DIVA-GIS (http://www.diva-gis.org). The cherimoya distribution was divided in units of measurement that allow comparison between different zones and sites. Taking into account the replicability by local partners all analyses were carried out based on free software.

Individual countries collected data (georeferenced cherimoya sites) at three levels: collection sites, collection sites with *in situ* morphological characterisation (as input for morphological diversity studies, cf WP 1, Task 1.1) and collection sites with molecular characterisation data based in the 20 standard SSRs (molecular studies diversity studies, cf WP 2, Tasks 2.3 and 2.4).

A total of 1234 trees with complete correctly georeferenced molecular data have been included in explorative analysis of the diversity. This analysis is based on the total allelic richness that is found in a specific population/unit of measurement. Results of this analysis are given in Figure 1a and Figure 1b.

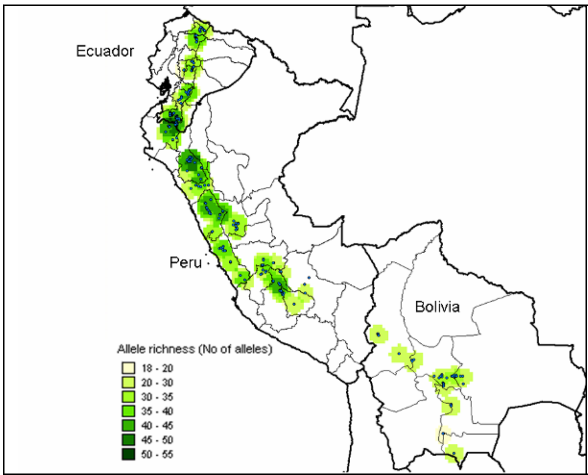


Figure 1a - Allelic richness (number of alleles) in the study area

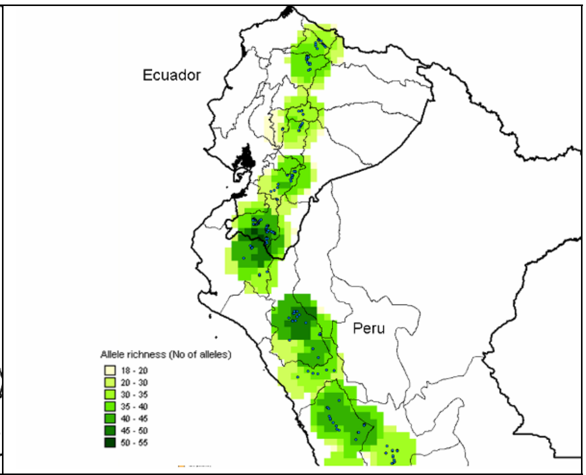


Figure 1b - Allelic richness (number of alleles) in part of the study area with the highest diversity

The allelic richness maps clearly confirm that the highest diversity (based on number of alleles) can be found in northern Peru and southern Ecuador. Diversity levels in Bolivia are significantly below the levels observed in Peru and Ecuador.

Unit	Number of sampled trees	Average number of alleles per locus (N_a)	Average number of effective alleles per locus (N_e)	Private alleles
Bolivia	381	4.3	2.2	2
Ecuador	213	5.9	2.4	8
Peru North	406	5.8	3.4	5
Peru South	234	5.7	2.9	2

The Structure software looks at the allelic composition (over the 9 analyzed SSR markers) of each of the individuals. Results of this analysis (based on 4 putative populations), given in Figure 3, clearly differentiate the individuals from Ecuador, northern Peru, southern Peru and Bolivia. While in the Ecuadorian materials some accessions similar to those in northern Peru (red color) can be found, there is hardly any material in northern Peru that shows similarity to the major Ecuadorian group (blue color). In Peru the material collected in the south is less diverse than in the north of the country. The individuals that show a 100% yellow bar belong to the widely cultivated Cumbe variety. Further analysis has to show whether the accessions in the north that are similar to the southern material (the material with significant yellow bars) are also cultivated (Cumbe) material. Again the somewhat different composition of the Bolivian material (although with limited diversity) is confirmed. Surprisingly the material in Bolivia shows more similarity to the Ecuadorian material (more blue that red bars) than to the material from northern Peru (as could be observed from the PCA plot as well).

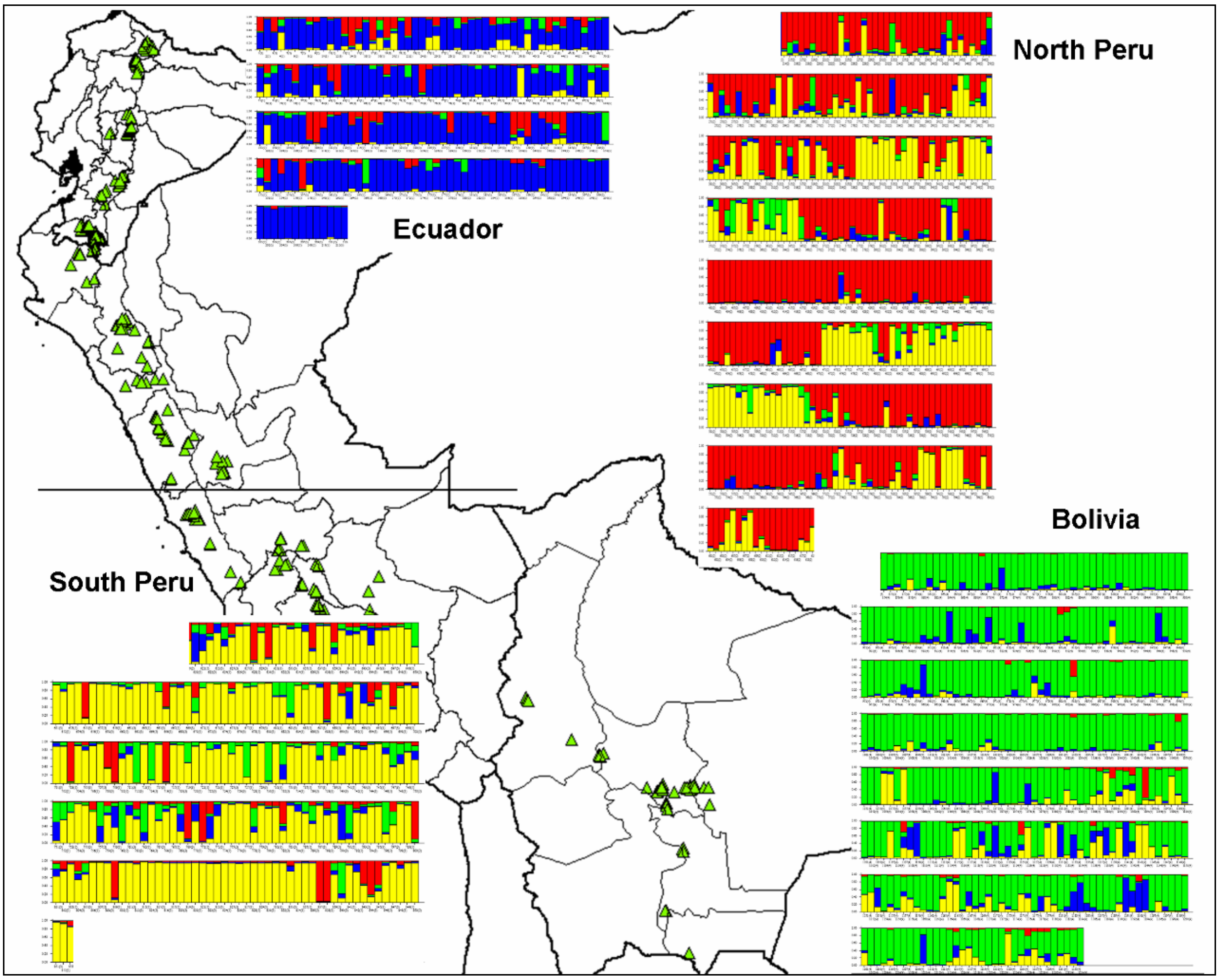


Figure 3 – Results of population structure analysis based on 4 putative populations. The vertical bars represent the individuals. The colors indicate the probability that an individual belongs to one of the 4 populations.

The Geocluster option in DIVA GIS allows analyzing similarities between allele composition at grid cell level (independently from the individuals). Figure 6 shows the result of this analysis, clearly differentiating materials from the three different countries. The dendrogram in Figure 6a again shows that there seems to be more similarity between the material in Bolivia and Ecuador than between Bolivia and Peru. Possibly this differentiation could be due to the large amount of common alleles that only occur in Peru.

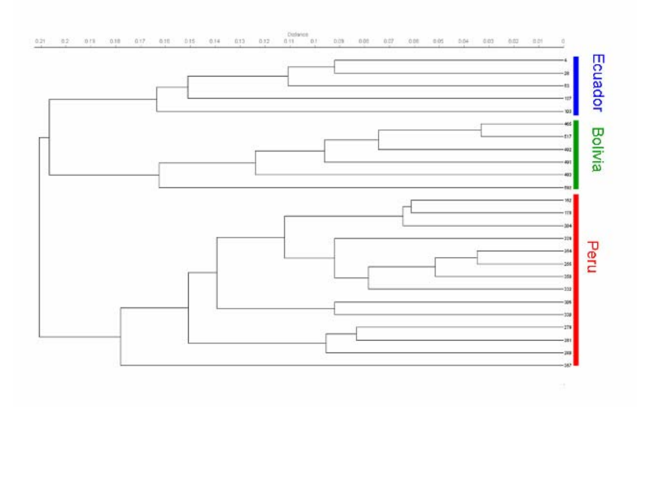


Figure 6a – Geocluster analysis showing different countries

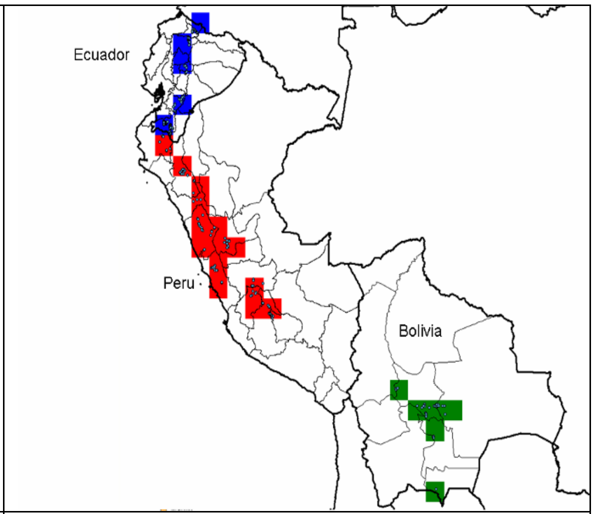


Figure 6b – Map of Geocluster analysis with 3 clusters

One of the objectives of a spatial analysis of the diversity is to provide inputs in the formulation of *in situ* strategies. These strategies aim to conserve as much diversity as possible at a minimal cost. Besides capturing the maximal diversity the selection of conservation sites also has to take into account existing redundancies and include both areas with high diversity and also those of unique diversity. The Reserve Selection tool in DIVA GIS identifies the cells that need to be prioritized for conservation taking into account the need to capture as much as the diversity in a minimal number of cells. Results of the analysis are given in Figure 7.

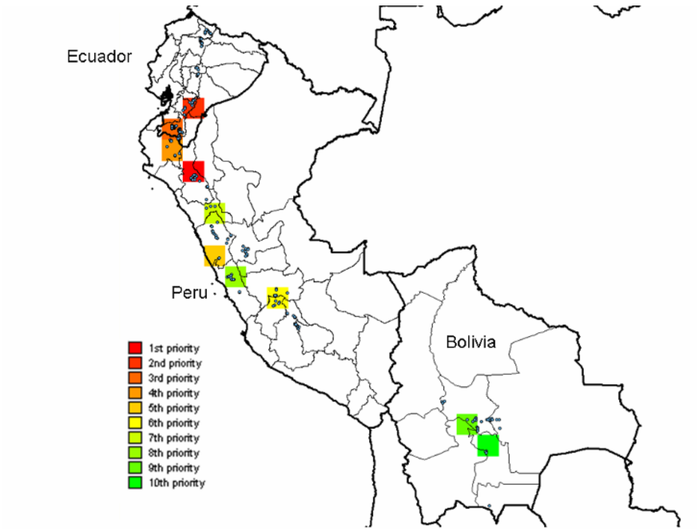


Figure 7 – Result of Reserve Selection analysis, showing those cells that need to prioritize to maximize conservation of the cherimoya genepool

The answer to the question of where the highest diversity of cherimoya can be found will depend on what is exactly meant with the highest diversity. Looking at the absolute number of observed alleles the area would be located in southern Ecuador and northern Peru (zone between Loja and Cajamarca). Whereas the molecular cherimoya diversity in southern Ecuador is characterized by the presence of some (very) rare alleles combined with very common ones (found from Ecuador down to Bolivia) the diversity in Peru is more characterized by the presence of common alleles only found in northern Peru. In general terms diversity in northern Peru is higher than in Ecuador as the alleles that compose the diversity are more common ones. The southern part of Peru seems to be less diverse with most of its diversity also present in the northern part of the country. Cherimoya in Bolivia is significantly less diverse than in Peru and Ecuador, yet some found alleles turned out to be unique for the country. The fact that different

analysis showed more links between material from Bolivia and that of Ecuador than that between Bolivia and its neighbor country Peru warrants further analysis.
In general the molecular and spatial analyses confirm the results found in literature which are based on observations, including from early Spanish chroniclers.

Task 3.2. Decision on the location and number of wild trees from which seeds will be collected

Partner 3 (UNIVIE) has revised the general geographical distribution of *Annona cherimola* on the basis of new material collected and holdings of 8 international herbaria. Since the final analyses are still on their way for Task 3.1 (hotspots of diversity) and Task 5.1 (*in situ* conservation strategy for wild populations), the decision will be made when the final results are obtained.

Workpackage number: WP4

Ex situ conservation of cherimoya genetic resources

Objectives

General objective: To optimize the resources devoted to management of cherimoya germplasm collections

Specific objectives: i) Development of an updated inventory of the genetic resources of cherimoya currently conserved in the different countries. ii) Consider the establishment of local collections and national core collections that represent most of the diversity conserved in the different germplasm collections. iii) Study of the necessity of increasing the number of genotypes conserved in the different collections taking into account previously non-prospected areas.

Description of work

- Task 4.1. **Inventory of current ex situ germplasm collections**. During this task a complete inventory of all the genotypes conserved including passport data was carried out. This inventory was disseminated through the web page and was updated as new material is introduced into the *ex situ* collections and as the results of WP1 and WP2 were analysed.

- Task 4.2. **Establishing local collections and national germplasm core collections**. The data obtained in WP1 and WP2 was used to decide the necessity of establishing core collections as well as the number of accessions needed to represent the diversity conserved. These core collections could be studied with further detail in the future. Similarly, the necessity of establishing sustainable local collections and the number of accessions was evaluated.

- Task 4.3. **Evaluation of the necessity of incorporating additional genotypes into the collections**. The necessity of incorporating new accessions into the existing and/or new collections will be evaluated.

- Task 4.4. **Establishment of seed banks from wild cherimoya stands**. In order to preserve the cherimoya variability in the hotspots located in WP3, seeds will be collected from designated areas and genotypes and conserved under low temperatures. Recommendations for the maintenance of this material after the end of this project will be discussed and established considering the possibility of collecting seeds again at 5-10 years intervals from the same areas (assuming they have been conserved) instead of the costly process of regenerating the plants from seeds.

Deliverables
D7. Inventory of current *ex situ* germplasm collections.
D8. Establishment of core collections and additional incorporations into local and national collections
D9. Establishment of seed banks of wild material.

Results

Task 4.1. Inventory of current *ex situ* germplasm collections.

In Spain, an inventory of all the accessions conserved in the Spanish cherimoya germplasm bank was developed during the two first years of the project. This is the only active *ex situ* cherimoya collection in Spain holding currently 271 cherimoya accessions from 14 countries: Australia (13 accessions), Bolivia (18 accessions), Chile (12 accessions), Colombia (5 accessions), Costa Rica (3 accessions), Ecuador (50 accessions), Italy (3 accessions), Japan (1 accession), Mexico (6 accessions), Peru (137 accessions), Portugal (31 accessions), Spain (23 accessions) and USA (30 accessions). Since some of them were collected directly in the field in Andean countries a replica was made at the time of the collections and some of the accessions are also conserved in germplasm banks in Peru and Ecuador. Repatriation of the accessions from those countries is taking place in the framework of this project. These accessions will be combined to the collections made in each country to establish national and local cherimoya germplasm collections. Additionally the collection contains 4 other *Annona* species, 5 atemoyas (hybrids between A. cherimola and A. squamosa) and 6 Rollinias.

In Ecuador an inventory of the existing *ex situ* cherimoya collections was carried out and, currently, the only active *ex situ* collection is the INIAP collection maintained by Partner 5 (INIAP) in Tumbaco (Quito). This collection holds126 trees representing 42 accessions.

In Peru, in this task the recompilation and verification of the documentation of the different *ex situ* cherimoya collections have been carried out in five regions (Cajamarca, Piura, La Libertad, Ancash and Lima) by Partner 6 (INIEA). A complete inventory of the accessions conserved has been performed in the different collections: Sub EEA Paijan de la EEA Vista Florida de Chiclayo, Universidad Nacional Hermilio Valdizan de Huanuco, CE La Molina and Anexo Huanchacc de la EEA Canaán. The following table describes de location of the collections and the number of accessions.

Department	Province	District	Place	No. accessions	Latitude	Longitude	m.a.s.l.
Ayacucho	Huanta	Luricocha	Huanchacc	340	12º 55` 50.0"	74º 17`37.0"	2361
Huanuco	Ambo	Tomayquichua	Quiscan	110	10º 05`28.3"	76º 12`27.1"	2077
La Libertad	Ascope	Paijan	Chuin Alta - Anexo EEA Vista Florida	50	07°05'11.9"	79°16'20.4"	1773
Lima	Lima	La Molina	Centro Experimental-INIA	219	11º07'57.7"	77º02'05.4"	1609

A total of 18 Bolivian accessions conserved in the Spanish Germplasm Bank have been incorporated into the germplasm collection. Fifteen additional accessions previously collected in Bolivia are also being preserved in a farmer’s orchard in Los Bañados de la Cruz in Comarapa and 18 additional promising accessions collected in 2006-2007 are also being conserved. Two replicas of the collection have been estrablished to guarantee the conservation of cherimoya genetic resources in Bolivia.

a) ***Comarapa***.- The collection has been established in an orchard of the Escuela Técnica Agropecuaria "Campagnat", in the area of Río Arriba, 3 km from the village of Comarapa. The long term conservation of this collection is guaranteed by the signature of an agreement among the Local Government of Comarapa, the Escuela Técnica Agropecuaria "Champagnat", the Asociación Integral de Productores Frutihortícolas de la Provincia Caballero AIP and PROINPA.

b) ***Cochabamba***.- In the orchards of PROINPA a replica of the collection has been established.

Additionally, 97 seed samples from different accessions have been preserved in Comarapa.

Task 4.2. **Establishing local collections and national germplasm core collections**.

Partner 1 (CSIC) has developed a core collection with 40 genotypes that represent the diversity conserved in the whole germplasm bank maintained at the E.E. la Mayora (Spain) comparing different methods. This set of 40 genotypes could serve as a basis to exchange germplasm to other countries interested in cherimoya cultivation and the approach followed could also be used by other groups in CHERLA in order to develop national core collections. For the other countries this goal will be postponed until all the information has been analyzed. The results have been published:

Escribano, P., M.A. Viruel, J.I. Hormaza. Comparison of different methods to construct a core germplasm collection in woody perennial species with SSR markers. A case study in cherimoya (*Annona cherimola* Mill., Annonaceae), an underutilized subtropical fruit tree species. Annals of Applied Biology.

In Bolivia, local germplasm collections have been established in three different areas in order to guarantee the long-term *ex situ* conservation of the most interesting national germplasm: Comarapa, Mizque and Cochabamba. In both Comarapa and Mizque, interinstitutional agreements were signed among PROINPA, local governments, production associations, and a Technical Agricultural School in order to guarantee a long-term conservation of the collections.

a) Comarapa. This collection is being established in an orchard of the Technical School Campagnat in the Río Arriba area, about 3 Km. from the town of Comarapa. The maintenance of this collection is guaranteed thanks to the signature of an agreement between the local city council, the technical school, the Association of producers of the Caballero Province (AIP) and PROINPA (Partner 7).

b) Mizque. Under the framework of the National System for the conservation of genetic resources "Sistema Nacional de Recursos Genéticos para la Alimentación y la Agricultura" (SINARGEAA), in the Fruits program a replica of the collection is being established in the Maira Experimental Station in Mizque; this station belongs to the Cochabamba Department authorities. An interinstitutional agreement will also be signed in this case to guarantee the long term conservation of the collection.

c) Cochabamba. In the orchards of PROINPA an additional replica is being established also with the objective of ensuring a long term conservation of the material.

Task 4.3. **Evaluation of the necessity of incorporating additional genotypes into the collections**.

Since the cherimoya germplasm bank in Spain can be considered as the collection of reference worldwide for cherimoya, new accessions from different countries are being incorporated continuously. Thus, during 2007, 8 new accessions from USA, Costa Rica and Mexico were added to the collection and during 2008, 7 new accessions from USA, Costa Rica and Mexico were added to the collection.

NCI has characterized previously selected cherimoya accessions which had been lost and new collections have also been carried out mainly in Espíndola. A total of 11 promising accessions were selected. In the north of Peru, most of the work of NCI has involved collection and characterization in the province of Ayabaca (districts of Ayabaca, Sigchez and Montero) selecting 27 promising accessions. After the molecular analysis a total of 42 accessions were selected to be included in the collection: 29 semicultivated, 11 cultivated and 3 wild. These accessions represent a large geographic range in the country and they have been grafted in the collection in Tumbaco.

A total of 29 accessions collected in Bolivia in the 1980s and 1990s and conserved in the Spanish cherimoya germplasm bank were replanted in the three collections in Comarapa, Mizque and Cochabamba during 2007 together with 18 additional promising selections collected during the prospection trips (2006-2007). All those selections have been grafted and they will flower during 2009 or 2010

Task 4.4. **Establishment of seed banks from wild cherimoya stands**

Since no previous results were available on the duration of seeds under cold temperature conservation, an experiment was initiated in Peru in the frame of the CHERLA project to conserve seeds in polyester bags in a cold chamber (3 °C and 70 % relative humidity). After one year the viability is higher than 85% so this approach will be recommended to the other partners to conserve seeds from wild cherimoya stands.

Workpackage number: WP5

In situ conservation of cherimoya genetic resources

Objectives

General objective: To establish guidelines and recommendations for *in situ* germplasm conservation in wild forests and traditional cultivated and semicultivated situations.

Specific objectives: i) Recommend the main areas of wild populations that should be preserved. ii) Establish main areas of semi-domesticated populations that should be preserved through participation of local communities and their official representatives. iii) Develop the necessary legal tools/environment to allow for sustainable conservation and protection of the conservation areas.

- Task 5.1. **Inventory of wild populations and definition of conservation measures**. This will allow us to establish guidelines to conserve the areas where the highest diversity exists.

- Task 5.2. **Inventory of semi-domesticated populations and definition of conservation measures**. This task included field trips to make an inventory of the genotypes present in semi-domesticated conditions in the areas not prospected in the past.

Deliverables

D10. Strategies to conserve genetic resources in cultivated and semi-cultivated germplasm.
D11. Strategies to conserve genetic resources in wild populations.

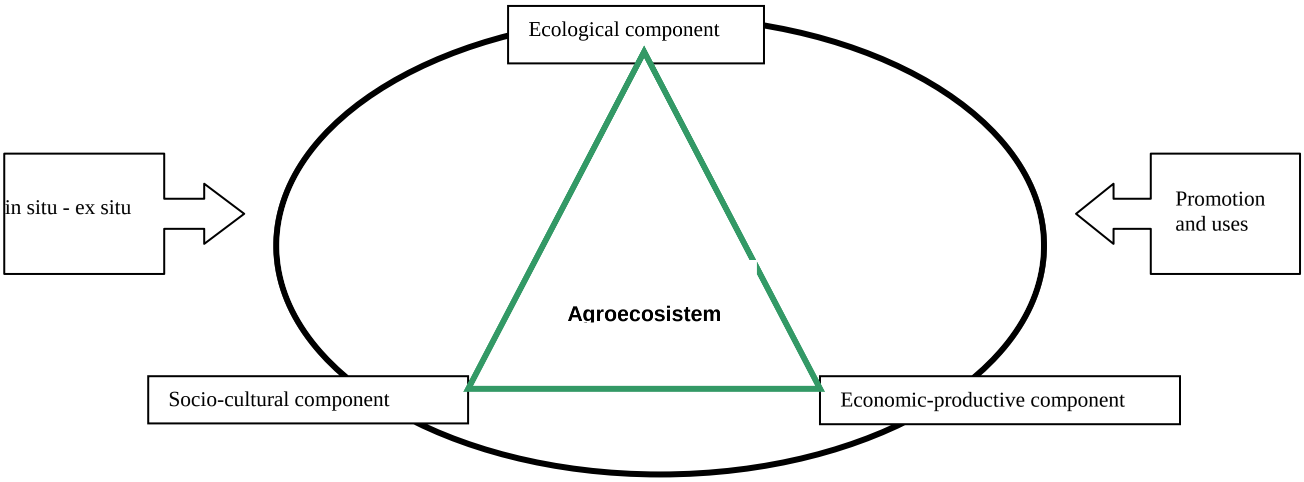
Results

Task 5.1. Inventory of wild populations and definition of conservation measures

A final proposal for the conservation of wild populations has been developed and released.

Task 5.2. Inventory of semi-domesticated populations and definition of conservation measures

A proposal for the *in situ* conservation of cultivated and semi-cultivated populations has been developed and released. The approach proposed involves three different components: economic-productive, socio-cultural and ecologic and two transversal components: *in situ* - *ex situ* interaction and promotion and uses of the material.



This strategy is being implemented in Bolivia, Ecuador and Peru resulting n the following activities:

1.1 Ecological component

The ecological component is oriented towards a rational use of the natural resources.

It comprises the following activities:

- Zonification and espatiation of the micrcenters of diversity.
- Inventroy and documentation of diversity in a database based in populations studies and phenotipic and genotipic caracterization.
- Identification and conservation of diversity in local orchards.

1.2 Economic-productive component

The economic-productive component is oriented towards agronomic imporvement and the comercialization of the higher possible number of local genotypes to avoid genetic erosion.

It comprises the following activities:

- Socioeconomic characterization of the micrcenters of diversity.
- Promotion of cherimoya diversity through the cmmercialization of a diverse product.
- Establishment and/or selection of pilot cherimoya orchards.
- Research, promotion and dissemination using a participative approach.
- Networking strenghtening (associations, cooperatives…).
- Promotion of rural microenterprises and establishment of commercial relations with private players for harvesting and commercialization.

1.3 Socio-cultural component

This component is directed towards the dinamization and sensibilization of the local environment to favor the conservation of diversity while maintaining local traditions.

It comprises the following activities:

- Ethnobotanic documentation of diversity.
- Organization of local fairs and markets.
- Capacitation to local farmers and groups of farmers.
- Dissemination to local politicians, cooperatives, unions, etc. of the advantages of conserving and using local diversity

1.4 Transversal component: **ex situ – in situ**

- In order to promote ex situ and in situ conservation and exchange of experiences visits of farmers to national and local germplasm collections are organized

1.5 Transversal component: promotion and use

A main goal of this activity is to promote cherimoya consumption both as fresh and processed products.

Workpackage number: WP6

Identification, evaluation and control of pest damages in cherimoya fruits

Objectives

General objective: To evaluate the damage caused to cherimoya fruits by different insect pests and design sustainable control strategies

Specific objectives: i) Identification of the main pests that affect cherimoya production in the different countries. ii) Evaluation of the economic damage caused by the different pests. iii) Development of biological control and participative selection strategies.

Description of work

- Task 6.1. Identification of the main pests and their relative importance. Previous results have shown that the main pests that affect cherimoya fruits in the Andean countries involved in CHERLA are species of the genera *Anastrepha* and *Ceratitis*. During this task, the main pests affecting cherimoya fruits were evaluated in experimental orchards in different areas of the INCO countries participating in the project. The relative importance of each of them was assessed to establish possible differences in control strategies among countries and/or areas.

- Task 6.2. **Analysis of economic damage**. Taking into account the number and extension of the damages a first estimation of the overall consequences caused by the different pests to cherimoya fruits has been carried out. Damages were expressed as yield, quality and production losses, and financial/economic losses incurred by producers/merchants along the production/commercialisation chain.

- Task 6.3. **Strategies of control: Biological control and participative germplasm selection**. During this task, the possible control strategies have been analysed. Biological and integrated control mechanisms were introduced in pilot cherimoya growing communities

Deliverables

D12. Identification of main pests affecting fruits.
D13. Evaluation of financial economic damage due to pests.
D14. Design of strategies of biological control and participative germplasm selection.

Results

Task 6.1. Identification of the main pests and their relative importance

In Spain, undoubtedly the most important pest is the fruit fly *Ceratitis capitata*.

In Ecuador, the main species that affect cherimoya fruits belong to the genera *Anastrepha* and *Ceratitis* and the data obtained indicate that the most important species in cherimoya is *Anastrepha fraterculus.*

In Peru, INIEA has studied the main pests that affect cherimoya in the germplasm bank of Ayacucho. Fruit fly (*Anastrepha* sp.) is the main pest affecting up to 80% of the fruits but other pests have also been found:

- Migratory locust (*Shistocerca piceifrons peruviana*)
- Lorito (*Diabrotica*)
- Leafminers (*Phyllocnistis* sp.)
- Queresa Marrón (*Parassetia nigra*).
- Coqui (*Atta* sp.)

In Bolivia, the identification of the main pests was carried out in different communities in Comarapa and Saipina. The first results obtained indicate that the following are the main pests found classified according to their relative importance:

1st. Seed borer: *Bephratelloides sp*

2nd. Fruit flies: *Ceratitis capitata* and *Anastrepha* spp.

3rd. Fruit rot: *Cladosporium carpophilum.* This pest causes damage in the fruits especially in regions with a high relative humidity.

4th. Leafminer: *Lyonetia* spp. Their identification is currently being carried out. It seems that the incidence of this pest is increasing

In a literature study, 76 different globally occurring pests of cherimoya were identified. In the process of editing botanical descriptors for cherimoya, the initial list of reported pests has been reduced to the following 37 based on the advice of experts from Bioversity:

Group + Scientific name	Vernacular name	Pest reported in cherimoya by
Scale insects		
Aspidiotus spp.	Coconut scale	Peña & Bennett (1995)
Ceropute yuccae	Coccid	Nava Diaz *et al*. (2000)
Icerya purchasi	Cottony cushion scale	Peña & Bennett (1995)
Lepidosaphes beckii	Purple scale	Peña & Bennett (1995)
Parasaissetia nigra	Nigra scale	Sanewski (1991), Scheldeman (2002)
Parthenolecanium corni	European fruit lecanium	Peña & Bennett (1995)
Pinnaspis aspidistrae	Fern scale	Peña & Bennett (1995)
Planococcus citri	Citrus mealybug	Sanewski (1991)
Pseudococcus filamentosus	Citrus mite	Peña & Bennett (1995)
Saissetia spp.		Sanewski (1991)
Selenaspidus articulatus	Rufous scale	Peña & Bennett (1995)
Unaspis citri White louse scale	Peña & Bennett (1995)	
Whiteflies		
Aleurotrachelus trachoides	Whitefly	Peña & Bennett (1995)
Aphids		
Aphis gossypii	Cotton aphid	Peña & Bennett (1995)

Fruit flies		
Anastrepha spp.	Fruit fly	Bridg (2000), León Fuentes (1999)
Bactrocera tryoni	Queensland fruit fly	Sanewski (1991)
Ceratitis capitata	Mediterranean fruit fly	Morton (1987), Peña & Bennett (1995)
Coleopteran		
Apate monachus	Black borer	Bridg (2000)
Conoderus spp.		Peña & Bennett (1995)
Diabrotica spp.	Corn rootworm	Peña & Bennett (1995)
Lepidoptera		
Cerconota anonella	Annona fruit borer	Bustillo & Peña (1992), Peña & Bennett (1995)
Cocytius antaeus	Giant sphinx	Nava Diaz *et al*. (2000)
Graphium spp.		Sanewski (1991)
Lyonetia sp.	Leaf miner	Peña & Bennett (1995)
Talponia batesi	Seed borer	Nava Diaz *et al*. (2000)
Thecla sp.		Morton (1987), Peña & Bennett (1995)
Wasps		
Bephrata maculicollis	Soursop wasp	Morton (1987), Peña & Bennett (1995)
Bephratelloides spp.	Annona seed wasp	Peña & Bennett (1995)
Thrips		
Thrips tabaci	Onion thrips	Peña & Bennett (1995)
Mites		
Brevipalpus californicus	False spider mite	Peña & Bennett (1995)
Brevipalpus chilensis	Chilean false red mite	Peña & Bennett (1995)
Tetranychus urticae	Two-spotted spider mite	Peña & Bennett (1995)
Nematodes		
Helicotylenchus spp.	Spiral nematode	Bridg (2000)
Meloidogyne incognita	Root-knot nematode	Bridg (2000)
Pratylenchus spp.	Lesion nematode	Bridg (2000)
Tylenchorhynchus spp.	Stunt nematode	Bridg (2000)
Xiphinema americanum	Am. dagger nematode	Bridg (2000)

Task 6.2. Analysis of economic damage

Ceratitis capitata (Villena & Arata, 2004)

Anastrepha fraterculus (Villena & Arata, 2004)

The main conclusions from the analysis are:

i. economic damage to the cherimoya subsector in Ecuador, Peru and Bolivia is at least € 3.9 million;

ii. economic damage to the cherimoya subsector in Spain is at least € 2.3 million

iii. proportional distribution of economic damage between producers and traders is unequal in each of the countries under consideration;

iv. reduction of fruit damage to 12 % by means of better fruit fly control measures, would yield a gross benefit (i.e. not taking production and transaction costs into account) of € 2.2 million, and would reduce economic damage to the cherimoya subsector in the 3 countries under consideration to € 1.7 million;

v. of all 3 Andean countries under consideration, Peru represents the highest share in gross value of cherimoya production and even more in economic damage for producers.

If fruit fly infestation in cherimoya would be reduced dramatically, it is likely to initiate a market reaction in which farm gate and final market prices will rise beyond current levels. This increases potential benefits of control/eradication programs. Moreover, cherimoya is only one of the many Andean fruit crops that suffer from pest infestations. Control and/or eradication of pests will yield economic benefits far beyond € 2.2 million stated in this study, since cherimoya rarely is a monoculture and producers would benefit from improved marketability of other fruit crops as well.

NGO and governmental institutions have the knowledge and skills to implement (improved) pest management programmes.

Results were released as Deliverable 13 “Evaluation of financial economic damage due to pests”.

Task 6.3. **Strategies of control: Biological control and participative germplasm selection**

The whole strategy includes and intensive control of the pest in the whole working area; this includes:

a) Monitoring the populations of the pest.

b) Intensive communication with farmers and capacitacion events.

c) Quarantine protection in some orchards.

d) Control during 12 consecutive weeks (tramps, collection of affected fruits…)

e) Focalized application of tramps and control of larval focuses.

In the case of Peru, at the beginning of the project, a loss of about 56% of the final product due to fruit flies was observed. After the first two years of the project this loss has been reduced to 33% allowing an increase in the amount of good quality fruits in national markets.

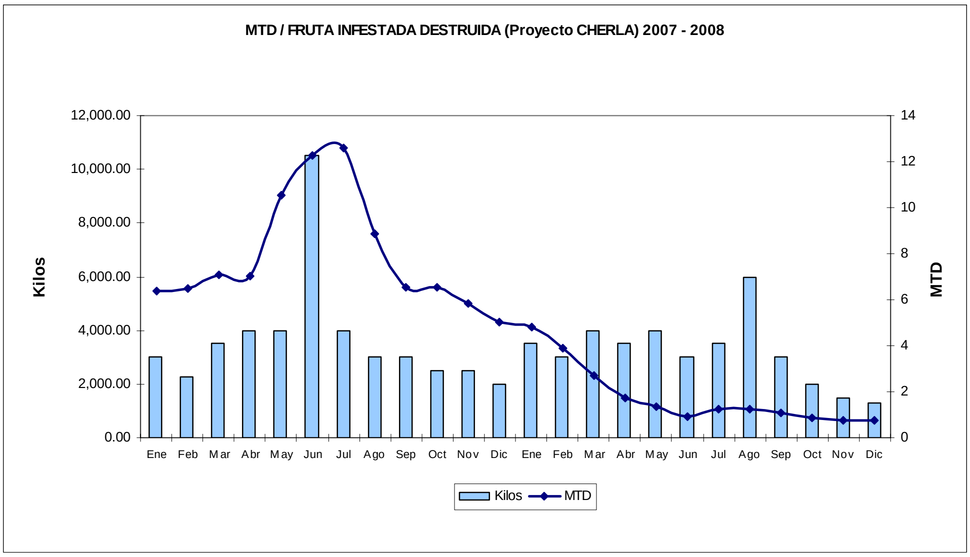
In Bolivia training workshops have been implemented in Independencia, Aiquile and Comarapa in order to familiarize the growers and technicians with the biology and behavior of the different fruit flies species. Integrated management is being implemented mainly: collection and destruction of affected fruits in the soil, control of weeds, tramping with McPhail type tramps and specific controlled chemical treatments in some cases.

In Ecuador an observation made during the development of this wp has been the fact that the tramps used also capture a high proportion of beneficial insects: only about 35% of the insects captured belong to fruit flies. As a consequence, an alternative is under experimentation:

bagging individual fruits. During 2007, about 6000 fruits have been bagged and these fruits will be commercialized during 2008. Additionally, a product approved for organic production (Spinosad) is being studied. All this complemented with better cultural and sanitary techniques can help to highly reduce the incidence of the pest.

In Bolivia, chemical control and packing of fruits with different materials (fabric, paper, plastic) were the two activities that were applied for fruit-fly control. Fruit packing gave the best control. In Bolivia training workshops have been implemented in Independencia, Aiquile and Comarapa in order to familiarize the growers and technicians with the biology and behavior of the different fruit flies species. Integrated management is being implemented mainly: collection and destruction of affected fruits in the soil, control of weeds, tramping with McPhail type tramps and specific controlled chemical treatments in some cases.

In Peru, survey of the pest was done with 71 multilure traps, which attract adults using putrescine, trimetalamine and ammonium acetate as food. Dynamics of the pests relative to that of its host was evaluated during two consecutive years. Only species of the genera *Anastrepha* were reported in Peru, those being *A. fraterculus*, *A. distincta*, *A. atrox* and *A. schultzi*. During the first year the populations increased from May to June because fruits were at maturation, and started to decrease in the following months. In the second year fruit flies density was kept at low levels thanks to the Integrated Pest Management measures applied to the area. In Peru Integrated Management was implemented in 100 ha, and consisted in mechanical control during 18 months, which resulted in a reduction of the pest populations, followed by chemical control with which low values of mtd were obtained.



MTD for the genera *Anastrepha* vs. buried fruits (Kg) in Apurimac – Perú, during 2007 and 2008

In Ecuador an observation made during the development of this wp has been the fact that the tramps used also capture a high proportion of beneficial insects: only about 35% of the insects captured belong to fruit flies. As a consequence, an alternative is under experimentation: bagging individual fruits.

Workpackage number WP7

Cultural techniques and fruit production

Objectives

General objective: To study the main problems in cherimoya reproductive biology and optimize cultural techniques to improve yield and quality of the final product

Specific objectives: i) To study the reproductive biology in cherimoya from pollination to fertilization. ii) To establish the main problems that affect fertilization and yield. iii) To optimize current cultural techniques (pruning, soil management, nutrition, irrigation). iv) Participative selection of the best adapted germplasm to particular conditions.

Description of work

- Task 7.1. Fruiting mechanisms. During this task the reproductive biology of cherimoya was studied in the cultivar Fino de Jete. The results were linked to WP9 that includes the dissemination and training activities.

- Task 7.2. Optimization of cultural techniques and fruit production. With the results obtained in Task 7.1. and with the accumulated knowledge of the different partners of this project it has been possible to recommend the best approaches to optimize sustainable cherimoya production in the different countries. These optimal cultural techniques were applied in pilot cherimoya growing communities, both in existing cherimoya orchards as well as in demonstration plots allowing to fine-tune them to local conditions. The successful introduction of these improved cultural practices also served as an example for other cherimoya growing communities in the neighbourhood. This task was linked to WP9 that includes the dissemination and training activities.

- Task 7.3. **Participative selection**. This task involved the active participation of the local communities in the selection of the best germplasm adapted to the local conditions taking into account the traditional knowledge of the communities involved. Two main producers organizations were involved: the Asociación Integral de Productores Frutícolas AIP-Caballero in Comarapa (Bolivia) whose members had some experience in cherimoya cultivation and PROCAFEQ (Asociación de pequeños productores de café de altura de Espíndola y Quilanga), a producer cooperative in the Loja province in Ecuador close to the Peruvian border that mainly produces organic coffee but that showed a great interest in diversifying their production with crops such as cherimoya.

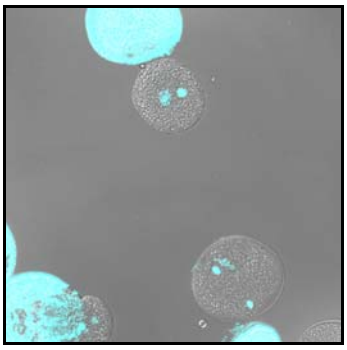
Deliverables

D15. Establishment of main problems in reproductive biology.
D16. Optimization of cultural techniques.
D17. Design of strategies for participative selection of the best adapted genotypes.
D18. Selection of elite material to be introduced in pilot cherimoya growing communities in each country

Results

Task 7.1. Fruiting mechanisms

Pollen viability and pollen tube growth in the pistil was studied in order to establish the main bottlenecks for a good yield. Results indicate that cherimoya produces both bicellular and tricellular pollen grains and that an overlap between the female and male phases can be observed depending on the environmental conditions. The results obtained will have immediate applied consequences to improve cherimoya production.



Bicellular and tricellular cherimoya pollen grains at anthesis

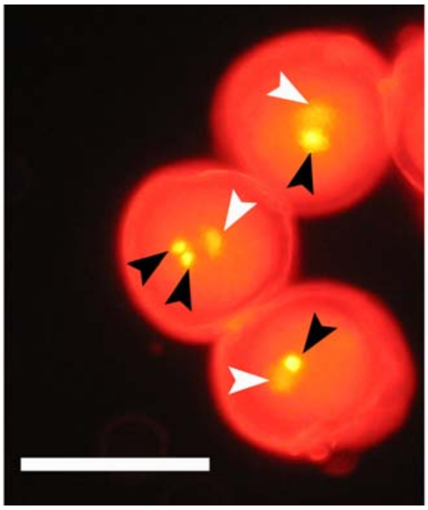
Germinating bicellular cherimoya pollen

The main problems in reproductive biology have been established. For this purpose in a first step reproductive biology has been studied. Pollen and pistil development have been sequentially examined and changes along development have been histochemically characterized. Also the process that goes from pollination to fertilization has been documented. Finally, the influence of environmental conditions -namely temperature and relative humidity- on these processes has been evaluated. These results have been analyzed in relation to two potential scenarios in relation to pollination: with or without pollinator insects. The results have been released as Deliverable 15 "Establishment of main problems in reproductive biology".

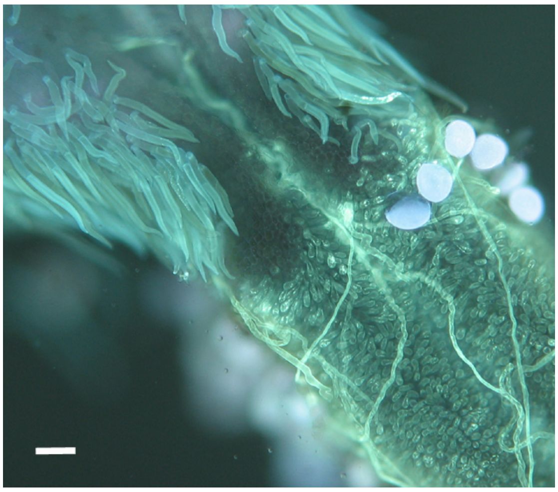
The cherimoya flower -as other Annonaceae flowers- is formed by three external and tree internal petals that host a conical receptacle that holds some 150 pistils surrounded by a whorl of stamens. Also, as in other Annonaceae, cherimoya flowers present protogynous dichogamy. The flower opens as female with receptive pistils and remains in this stage until the next day in the afternoon, when it changes to the male stage: the anthers shed the pollen grains as the petals further widen apart as the pistil is no longer receptive.

Flower development starts 40 days prior to flowering and pollen development elapses from 23 days prior to anther dehiscence. Following meiosis, the young pollen grain experiments a number of changes that are highly conserved in Angiosperms. However, cherimoya pollen has two peculiarities: the first one -shared with other Annonaceae- is that pollen is shed from the anthers in groups of four pollen grains reminiscent of the pollen tetrad. The second one is that upon pollen shedding, pollen is not completely dehydrated and pollen development continues around the time and following anther dehiscence. This together to the fact that the second pollen mitosis occurs close to anther dehiscence, results in the production of a mixed population of bi and tricellular pollen. This proportion appears to be variable depending on the days. An experiment evaluating the effect of temperature in this process shows that pollen mitosis II is temperature dependent and that optimum temperatures are 20-25ºC. Temperatures below 25ºC result in bicellular pollen. The stage of pollen is relevant on its subsequent performance along the reproductive process. The advance developmental stage of tricellular pollen results in a more rapid pollen germination and fertilization. But, also the hydrated condition of pollen difficults pollen conservation, which is a prerequisite for hand pollination. The female part starts development 19 days prior to anthesis. A conical shape receptacle hosts some 150 pistils. At maturity these secretory papillae form a continuous layer recovering the stigmatic surface and continuing down over the style margins down to the ovary through and open stylar canal, forming a continuous conductive surface that guides the pollen tubes in their way to the ovule. The embryo sac is formed at anthesis. The pollen tube reaches the ovule 9 hours after pollination and the first cellular endosperm is apparent three days after pollination. The flowers are receptive in the morning when they attain the female stage and remain receptive until the next day when they are close to change to the male stage. At this point the stigmas are no longer receptive to pollen germination. An evaluation of the effective pollination period though fruit set following sequential hand pollination shows that the effective pollination period last until a few hours prior to change to the male stage. However, stigmatic receptivity is dependent on the prevailing humidity conditions and high humidity of 95 % prolongs stigmatic receptivity for one hour.

While no difficulties have been encountered following pollination a clear problem exists in relation to pollen transport. Two different scenarios have been faced in the different countries and locations of cherimoya cultivation examined in this consortium. The first one refers to areas where cherimoya is native or is well established in semi wild conditions as it can be in areas of Ecuador, Peru and Bolivia. The other refers to areas where this crop is new and has been extended without its natural pollinating agent, as it can be in Spain or in new growing areas in Ecuador, Bolivia and Peru. In these circumstances hand pollination is required to get an adequate crop. This is due to the heterodichogamy of this species with a different maturation of the female and male parts. In this sense it would be worth to evaluate the germplasm gathered in this consortium for possible genotypes with a certain overlap between the male and the female phases. These genotypes would be worth to evaluate in these new growing areas. The other scenario refers to areas where cherimoyas are native or are growing as semi wild. In these areas fruit set occurs in a natural way. However, there is a paucity of information on the possible pollinating agent. In other Annonaceae, Nitidulidae, Curculionidae, Chrysomelidae or Dynastinae beetles have been reported to be the pollinating insect and within this family different species appear to have coevolved with different Annona species depending on the size of the flower. But so far no information is available on the possible pollinating insect for *Annona cherimola* in its natural habitat. Identified this difficulty, an initiative has been launched within the consortium to identify this pollinating agent in the three countries where cherimoyas grow and set fruit without the requirement of hand pollination. A further difficulty has been identified that conflicts this work with another proposed work in this project and this is the fight against the fruit fly that causes fruit rottenness. For this purpose insect traps were used to capture fruit flies. But the identification of the insects that fail in these traps shows that a considerable proportion of beneficial fauna are also captured. More alarming is the fact that a good number of Nitidulidae beetles, which could be the potential pollinating agents, also fall in these traps. Identified this difficulty, an initiative has been launched to further characterize these insects and their potential use as pollinator agents. Whether this information is confirmed an effort will be made to redirect different management practices, as fruit bagging that prevents fruit fly visits, while no interfering with pollinating insects.



The coexistence of bi-tricellular pollen at anther dehiscence



Pollen tubes growing in the stigma to reach the open canal stylar

Task 7.2. Optimization of cultural techniques and fruit production

Most of the activities included in this Task were also included in WP 9 (Training and dissemination of project activities).

In Spain a thorough study of cherimoya cultivation has been made. This can be used as a model for other countries (Ecuador, Peru and Bolivia) where cherimoya cultivation is still little developed. The main points of the study are the following:

- Spain is the first world producer of cherimoyas with about 2700 Has. All the orchards are situated below 600 m.a.s.l. and from 200 meters to 18 km from the coast. Cultivation of cherimoya in Spain takes place in the Southern Spanish Coast between Torrox (province of Malaga) and Motril (province of Granada). In the province of Granada more than 90% of the cultivated extension is present.
- Spanish production peaks between the end of September and the end of December. A small production of low quality takes place in January and February. In March and April there is an additional production of similar quality to that of September-December.
- Total Spanish cherimoya production is about 30000 Tm and most of the production is for the local Spanish market. Exports to the rest of Europe are small (about 8% of the production.
- The productive sector is not well organized and only 5% of the production is commercialized through producers' cooperatives.
- All the producers use advanced techniques such as pruning or hand-pollination. Hand-pollination has allowed to increase yields from less that 5 Tm/ha to more than 12 Tm/ha.

- The main problem of cherimoya production in Spain is the concentration of the production from beginning of October to the end of November, where 75% of the production takes place. This is mainly due to the following causes:
    - Cultivation of just a single variety, Fino de Jete.
    - Short shelf-life of the fruits that complicates storage.
    - The fruits are sold in a low number of markets
    - Variable quality in the commercialized fruits.

In Ecuador, a first study of the current status of cherimoya cultivation has been performed with the following main conclusions:

- Most of the production is obtained with minimum technical inputs with the exception of an orchard in Guayllabamba. The production is mainly consumed in the family of the producer and a low percentage of the fruits (the best) can be sold in local markets.
- Most of the orchards are old and are situated in marginal areas.
- Most of the producers do not know the origin of the trees present in their orchards.
- The health status of the plant is bad and most of the farmers do not have any knowledge on pests and diseases and on how to deal with them.
- Most of the plants are seedlings and grafting is mainly unknown.

In Peru, a first diagnosis of the cultivation conditions has been performed in different orchards in Santa Eulalia, Callahuanca and Cumbe – Lima by Partner 6 (INIEA). The main limitations found are the following:

- Propagation of plants without disinfestation of the material (seeds or the rootstocks, buds of the cultivar, soil).
- Nurseries do not apply sanitary measures to avoid transmission of pests and diseases.
- 90% of the producers in Callahuanca have incorporated pruning technologies, hand pollination and control of fruit fly whereas in Cumbe only 25% have incorporated those strategies.

In Peru, a first pilot orchard has been established in Callahuanca by Partner 6 (INIEA) in order to transfer and demonstrate improved cherimoya cultivation techniques. In collaboration with partner 1 (CSIC) three workshops on cherimoya production techniques with producers and technicians were held in July in Ayacucho, Cajamarca and Cumbe-Lima.

In Bolivia, different training courses were held. Mean topics dealt with included production techniques, production of plants in nurseries, pruning, hand pollination, pest control, commercialization … Courses were organized in 6 communities of the Caballero Province (Pie de la Cuesta, San Juan del Potrero, Bañados del Rosario, Río Juntas, Río San José y San Isidro) with the participation of 66 cherimoya producers of the AIP.

Similarly, in collaboration with partner 1 (CSIC), two workshops on cherimoya production techniques with producers and technicians were held in Comarapa and Aiquile. Producers of other regions were also present at those workshops: Mizque and Independencia (Department of Cochabamba), Vallegrande, Moro Moro, Saipina and El Trigal (Department of Santa Cruz). Moreover, in coordination with local governments, different pruning trials have been performed in Independencia, Aiquile, Comarapa and Saipina on a total of 14000 trees in 34 Ha. Similarly, hand-pollination demonstrations were also performed in Independencia, Mizque, Aiquile, Comarapa and Saipina on a total of 2066 flowers in 474 trees.

The results were released as Deliverable 16 “Optimization of cultural techniques

Task 7.3. **Participative selection**

In Ecuador, in order to encourage participation of the producers in CHERLA, different visits have been performed to the Doña Ana cooperative (Guayllabamba, Pichincha) and to Ayabaca by the local communitarian leaders of Espíndola and Ayabaca (Loja) coordinated by Partner 9 (NCI). Producers and technicians involved in CHERLA have also participated in the workshop that took place in Cajamarca (Peru) on advanced cultural techniques for cherimoya by Partner 6 (INIEA) and 1 (CSIC). These experiences have allowed starting the production of plants in nurseries and the application of pruning techniques by local communities in different areas of the province of Loja.

In Peru, seven promising accessions identified in the germplasm bank and characterized by good yield, good fruit quality and resistance/tolerance to pests and diseases, were introduced in 2004 in farmers orchards in three different agroecological areas in Ayacucho (Luricocha, San Miguel and Río Pampas) coordinated by Partner 6 (INIEA). Evaluations on phenotypical and productive traits are being carried out. The first analyses indicate that statistical differences are present among years, genotypes and genotype x year interactions, rootstock diameter and number of fruits. Genotypes PCHI-236 and PCHI-238 seem to present a better adaptation to the agroecological conditions of Huanta.

In Bolivia, in collaboration with local governments in Independencia, Mizque, Aiquile, Comarapa and Saipina, seven pilot orchards have been identified to perform research and transference of the results involving the local producers and coordinated by Partner 7 (PROINPA).

In Peru seven promissory accessions are being evaluated by INEA (Partner 6); those accessions have been planted in farmer’s orchards in three different agroecological regions in Ayacucho (Luricocha, San Miguel and Río Pampas); the farmers are performing the evaluation and selection of the best materials. Three of them have been selected by the local farmers (PCHI-236, PCHI-238 and PCHI-169). PCHI-238 seems to be the best of the three in agronomic behavior and fruit quality. In northern Peru, prospection and characterization of 190 local genotypes based on the local knowledge was performed by NCI. From those, 28 genotypes were selected for further testing with local producers.

In Bolivia, in order to complement the conservation of the local diversity, an important effort has been devoted during 2007 (PROINPA, Partner 7) to the improvement of cultural techniques (pruning, pollination and pest control) in local orchards with high genetic diversity.

In order to increase the sustainability of the production, in Bolivia (PROINPA, Partner 7) has developed studies in the combined production of cherimoya and annual crops such as beans, tomatoes, maize and other cereals. Additionally, some work has been performed on the production of cherimoya with combined with other fruit tree species such as avocado, citrus, papaya, etc. The main objective of this strategy is to optimize the production in empty spaces, improve the soil texture and an efficient water use, increase the economic profitability of the orchards and maintain the ecological balance. In order to identify the most promising local cherimoya genotypes, participative selection has been performed with local producers in Bolivia with the communities of Comarapa and Saipina. The most discriminant traits evaluated were: lisa or impressa skin, low seed index, high flesh quality, high yield, ºBrix, resistance to penetration and resistance to abrasion.

In Ecuador, this approach has been performed by NCI (Partner 9) with the "Asociación de productores de café de altura de Espíndola y Quilanga" (PROCAFEQ) with the goal of harvesting cherimoyas from trees that provide shadow to the organic café produced in that area.

Similarly, in Ecuador, a detailed prospection in the areas where prospections had been performed in the past was carried out by INIAP (Partner 5) and NCI (Partner 9). A total of 104 additional accessions were studied and, from those, 17 accessions have been selected for further testing with local producers.

In Southern Ecuador and northern Perú two cherimoya production nurseries have been implemented with the local communities by NCI (Partner 9) with the objective of grafting the most promising local accessions. A total of 2500 plants have been produced and given to the local producers.

Despite the short time available to carry out activities on selection, multiplication and introduction of elite material in perennial fruit species, progress during the 3 years of project execution has been significant. All countries have effectively introduced elite material in the selected pilot communities. Over 4000 elite plants have been introduced in 40 communities in the three Andean project countries (Bolivia, Ecuador and Peru). Because of the short time period on which this selection was based in most of the cases (in some areas the selection has been done based on in situ characterisation data) further characterisation and evaluation, optimally with participation of local producers will need to be carried out to confirm the evaluation of the elite material and if needed to eliminate underperforming materials. All project partners have made commitments to follow up on the performance of the introduced material, if possible with new funds, but if needed with institutional funds.

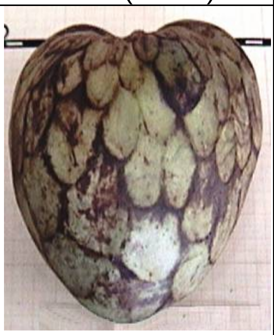
The further introduction and distribution of the selected material will be a good, and easily measurable, parameter to evaluate the impact of this activity and of the CHERLA project in general.

Details from INIA's (Peru) elite material:

Details of PROINPA's (Bolivia) elite material

Accession number	Short Description
Bol 002	Umbonata, little infestation with fruit flies, seed index: 4.2, thin peel, white pulp, loose seeds, soluble solids content: 26 °Brix
Bol 004	Impressa, loose seeds, resistant to abrasion, pulp without defects
Bol 007	Impressa, no infestation with fruit flies, loose seeds, resistant to abrasion, pulp without defect
Bol 008	Impressa, no infestation with fruit flies, loose seeds, resistant to abrasion, pulp without defects, seed index: 3.2, soluble solids content: 20 °Brix
Bol 050	Impressa, no infestation with fruit flies, loose seeds, resistant to abrasion, pulp without defects, soluble solids content: 26 °Brix, seed index: 6, excellent taste
Bol 075	Impressa, loose seeds, resistant to abrasion, pulp without defects, soluble solids content: 27 °Brix, seed index: 4, buttery pulp with good taste
Bol 079	Impressa, no infestation with fruit flies, loose seeds, highly resistant to abrasion, soluble solids content:31 °Brix, seed index: 4.8
Bol 078	Impressa, loose seeds, sensitive to abrasion soluble solids content:22 °Brix, seed index: 4
Bol 038	Umbonata, with fruit flies infestation, semi-cloaked seeds, sensitive to abrasion, thin peel, juicy and white pulp, soluble solids content:25 °Brix, seed index: 8

Details of INIAP's (Ecuador) elite material

CH37 (17503)	CH50 (17507)	CH11 (17514)	CH113 (17461)
			

Details of NCI's (Ecuador) elite material

Code	Photograph
con 4	
con 6	
mac 1	
con 7	
bel 6	
gua 5	
yur 1	
tai 1	

Code	Photograph	Code	Photograph
mus 1		chi 2	
gua 21		bel 4	
caz 8		tai 6	
tai 7		cec 1	
gua 16		con 8	
con 1		mac 2	
cec 10		mus 2	

Workpackage number: WP8

Commercialization and marketing

Objectives

General objective: To improve the approaches used in INCO countries in every step of cherimoya production

Specific objectives: i) Improvement of plant material production in nurseries. ii) Improvement of postharvest and processing procedures. iii) Improvement of strategies of commercialization and fruit production.

Description of work

- **Task 8.1**. **Vegetative propagation and commercialization of selected trees in nurseries**. This activity included the evaluation and improvement of the conditions for vegetative propagation of cherimoya plants. To start with proper plant material including phytosanitary quality conditions is the first step for an optimal and sustainable orchard management and production. The current methods used for vegetative propagation and commercialization of trees in nurseries were evaluated and recommendations for improvement were made. As in Task 7.2 and 7.3 the pilot cherimoya growing communities implemented these cherimoya nurseries and the example is being followed by neighbouring communities.

- **Task 8.2**. **Harvest, postharvest and processing**. During this activity appropriate harvest, postharvest and processing protocols were established and tested in the pilot communities. Keeping in mind the sustainability of the overall production processes, the use of sophisticated equipment (such as controlled atmosphere/temperature chambers) was avoided. Instead the project focused on low-cost and easy-to-manage infrastructure and methods trying to establish minimum basic protocols that can improve substantially the quality of the final product.

- **Task 8.3**. **Fruit commercialization and marketing**. Depending on the areas/countries involved fruit commercialization and marketing took place at the very local or regional level. The situation was evaluated in a case by case basis and specific recommendations were made for each area trying to strengthen cherimoya producer associations and their connexion to selling points at the local and national levels. Bottlenecks were identified and strategies to improve commercialisation were defined and tested.

Deliverables

D19. Improved techniques for nurseries.
D20. Establishment of minimum protocols for appropriate harvest, postharvest and processing.
D21. Optimization of fruit commercialization and marketing.

Results

Task 8.1. **Vegetative propagation and commercialization of selected trees in nurseries**.

World-wide information on cherimoya rootstocks is very scarce. Traditionally, Spain has used the seedling rootstocks of the Spanish most common cultivar (98 % of the cultivated area), i.e the cv. Fino de Jete. The production of grafted plants of cherimoya used to be done in the field, mainly at the edges of the commercial plots. Growers used to transplant trees from seeds that had sprouted underneath the adult tree. When these sprouts reached the adequate diameter size, they were grafted and, once they had rooted after winter of the next year, they were transplanted in the soil at the final location. In the last 30 years, the technique of plant production in polyethylene bags has been developed; this has permitted the commercial planting at any time of the year, it has reduced plantation costs and there is also a reduction in the number of plants lost.

Under the Spanish climatic conditions, the commercial propagation of cherimoya can be done outdoors. Nevertheless, it is advisable to do it under mesh or plastic because plants develop faster.

The substratum blend needs to be loose. Alter preparing the blend, it has to be disinfected. Solarization is the cheapest and simplest method to disinfect soils. Mesh of black polyethylene or polypropylene can be used to cover the soil of the nursery. Mesh of polypropylene lasts for longer. This will hamper the invasion of weeds and the rooting of the propagated plants. To facilitate the maintenance of nurseries, it is recommended to install fixed irrigation.

Once seeds are taken out from fruits, the empty seeds must be discarded. Seeds must be sowed at c.a 1 cm deep. After 3-4 weeks they will germinate.

It is absolutely necessary that bags are well drained.

The following year the plant will be ready to be grafted using any of the different grafting methods useful for cherimoya. All this is explained with more detail in Deliverable 19, D19 “Improved techniques for nurseries”.

Task 8.2. Harvest, postharvest and processing

In Bolivia with the AIP Caballero different packaging boxes have been studied. As a result, wood boxed of 10 Kg. have been selected as the best for local transportation. The AIP Caballero is now using this type of packaging in different local and national markets. The results have been transferred to the other countries participating in the project to perform a similar strategy.

In Bolivia different processing methods have been tried and they could be extended to other producing countries:

Frozen pulp

This process is already standardized and it is easy to perform.

Cherimoya jam

Although cherimoya jam has a good flavor, it is still necessary to improve the process since the color is brown; work is under way to optimize the process mainly by adding sugar when the pulp has at least 20 ºBrix.

Pasteurized pulp

The Appert method was used to obtain pasteurized pulp with a pasteurization time of 35 min. for 450 gr glass recipients. After the first trials the presence of microorganisms was detected and in the following trials the pH was reduced by adding citric and ascorbic acids; this resulted in the loss of the typical cherimoya flavor. Thus, work is under way to sterilize the product changing the recipients to withstand higher temperatures or, alternatively, playing with the acid composition.

Task 8.3. **Fruit commercialization and marketing**.

Details can be found in the Deliverable D21. The main conclusions of this work are the following:

Value chain analysis was approached by means of 4 topics: market channels, governance, quality performance and value added distribution. It was shown that within the Andean cherimoya value chain, two channel types are clearly distinctive: Cumbe cherimoyas, produced in the Lima region in Peru, and ‘domestic’ cherimoyas produced elsewhere. The Cumbe cherimoya value chain was identified as the most competitive of the two.

Regarding market channels, Cumbe cherimoyas are the exception to the rule that cherimoyas remain within the producing countries. Cumbe cherimoyas are found on markets in all considered Andean countries and there value chain is typically longer (including more intermediaries) than those of domestic cherimoyas. The latter are distributed to local village markets or to the nearest domestic city (wholesale and retail) markets.

Governance regimes are generally weak, but nevertheless stronger with Cumbe cherimoyas than with domestic cherimoyas. Trust in the Cumbe chain is higher, indicating that delivered quality of Cumbe cherimoyas better complies with quality standards set by traders and consumers than quality of domestic cherimoyas.

Consequently, quality performance of Cumbe cherimoyas is also better than quality of domestic cherimoyas. It was shown, however, that on farm quality is not distinctively better for Cumbe cherimoyas in comparison with domestic cherimoyas. Intensive grading and selection at wholesale markets (mostly in Lima), where vast volumes of Cumbe cherimoyas are trades, yields an outstanding quality further along the value chain. Organoleptic quality of cherimoya fruits is highly praises by Andean consumers, but they nevertheless have certain expectations towards various aspects of cherimoya quality.

Consumers have these expectations because cherimoya is among the most expensive fruits at all Andean markets. Consumers are willing to pay these elevated prices, but demand exquisite quality for it. Due to its distinctive quality, prices along the whole value chain of Cumbe cherimoyas are higher than prices of domestic cherimoyas. Distribution of value added is quite even; middlemen are not the supposed exploiters. For the Cumbe chain, highest share in value added is taken by producers. Nevertheless, also for them the general rule of uncertain markets is valid. Therefore, crop diversification is also for them a necessity.

In general, producers from the Lima region profit from different rents of which organisational (in this case 'location') rent. Proximity to the Lima metropolis entails a better education, better access to extension services and farm implements, stronger horizontal and vertical integration and reduced transport costs. Although producers from the Lima region are undoubtedly better of than other Andean producers, they should still be regarded as 'rural poor' since they continue to operate in a rural context in a developing country.

To overcome the identified constraints, value chain actors or chain service providers can engage in:

Producers

– associate in farmer groups to improve negotiation power in the chain and to have better access to farm implements and extension service;

– adopt knowledge on adequate cherimoya cultivation practices: irrigation, fertilization, pruning, crop protection and artificial pollination;

– improve genetic material of their cherimoya orchards by means of selected grafts; thereby taking into account that locally selected germplasm will most likely best adapt to local environmental conditions; thus avoiding genetic erosion;

– seek denominations of origin in order to protect local germplasm and to certify distinctive quality from a certain region;

Middlemen

– improve grading and selection in order to assure higher prices further along the value chain;

– provide adequate packaging for (long distance) transport to wholesale markets.

Wholesalers and retailers

– install a market information system in order to make quality standards more clear to middlemen and producers;

– improve cherimoya storage conditions (adequate packaging, covered storage houses and limited handling in order not to bruise the sensitive fruit peel);

Ice-cream parlours

– engage in product development, based on cherimoya (yoghurt, juice, pastries, etc.);

– seek possibilities for market development beyond Bolivia;

NGOs and governmental development institutes

It is clear that – especially for producers – the aforementioned recommendations can not be achieved without the help of NGO and/or government development institutes. It would be naïve to assume that actors in the cherimoya value chain will ever get to know or read the present report. NGOs and governmental institutes form a bridge between compiled scientific results and its application in the field. These organisations play a vital role in:

– extension to producers, in order to transfer innovative cropping technologies;

– allow access to improved planting/grafting material;

– assist in the formation of farmer groups;

– initiate pilot projects for improved cherimoya commercialization (organised collection, initial packaging, denomination of origin and set up of a financial system);

The Bolivian NGO PROINPA and the Ecuadorian NGO NCI prove that such pilot projects can have significant results.

Government

– enhance support to scientific research on underutilized species and local plant genetic resources;

– improve the legal framework for protection of denominations of origin;

– find a politically acceptable equilibrium between protection of plant genetic resources and assuring sufficient access to genetic resources for the benefit of the rural poor;

– invest in road and market infrastructure.

The optimization of the commercialization has allowed to develop specific labels linked to cherimoya origin in the different countries.

Spain:

Bolivia:

Ecuador:


chirimoya "Loja"
100% Orgánico
Con la compra de este producto está contribuyendo a mejorar las condiciones de vida de los pequeños productores de Espíndola, Quilanga y Paltas.
CHERLA
NATURALEZA & CULTURA
INFORMACION Y PEDIDOS
Naturaleza y Cultura Internacional • 2573691 • Procafeq: 092095905


Chirimoya "Loja"
pedidos: 2573691 / 092095905

Peru:


CHIRIMOYA
CUMBE

Workpackage number: WP9

Training and dissemination of project activities

Objectives

General objective: To disseminate information on the activities and results obtained in the project

Specific objectives: i) To maximise the exploitation of the results obtained, to ensure the circulation of reports, papers, methodology and guidelines. ii) Recommendations for farmers and technicians to enhance the technical, economical and social profitability and sustainability of cherimoya production. iii) Training of young professionals and students involved in the project.

Description of work

- Task 9.1. **Establishment and maintenance of the CHERLA web site**. This was carried out in Spanish and English for the permanent dissemination of the project progress and final results. The web page was updated at least every 6 months.

- Task 9.2. **Elaboration of support materials for training workshops**. These support materials were used in the field days and local workshops designed to transfer the applied results to farmers, associations, NGOs, local and regional authorities.

- Task 9.3. Elaboration of cherimoya management guides for farmers and technicians. These guides summarized the recommendations for efficient cherimoya sustainable production.

- Task 9.4. Specific training for young professionals and students. Young researchers and professionals were encouraged to join the different Research Centres of the Consortium for specific training in different subjects.

- Task 9.5. **Preparation and submission of scientific paper and communications**. Scientific papers were prepared and submitted to relevant peer-reviewed scientific and technical journals. Relevant results were also be presented in international meetings and workshops.

Deliverables

D22. Establishment and maintenance of the CHERLA internet web site.
D23. Elaboration of support materials for training workshops.

D24. Elaboration of cherimoya management guides for farmers and technicians.

D25. Training courses for young professionals and students.

D26. Preparation and submission of scientific papers and presentations in appropriate meetings and workshops.

Results

Task 9.1. Establishment and maintenance of the CHERLA web site

At the end of 2006 the CHERLA web site was already operative (www.cherla.com). Initially only in Spanish, in the second year two additional modules were constructed, one in English and the other in Quechua. This increased the impact of the project on the local communities.

During 2007, we have recorded (www.globalaudit.com) a total of 2490 external visits to the website, with an average of 13 visits per day. Visits from 45 different countries have been recorded; the country with the highest number of visits is Spain followed by Peru, Belgium, USA, Ecuador, Bolivia, Chile, Colombia and Argentina. During 2008, we have recorded (www.globalaudit.com) a total of 4317 external visits to the website with an average of 14 visits per day, compared to 2490 external visits in 2007, with an average of 13 visits per day. Visits from 45 different countries have been recorded; the country with the highest number of visits is Spain followed by USA, Ecuador, Peru, Bolivia, Italy, UK, France, Belgium and Chile.

Task 9.2. Elaboration of support materials for training workshops

Support materials have been elaborated and distributed during the different workshops and field days organized by the partners participating in the CHERLA consortium.

Task 9.3. Elaboration of cherimoya management guides for farmers and technicians

These support material have been elaborated for each of the training workshops performed.

The following describes the main courses and workshops that have been carried out:

2006.

Peru (Partner 5, INIEA):

1. Advanced techniques in cherimoya cultivation. Participation of about 200 producers and technicians. (in collaboration with Partner 1, CSIC). July 2006:

17.07.06	Huanta – Ayacucho, about 80 participants
19.07.06	Cajamarca – San Juan, about 40 participants
21.07.06	Santa Eulalia – Lima, about 120 participants

2. Training courses for producers, technicians and professionals in cherimoya producing areas of Ayacucho: nursery management, grafting, planting, irrigation, pruning, fertilization.

3. Training courses for producers, technicians and professionals in Ancash – Huaraz.

4. During the collections trips, talks and advice have been given to producers in 5 regions (Piura, Cajamarca, La Libertad, Lima and Ancash).

5. Training of producers and technicians in pruning, hand pollination, fertilization and pest and disease control in a pilot orchard in Callahuanca.

6. Training on molecular characterization of students of the Universities of La Molina, San Marcos, San Agustín - Arequipa, Pedro Ruiz Gallo – Lambayeque and Ricardo Palma.

7. Visit and training of a technician of INIEA in the E.E. la Mayora in Spain (Partner 1): cherimoya, avocado and mango cultivation techniques.

Bolivia (Partner 7, PROINPA):

Two local cherimoya fairs were organized in Comarapa and Independencia. These fairs were useful for the exchange of experiences among farmers, technicians and researchers. Six training courses were given to a total of 66 cherimoya producers of the AIP Caballero. Several talks and meetings were performed with local authorities and producers organizations.

Similarly, in collaboration with partner 1 (CSIC) two workshops on cherimoya production techniques with producers and technicians were performed in Comarapa and Aiquile. Producers of other regions were also present in those workshops: Mizque and Independencia (Department of Cochabamba), Vallegrande, Moro Moro, Saipina and El Trigal (Department of Santa Cruz). A total of 160 persons assisted to the 2 workshops and through radio media the information reached a large number of producers.

As a complement to these tasks, different fairs with local producers have been taken place with the objective of promoting local cherimoya diversity. These events result in an active exchange of experiences among different producers and in the exchange of plant material helping to conserve the local diversity.

In these fairs demonstration of fresh and processed (ice cream, jam, spirits, etc.) cherimoya products took place. One of the fairs was at the binational level (Ecuador and Peru) and took place in the Ecuadorian side of the border; during 2008 a similar fair will take place in the Peruvian side and the objective is to maintain this annual celebration.

Local fairs also took place in different areas in Bolivia:

2007.

Country	Event	Partner	Place	Participants
Ecuador	Workshop on cultivation techniques	INIAP CSIC	Hostería San Alejandro, Perucho	45 direct
	Exchange of experiences in cherimoya cultivation	NCI CSIC	Loja	58 direct
	Workshop on cherimoya fruit transformation	NCI	Catacocha, Amaluza, Conzapamba	100 direct
	Exchange of experiences in cherimoya cultivation	NCI	Vilcabamba Amaluza	30 direct
	I Binational Fair on cherimoya	NCI	Amaluza	84 direct 200 indirect
Peru	Exchange of experiences in cherimoya cultivation	NCI CSIC	Ayabaca	56 direct
	Workshop on cherimoya fruit transformation	NCI	Ayabaca	48 direct

	Exchange of experiences in cherimoya cultivation	NCI	Ayabaca	20 direct
Bolivia	Techniques to improve cherimoya production	PROINPA	Comarapa and Saipina	150 direct 300 indirect
	Techniques to improve cherimoya production	PROINPA	Aiquile and Mizque	80 direct 300 indirect
	Techniques to improve cherimoya production	PROINPA	Comarapa	6 direct
	Techniques to improve cherimoya production	PROINPA	Independencia	50 direct 200 indirect

As described below different fairs and workshops with local producers have been taken place in the different countries with the objective of promoting local cherimoya diversity and appropriate cultural techniques. These events result in an active exchange of experiences among different producers and in the exchange of plant material helping to conserve the local diversity.

2008.

Country	**Event**	**Partner**	**Place**	**Participants**
Bolivia	Capacitation to cherimoya producers in cherimoya cultivation techniques	PROINPA	Comarapa	20 direct
Bolivia	Capacitation course in integrated fruit fly management	PROINPA	Comarapa	30 direct 30 indirect
Bolivia	Meeting with local Comarapa Council to discuss proposals to continue CHERLA activities	PROINPA	Comarapa	15 direct
Bolivia	Meeting with the Program Alianza Rural PAR to discuss proposals to continue CHERLA activities	PROINPA	Santa Cruz	6 direct
Perú	Course “Spatial, morphological and molecular data analyses of the National Germplasm Collections of SUDIRGEB – INIA”	INIA	SUDIRGEB - INIA, Lima (23-28/06/08)	8 direct 20 indirect
Perú	Workshop: identification of main capacitation needs of cherimoya cultivation in the Callahuanca, Santa Eulalia and San Mateo de Otao valleys	INIA, SENASA	Municipalidad de Callahuanca y Agencia Agraria de Santa Eulalia	25 direct 120 indirect

Task 9.4. Specific training for young professionals and students

Some changes have taken place in the original schedule for courses. We have centered in performing workshops and courses with producers and technicians and delay the courses for young professionals and students to the last part of the project once results are available. Thus, a course was performed in Cali (Colombia) coordinated by partner 4 (IPGRI) with the goal of getting a consensus on the analyses of the diversity of cherimoya in the different countries participating in CHERLA.

In addition to these specific courses, training of professionals and students is also being carried out through the stay in different groups of the consortium. Thus, professionals and students from Peru (Partner 6, INIEA) and Ecuador (Partner 5, INIAP), have been trained in the E.E. la Mayora (Partner 1, CSIC) in cultivation techniques and molecular tools, respectively. This exchange of professionals and students will continue during the whole duration of the project.

Ph.D. theses

In Spain, one Ph.D. thesis was presented in 2007: “Molecular characterization and diversity studies in cherimoya”, Pilar Escribano and one Ph.D. thesis was presented in 2008: “Reproductive biology and fruit set in cherimoya (*Annona cherimola* Mill.), Jorge Lora.

Master theses

In Belgium, 2 master theses were presented in 2008 by Partner 3 (UGHENT):

- Aileen Dhondt. "Marketingplan voor cherimoya (*Annona cherimola* Mill.) in de centraal-oostelijke Andesvalleien van Bolivia"

- Annelies Vermeersch. "Ketenanalyse en marketingplan voor cherimoya (*Annona cherimola* Mill.) in de Altiplano en de noordoostelijke Andesvalleien van Bolivia"

Other theses

Bolivia:

Cáceres, A. 2008. Estudio de la diversidad genética de 198 accesiones de chirimoya (*Annona cherimola*) utilizando marcadores moleculares. Tesis de licenciatura de la Universidad Mayor de San Simón UMSS.

Peru:

Farfán N. 2009. Estudio comparativo de la variabilidad genética de 145 accesiones de chirimoyo cultivado “*Annona cherimola*” del banco de germoplasma del INIA y 122 muestras de chirimoyo silvestre de 5 regiones del país utilizando marcadores moleculares SSR. Tesis para optar el título de Ingeniero Agrónomo por la Universidad Nacional San Antonio de Abad del Cusco. Tesis sustentada satisfactoriamente.

Pinedo E. 2009. Análisis de Diversidad Genética de la Colección Nacional de Germoplasma de *Annona cherimola* Mill. conservada en la estación experimental Canáan -INIA mediante el uso de marcadores microsatélite”. Tesis de licenciatura en Biología de la Universidad Nacional Agraria La Molina.

Ecuador

Castillo, J. 2008. Evaluación de la entomofauna ligada a la producción de chirimoya, con énfasis a Mosca de la Fruta. UTPL – NCI

Robles, H., 2008. Caracterización morfológica de Chirimoya en la Provincia de Ayabaca. UNP – NCI.

Alguacil, G. (2008) Biología floral en chirimoyo. Ciclo floral e insectos polinizadores. U. Gante – NCI

Willemys, K. (2008) Evaluación de 3 sistemas de defoliación en la fructificación. U. Gante – NCI

Gonzaga, M. (2008) Manejo de cosecha y poscosecha de chirimoya. UNL – NCI.

Task 9.5. **Preparation and submission of scientific paper and communications**

Scientific papers

Chatrou, L.W., M. P. Escribano, M.A. Viruel, J.W. Maas, S. Wink, J.E. Richardson, J.I. Hormaza (in press) Microsatellite flanking regions provide a new source of data for plant species level phylogenetics. *Molecular Phylogenetics and Evolution*

Escribano, P., M.A. Viruel, J.I. Hormaza (2007) Molecular analysis of genetic diversity and geographic origin within an ex situ germplasm collection of cherimoya by using SSRs. Journal of the American Society for Horticultural Science 132: 357.367.

Escribano, P., M.A. Viruel, J.I. Hormaza (2008) Development of 52 new polymorphic SSR markers from cherimoya (*Annona cherimola* Mill.). Transferability to related taxa and selection of a reduced set for DNA fingerprinting and diversity studies. Molecular Ecology Resources 8: 317 – 321.

Escribano, P., M.A. Viruel, J.I. Hormaza (2008) Comparison of different methods to construct a core germplasm collection in woody perennial species with SSR markers. A case study in cherimoya (*Annona cherimola* Mill.), an underutilized subtropical fruit tree species. Annals of Applied Biology 153: 25 – 32

Lora, J., M. Herrero, J.I. Hormaza 2007) Germinación de polen de chirimoyo. Implicaciones para la optimización de la polinización manual. Actas de Horticultura 48: 134-136

Lora, J., M. Herrero, J.I. Hormaza (2009) The coexistence of bi and tricellular pollen in *Annona cherimola* Mill. (Annonaceae): Implications for pollen evolution. *American Journal of Botany* 96: 802-808.

Lora, J., M.P. Testillano, M.C. Risueño, M. Herrero, J.I. Hormaza (in press) Pollen development in *Annona cherimola* (Annonaceae). Implications for the evolution of aggregated pollen. *BMC Plant Biology*

Lora, J., J.I. Hormaza, M. Herrero (in press) Pollen tube pathway and pollen-pistil interaction in a primitive angiosperm, *Annona cherimola* Mill. (Annonaceae). *Annals of Botany*

Vanhove, W. & Van Damme, P. (in press). Marketing of Cherimoya in the Andes for the Benefit of the Rural Poor and as a Tool for Agrobiodiversity Conservation. Acta Horticulturae

Vanhove, W. & Van Damme, P. (submitted). Segmentation of Andean cherimoya (*Annona cherimola* Mill.) consumers as a strategy to relieve underutilization in its centres of diversity. *Fruits*.

Vanhove, W., Speelman, S., D'Haese, M. & Van Damme, P. (submitted). Technical Efficiency of Andean cherimoya farms and its determinants. *Agricultural Economics*.

Technical papers

Garcia, W., Guzmán, B., Hermoso, J., E. Guirado. 2008. Sistemas de poda el cultivo de chirimoyo En Revista de Agricultura. Año 60 No. 42. Separata Técnica Coleccionable 01-2008. Cochabamba, Bolivia. 4p.

Garcia, W., Guzmán, B., Hermoso, J., E. Guirado. 2008. Polinización en el cultivo de chirimoyo En Revista de Agricultura. Año 60 No. 44. Separata Técnica Coleccionable 02-2008. Cochabamba, Bolivia. 4p.

Lino, V., Guzmán, B., Barera, O., W. García. 2009. Plagas en el cultivo de chirimoyo En Revista de Agricultura. Año 60 No. 45. Separata Técnica Coleccionable 01-2009. Cochabamba, Bolivia. 4p.

García, W.; Guzmán, B.; Lino, V.; Hermoso, J.M.; Guirado. E.; Scheldeman, X. & J.I. Hormaza. 2009. Manual de Manejo Integral del Cultivo de Chirimoyo. Fundación para la Promoción e Investigación de Productos Andinos (PROINPA), Consejo Superior de Investigaciones Científicas (CSIC), Bioversity International, Proyecto "Fomento de sistemas sustentables de producción de chirimoyo en América Latina mediante la caracterización, conservación y utilización de la diversidad del germoplasma autóctono" (CHERLA), Unión Europea. Cochabamba, Bolivia, 52 p.

Tineo, J. 2009. Manejo del cultivo del chirimoyo en de sierra del Perú. Under review.

Valverde, B.; Guidi, A.; Guzman, B. & W. García. 2009. Recetario de chirimoya. Fundación para la Promoción e Investigación de Productos Andinos (PROINPA). Cochabamba, Bolivia, 12 p.

Contributions in scientific meetings

Cortez-Alvarez, R., A. Cáceres, W. García, B. Guzmán, J.A. Rojas (2007) Avance en la caracterización de la chirimoya (*Annona cherimola* Mill.). Evento: V Reunión nacional de Biotecnología – Red Bio Bolivia. La Paz, Bolivia.29-31 August 2007.

Escribano, P., J.I. Hormaza, M.A. Viruel (2007) A first SSR partial linkage map in cherimoya (*Annona cherimola* Mill., Annonaceae). The 2nd International Conference on Plant Molecular Breeding. Sanya, Hainan, China. March.

Escribano, P. M.A. Viruel, J.I. Hormaza (2007) Application of SSR markers for cultivar fingerprinting, diversity evaluation and breeding in cherimoya (*Annona cherimola* Mill.), Annonaceae. XV Plant & Animal Genome conference. Book of abstracts, pp. 133. San Diego, USA. January

Escribano, P., M.A. Viruel, J.I. Hormaza (2007) Caracterización molecular y diversidad genética del banco de germoplasma de chirimoyo de la E.E. la Mayora. XI Congreso Nacional de Ciencias Hortícolas. Libro de abstracts 52. Albacete, Spain. 24-27 April.

Escribano, P., M.A. Viruel, J.I.Hormaza (2007) Establishment of a core collection to optimise the conservation of cherimoya (*Annona cherimola* Mill.) genetic resources using SSR information. Eucarpia. XII Fruit Section Symposium. Book of abstracts, 30. Zaragoza, Spain, 16-20 September 2007

García, W. (2007) Producción de chirimoyo en Bolivia: recursos genéticos y técnicas de manejo del cultivo. Congreso Sudamericano de Agronomía y Seminario Nacional de Fruticultura. 10-12 October 2007. Universidad Mayor de San Simón, Cochabamba Bolivia.

González-Fernández, J.J., J.M. Hermoso, E. Guirado, A. Pérez de Oteyza, J.M. Farré, J.I. Hormaza (2008) Investigación con material vegetal de especies subtropicales en la E.E. la Mayora (CSIC). *VII Jornadas de Experimentación en Fruticultura*. La Tallada, Gerona. 28-30 may, 2008

Hormaza , J.I. (2008) Sociedad Española de Ciencias Hortícolas. Córdoba, Spain. 12 june. Estudios de genómica de chirimoyo. Conference and participation in the roundtable "El papel de la genómica en la mejora de especies hortofrutícolas cultivadas".

Hormaza , J.I. (2008) Mauritius University. Réduit. Mauritius. 9 october. Molecular studies in orphan fruit crops. A case study in cherimoya (*Annona cherimola* Mill.)

Lora, J., M. Herrero, J.I. Hormaza (2007) Germinación de polen de chirimoyo. Implicaciones para la optimización de la polinización manual. XI Congreso Nacional de Ciencias Hortícolas. Libro de abstracts 53. Albacete, Spain. 24-27 April.

Lora J., M. Herrero, J.I.Hormaza (2007) Outcrossing rate and stigmatic receptivity in cherimoya (*Annona cherimola* Mill., Annonaceae) a dichogamous subtropical fruit tree species. Eucarpia. XII Fruit Section Symposium. Book of abstracts, 154. Zaragoza, Spain. 16-20 September.

Lora, J., J.I. Hormaza, M. Herrero (2008) Final pollen development and pollen-pistil interaction in a primitive angiosperm, *Annona cherimola* Mill. (Annonaceae). *XX International Congress on Sexual Plant Reproduction*. Brasilia, Brazil. Documentos 259: 176-177. 4-8 august, 2008

Pinedo, E.; Aquino, Y.; Rimachi, F.; Sigüeñas, M.; Benavides, J.; Vivanco W. 2008. Artículo Distribución geográfica de la variabilidad genética de *Annona cherimola* Mill. Presente en la colección nacional de germoplasma- INIA. 13vo Congreso Latinoamericano de Genética, 4-7 may 2008. Lima – Perú. http://www.13congresolatinoamericano.spg.org.pe/martes6.html

Rimachi, F.; Aquino, Y.; Sigüeñas, M.; Benavides, J.; Vivanco W. 2008. Análisis de la diversidad genética de poblaciones semicultivadas de Annona Cherimola Mill ‘Chirimoyo mediante marcadores SSR.13vo Congreso Latinoamericano de Genética, 4-7 may 2008. Lima – Perú. http://www.13congresolatinoamericano.spg.org.pe/miercoles7.html

Rimachi, F.; Aquino, Y.; Pinedo E. y Sigüeñas, M. (2009). Análisis de la diversidad genética de poblaciones semicultivadas de *Annona cherimola*. Mill “chirimoyo” mediante marcadores SSR. Encuentro Científico Internacional 2009 de verano, ECI 2009v., 6 – 9 january 2009. Consejo Científico Tecnológico Internacional del ECI. http://www.encuentrocientificointernacional.org/ECI2009v/20081208ECI2009vresumenesaceptados.html#Biologicas

Vanhove, W. & Van Damme, P. (2007). Value Chain Analysis of Cherimoya in Ecuador, Peru and Bolivia. Poster presented at the congress: Value Chains for Pro-Poor Development. GTZ Conference, 30-31 May, Berlin.

Vanhove, W. & Van Damme, P. Marketing of cherimoya in the Andes for the benefit of the rural poor and as a tool for agrobiodiversity conservation. ICUC/Bioversity-organized International Symposium *Underutilized plants for food, nutrition, income and sustainable development*, held in Arusha, Tanzania, 3-7 March 2008

Viruel M.A., P. Escribano, J.I. Hormaza (2008) Genetic diversity and molecular breeding in cherimoya (*Annona cherimola* Mill.): an underutilized subtropical fruit tree species. *XVI Plant & Animal Genome conference*. San Diego, EE.UU. Book of Abstracts W203. 12-16 january, 2008

Books

Bioversity International y CHERLA (2008) Descriptors for cherimoya (*Annona cherimola* Mill.). Descriptores para chirimoyo (*Annona cherimola* Mill.) (Bioversity International, Roma, Italia; CHERLA project, Málaga Spain. ISBN: 978-92-9043-779-6 (english) and 978-92-9043-780-2 (spanish).