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DISTRIBUTION AND POTENTIAL OF CHERIMOYA
(*ANNONA CHERIMOLA* MILL.) AND HIGHLAND
PAPAYAS (*VASCONCELLEA* SPP.) IN ECUADOR

VERSPREIDING EN POTENTIEEL VAN CHERIMOYA
(*ANNONA CHERIMOLA* MILL.) EN
HOOGLANDPAPAJA'S (*VASCONCELLEA* SPP.) IN
ECUADOR

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TABLE OF CONTENTS

| | |
|---|----------|
| Abbreviations and Acronyms..... | VIII |
| Summary | X |
| Samenvatting..... | XIV |
| Resumen | XIX |
| | |
| 1. Introduction | 1 |
| | |
| 2. Literature Review | 4 |
| 2.1 Study Area..... | 5 |
| 2.1.1 Ecuador | 5 |
| 2.1.1.1 General Characteristics | 5 |
| 2.1.1.2 Resources..... | 7 |
| 2.1.1.3 Biodiversity | 8 |
| 2.1.2 Loja Province..... | 9 |
| 2.1.2.1 General Characteristics | 9 |
| 2.1.2.2 Geology and Geomorphology | 10 |
| 2.1.2.3 Pedology..... | 11 |
| 2.1.2.4 Climate..... | 12 |
| 2.1.2.5 Biotic environment | 15 |
| 2.1.2.6 Economy and Agriculture | 18 |
| 2.2 Cherimoya (<i>Annona cherimola</i> Mill.)..... | 20 |
| 2.2.1 Botany | 20 |
| 2.2.1.1 Family Annonaceae..... | 20 |
| 2.2.1.2 Genus <i>Annona</i> | 22 |
| 2.2.1.3 <i>Annona cherimola</i> Miller | 23 |
| 2.2.2 Origin | 25 |
| 2.2.3 Cultivation..... | 25 |
| 2.2.3.1 Propagation | 25 |
| 2.2.3.2 Cultivation Practices and Cropping | 27 |
| 2.2.3.3 Pollination | 29 |
| 2.2.3.4 Pests and Diseases..... | 30 |
| 2.2.3.5 Cultivars..... | 31 |
| 2.2.3.6 Breeding | 32 |
| 2.2.3.7 Post-Harvest..... | 33 |
| 2.2.4 Crop Ecology..... | 34 |
| 2.2.5 Commercialisation..... | 35 |
| 2.2.6 Nutritional composition..... | 36 |
| 2.2.7 Uses | 37 |
| 2.2.8 Situation of Cherimoya in Ecuador | 37 |

| | |
|---|-----------|
| 2.3 Highland Papayas (<i>Vasconcellea</i> spp.) | 39 |
| 2.3.1 Botany | 39 |
| 2.3.1.1 Family Caricaceae | 39 |
| 2.3.1.2 Genus <i>Vasconcellea</i> | 40 |
| 2.3.1.3 <i>Vasconcellea cundinamarcensis</i> V.M. Badillo | 41 |
| 2.3.1.4 <i>Vasconcellea stipulata</i> (V.M. Badillo) V.M. Badillo | 42 |
| 2.3.1.5 <i>Vasconcellea</i> × <i>heilbornii</i> (V.M. Badillo) V.M. Badillo | 43 |
| 2.3.1.6 Interspecific Hybridisation | 44 |
| 2.3.2 Origin | 45 |
| 2.3.3 Cultivation | 46 |
| 2.3.3.1 Propagation | 46 |
| 2.3.3.2 Cultivation Practices and Cropping | 48 |
| 2.3.3.3 Pollination | 50 |
| 2.3.3.4 Pests and Diseases | 50 |
| 2.3.3.5 Cultivars | 51 |
| 2.3.3.6 Breeding | 51 |
| 2.3.3.7 Post-Harvest | 52 |
| 2.3.4 Crop Ecology | 53 |
| 2.3.5 Commercialisation | 54 |
| 2.3.6 Nutritional composition | 55 |
| 2.3.7 Papain | 56 |
| 2.3.8 Uses | 58 |
| 2.3.9 Situation of <i>Vasconcellea</i> in Ecuador | 59 |
| 3. Problems and Objectives | 61 |
| 3.1 Problem Formulation | 62 |
| 3.1.1 Importance of Plant Genetic Resources Worldwide | 62 |
| 3.1.2 Importance of Plant Genetic Resources in the Andes | 64 |
| 3.2 Objectives | 65 |
| 4. Materials and Methods | 68 |
| 4.1 Ethnobotanical Survey | 69 |
| 4.2 Crop Ecology | 72 |
| 4.3 Germplasm Collection and Characterisation | 75 |
| 4.4 Generative Propagation | 80 |
| 4.5 Alternative Uses | 84 |
| 5. Results | 85 |
| 5.1 Cherimoya (<i>Annona cherimola</i> Mill.) | 86 |
| 5.1.1 Ethnobotanical Survey | 86 |
| 5.1.1.1 Nomenclature and Use | 86 |
| 5.1.1.2 Agricultural practices | 86 |

| | |
|--|------------|
| 5.1.1.3 Commercialisation | 88 |
| 5.1.1.4 Major Crop Constraints..... | 89 |
| 5.1.1.5 Conclusions | 89 |
| 5.1.2 Crop Ecology | 89 |
| 5.1.2.1 <i>In Situ</i> Evaluation of Growing Conditions | 89 |
| 5.1.2.2 Prediction of Suitable Cultivation Zones..... | 90 |
| 5.1.2.3 Conclusions | 93 |
| 5.1.3 Germplasm Collection and Characterisation | 94 |
| 5.1.3.1 Collection and <i>In Situ</i> Pomological Characterisation | 94 |
| 5.1.3.2 Selection of Promising Accessions | 98 |
| 5.1.3.3 Preliminary Comparison with Commercial Cultivars | 98 |
| 5.1.3.4 Conclusions | 101 |
| 5.1.4 Generative Propagation | 102 |
| 5.1.4.1 Seed Structure..... | 102 |
| 5.1.4.2 Germination | 102 |
| 5.1.4.3 Conclusions | 105 |
| 5.1.5 Overall Discussion and Conclusions..... | 106 |
| | |
| 5.2 Highland Papayas (<i>Vasconcellea</i> spp.)..... | 108 |
| 5.2.1 Ethnobotanical Survey | 108 |
| 5.2.1.1 Knowledge, Nomenclature and Use..... | 108 |
| 5.2.1.2 Agricultural Practices..... | 111 |
| 5.2.1.3 Commercialisation | 113 |
| 5.2.1.4 Conclusions | 114 |
| 5.2.2 Crop Ecology..... | 115 |
| 5.2.2.1 <i>In Situ</i> Evaluation of Growing Conditions | 115 |
| 5.2.2.2 Prediction of Suitable Cultivation Zones..... | 117 |
| 5.2.2.3 Conclusions | 118 |
| 5.2.3 Germplasm Collection and Characterisation | 119 |
| 5.2.3.1 Collection and <i>In Situ</i> Characterisation | 119 |
| 5.2.3.2 Preliminary Selection..... | 123 |
| 5.2.3.3 Conclusions | 125 |
| 5.2.4 Generative Propagation | 127 |
| 5.2.4.1 Seed Structure..... | 127 |
| 5.2.4.2 Germination | 129 |
| 5.2.4.3 Conclusions | 134 |
| 5.2.5 Alternative Uses | 135 |
| 5.2.6 Overall Discussion and Conclusions..... | 137 |
| | |
| 6. General Conclusions | 139 |
| | |
| References..... | 144 |

| | |
|-------------------------------|------------|
| Annex | 156 |
| Curriculum Vitae | 159 |

ABBREVIATIONS AND ACRONYMS

| | |
|-----------------|--|
| AFLP | Amplified Fragment Length Polymorphism |
| ANOVA | Analysis of Variance |
| APG | Angiosperm Phylogeny Group (Missouri Botanical Garden, U.S.A.) |
| BAPNA | N- α -benzoyl-DL-arginine-p-nitroanilide |
| BOF | Bijzonder Onderzoeksfonds (Special Research Fund, Ghent University) |
| CAF | Corporación Andina de Fomento (Andean Development Corporation) |
| CATER | Centro Andino de Tecnología Rural (Andean Centre for Rural Technology, Ecuador) |
| CEC | Cation Exchange Capacity |
| CGIAR | Consultative Group on International Agricultural Research |
| CIA | Central Intelligence Agency (U.S.A.) |
| CIF | Cost, Insurance and Freight |
| CIRAD | Centre de Coopération Internationale en Recherche Agronomique pour le Développement (International Centre for Rural and Agricultural Development Research, France) |
| CIRAD-FLHOR | Département Productions Fruitières et Horticoles (Department of Fruit and Horticultural Production) |
| CORPEI | Corporación de Promoción de Exportaciones e Inversiones (Export and Investment Promotion Corporation, Ecuador) |
| CTIFL | Centre Technique Interprofessionnel des Fruits et Légumes (Interprofessional Technical Centre for Fruits and Vegetables, France) |
| DENAREF | Departamento Nacional de Recursos Fitogenéticos y Biotecnología (National Department of Plant Genetic Resources and Biotechnology, Ecuador) |
| DGIC | Directorate General of International Cooperation (Belgium) |
| DNA | deoxyribonucleic acid |
| FAO | Food and Agriculture Organisation of the United Nations |
| FOB | Free on Board |
| GA ₃ | Gibberellic Acid |
| GDP | Gross Domestic Product |
| GIS | Geographic Information System |
| GPS | Global Positioning System |
| IBA | indole-3-butyric acid |
| IFC | Internet Food Channel |
| IICA | Instituto Interamericano de Cooperación para la Agricultura (Inter-American Institute for Cooperation in Agriculture) |
| INEC | Instituto Nacional de Estadística y Censos (National Institute of Statistics and Census, Ecuador) |

Abbreviations and Acronyms

| | |
|----------|--|
| INIA | Instituto Nacional de Investigación Agraria (National Agrarian Research Institute, Peru) |
| INIAP | Instituto Nacional Autónomo de Investigaciones Agropecuarias (National Institute for Agricultural and Livestock Research, Ecuador) |
| IPGRI | International Plant Genetic Resources Institute |
| ISHS | International Society for Horticultural Science |
| IU | International Unit |
| masl | meters above sea level |
| NGO | non-governmental organisation |
| PCA | Principal Component Analysis |
| ppm | parts per million |
| PREDESUR | Programa de Desarrollo para la Región Sur (Regional Programme for the Development of Southern Ecuador, Ecuador) |
| PROSEA | Plant Resources of South-East Asia |
| RAPD | Random Amplified Polymorphic DNA |
| SICA | Servicio de Información Agropecuaria (Agricultural Information System, Ecuador) |
| UPGMA | Unweighted Pair Group Method using Arithmetic Averages |
| USDA | United States Department of Agriculture |
| VLIR | Vlaamse Interuniversitaire Raad (Flemish Interuniversity Council, Belgium) |
| VVOB | Vlaamse Vereniging voor Ontwikkelingssamenwerking en Technische Bijstand (Flemish Association for Development Cooperation and Technical Assistance, Belgium) |

SUMMARY

Among scientists, Ecuador is well-known for its biological richness. Due to the wide variability of climates, ranging from polar to tropical, different ecological zones, from desert to rain forest, are present. From a point of view of biodiversity per area unit, Ecuador ranks among the highest in the world. As several agricultural crops, or their relatives, can be found in wild in Ecuador, this wide plant biodiversity offers unique possibilities to study known or new crop species in their natural environment.

Despite this biological richness, Ecuador suffers major economic problems, mainly due to the negative local and regional economic situation and fiscal mismanagement but aggravated by natural disasters as the 'El Niño' phenomenon. In the year 2000, 88 % of the rural population was estimated to live in poverty. Agriculture remains an important source of income for the country, e.g. Ecuador is the world's main exporter of bananas. Most of this income is generated by the so-called agribusiness while resource poor farmers remain at the level of subsistence farming.

This study presents the results of the research and development project '*Conocimientos y prácticas culturales sobre los recursos fitogenéticos nativos en el austro Ecuatoriano*' (Knowledge on and cultural practices related to the native plant genetic resources in southern Ecuador). The project aimed at demonstrating the potential of native plant species and preparing their cultivation. The wide scope of the project asked for a long-term research approach which is typical for perennial crop species. Therefore, the results presented herewith and which were obtained after only four years of investigation, must be considered as preliminary. They are aimed at raising interest with local and international scientists and governmental and non-governmental organisations to continue research. The ultimate objective of this study is to help create a better economic situation for local farmers by cultivation of selected local plant species using locally adapted cultivation practices.

An ethnobotanical survey in southern Ecuador revealed 334 native edible plant species to be present in the area. Based on abundance, local acceptance and market possibilities, cherimoya (*Annona cherimola* Mill.) and the complex of highland papayas (*Vasconcellea* spp.) were selected for a more detailed study of their cultivation potential. At international level they are as well considered as under-utilised Andean crops that need further research. This research was the first extensive investigation on the selected species in their centre of origin.

Cherimoya (family Annonaceae) originates from southern Ecuador and northern Peru, where it can be found in wild forest stands or tolerated, without farmers' intervention, in fields. In southern Ecuador, cherimoya is not cultivated at a commercial scale. Worldwide however, Spain is the main producer with Peru, Chile, Bolivia and Australia being other important cherimoya growing countries.

Highland papayas (family Caricaceae) is a general term used for *Vasconcellea* species that can be found in tropical regions, mainly in South America, at altitudes above 1,000 masl. Ecuador accounts for 15 of the 21 described *Vasconcellea* species. Nine species can be found in southern Ecuador. So far this region has been poorly studied. The latter is illustrated by the recent description of the new species *V. palandensis* (V.M. Badillo *et al.*) V.M. Badillo that was found in the region. Nevertheless, the region is the centre of origin of two of the most important species, *V. stipulata* (V.M. Badillo) V.M. Badillo and *V. × heilbornii* (V.M. Badillo) V.M. Badillo. In Loja Province, seven known *Vasconcellea* species have been observed up to this moment. The presented results only deal with the most important and most common species: *V. stipulata* (V.M. Badillo) V.M. Badillo, *V. cundinamarcaensis* V.M.

Badillo and the natural hybrid between these two species *V. × heilbornii* (V.M. Badillo) V.M. Badillo. On a world level, *V. cundinamarcensis* is being cultivated on a small scale in and exported from Chile, while one variety of *V. × heilbornii*, known as babaco (*V. × heilbornii* 'Babacó') was introduced and is now cultivated in different countries such as New Zealand, Spain and Italy. Up till now its consumer's acceptance has been low, mainly due to unfamiliarity with the fruit and the large size of its fruits.

For both cherimoya and highland papayas, multi-disciplinary studies were realised. First, an ethnobotanical study describes the actual status, local knowledge, cultivation practices and commercialisation. Second, a study of crop ecology assesses preferences for soil and climate, mostly using data obtained in wild and tolerated stands, thus evidencing natural growth conditions. A zonification, combining the preferences obtained in a GIS, gives a preliminary idea on of the best cultivation zones for Loja Province. Third, germplasm collection and *in situ* characterisation evidences the wide morphological variability found in the centre of origin. This should offer good selection possibilities and should eventually lead to outstanding accessions for future cultivation. Fourth, results of a study on generative propagation should lead to a better understanding of the germination process, which is essential for future seed conservation and management. Finally, for highland papayas the potential use of latex as a source of crude papain is examined in order to widen the crop's possibilities.

The ethnobotanical study shows that cherimoya indeed is a species that occurs commonly in the area but that is hardly ever propagated by the local farmers. Most plants (83 %), although owned by the farmers, became established as a result of natural seed dispersion. As cherimoya is an allogamous species, this passive propagation leads to a very heterogeneous stand of very different individuals and thus heterogeneous quality harvest. Local use of fertilisers, mostly organic fertilisation, is practically non-existent while application of pesticides is even completely absent. Other cultivation practices such as irrigation or pruning, although leading to considerable better yields, are, if applied, not targeted directly to the cherimoya plants. They are part of a general maintenance of the backyard garden. A little more than half of the surveyed farmers do sell a part of their cherimoya fruits. Prices received are very low in absolute and/or when compared to other agricultural products, approximately 1 – 5 euro for 100 fruits (year 2000 price level). Prices at retail level are up to 10 times higher, indicating the existence of commercialisation channels favouring middlemen and retail sellers. Cherimoya does not form an important source of income for local farmers. Phytopathological problems, mainly fruit fly (*Anastrepha* spp.), form, beside low fruit prices, the main production constraints for local farmers. All these constraints lead to a lack of interest in growing cherimoya by local farmers and to a high risk of genetic erosion because farmers find no interest in maintaining germplasm which seems useless or less interesting to them.

The abundance of wild cherimoya stands in Loja Province offers unique possibilities to study natural preferences of the germplasm at hand. Most trees are found between 1,500 and 2,000 masl, corresponding to mean annual temperatures between 14 and 24 °C. Temperature is the most important climatic factor for cherimoya and the mean annual temperature optimum lies between 16 and 20 °C. Trees benefit most from annual rainfall between 800 – 1,200 mm/year. Wild cherimoya trees prefer stands with light (sandy loam, loam or sandy clay loam), well-drained soils, slightly acid (pH 5.0 – 6.5) and with moderate organic matter content. A total of 27 % of Loja Province shows these edaphoclimatic conditions and can thus be considered as suited for future cultivation.

Presence of wild cherimoya stands combined with passive propagation methods by local farmers leads to a huge morphologic variability. Values of important pomological characteristics like fruit weight and seed index (number of seeds per 100 g fruit) exhibit very wide ranges. Despite a local traditional maxim which tells us that seed content can be

derived from the skin type, no significant relationship exists between both fruit characteristics. The huge pomological variability offers unique possibilities to select outstanding accessions for future cultivation and breeding. Preliminary selection based on *in situ* characterisation showed that some local accessions can easily compete with cultivars from important cherimoya exporting countries.

Cherimoya shows very erratic germination with germination continuing up to 900 days after sowing. Pre-application of gibberellic acid (GA₃) considerably accelerates and homogenises this germination. Soaking seeds for 24 h in a 1,000 ppm GA₃ solution was found to be the best pre-application treatment although soaking in water for 72 h also showed a significant positive effect on germination.

Although located in its centre of origin, cherimoya is not considered an important fruit crop in Loja Province. In contrast to other countries like Spain, cherimoya does not generate major income for local farmers. Nevertheless cherimoya cultivation could, given the presence of outstanding accessions and optimal edaphoclimatic conditions, form a good source of income. Careful selection of cultivation sites and accessions combined with vegetative propagation (grafting) and application of some low-cost general cultivation practices like fertilising, pruning and well-targeted application of insecticides should homogenise quality and increase quantity (yield) considerably and finally lead to higher prices. As low-resource farmers do not have the economic capacity to adapt this cultivation system, it is the role of governmental or non-governmental institutions to take the initiative and guide local farmers to a better use of local cherimoya potential, inter alia through better marketing, organisation, and exploitation of local resources.

The present situation of highland papayas in southern Ecuador is similar to that of cherimoya. Knowledge and vernacular nomenclature seem to be very much locally determined. In one region different species are often given the same name while the same species can get a different name in different regions. The ethnobotanical study showed a decrease in knowledge of highland papayas with the younger part of the population and a narrowing towards babaco. In regions where *V. stipulata* is abundantly present in the environment it is always greatly appreciated, often more than the well-known commercial babaco (*V. × heilbornii* 'Babacó'). Due to their low sugar content, *Vasconcellea* species need to be processed before consumption, mainly adding sugar to prepare juices, preserves or jams. *V. cundinamarcaensis* and *V. stipulata* do not receive much cultivation practices and are often the result of natural multiplication by seeds. The seedless or low seeded *V. × heilbornii* is propagated vegetatively by cuttings and receives more agricultural practices such as organic fertilisation and irrigation. A special case is babaco, which is often cultivated at commercial level with high input of fertilisers and fungicides. Most highland papayas are only marketed locally or not marketed at all. The exception is babaco which is commercially well-developed with relatively high selling prices at 0.36 euro/fruit (year 2000 price level). A clear trend towards investment in profitable babaco cultivation could be observed, implying a high risk of losing the wide variability of highland papayas. Only in more remote areas with still a significant presence of wild highland papayas and less dominance of babaco, consumers tend to hold on to other species or types.

Crop ecology of highland papayas is rather similar for the different *Vasconcellea* species. *V. cundinamarcaensis* shows more restricted preferences for temperature and thus altitude, with an optimal mean annual temperature between 12 and 14 °C, whereas *V. stipulata* adapts better to different altitude levels. It can be found between 1,000 and 2,900 masl, with an optimal mean annual temperature ranging 14 – 18 °C. *V. × heilbornii* prefers climatic conditions intermediate between its two presumed progenitors. Edaphic characteristics at highland papaya sites are very variable and seem, with the exception of *V. cundinamarcaensis*, not to play an important role. About 20 % of Loja Province shows edaphoclimatic characteristics associated with frequent *V. × heilbornii* and *V. stipulata*

presence. This is only the case for 6 % of the province for *V. cundinamarcensis*. Striking is the lack in overlap in the distribution of *V. cundinamarcensis* and *V. stipulata*, the supposed progenitors of *V. × heilbornii*.

Germplasm characterisation reveals once more a huge morphological variability, leading to difficulties in identification and the hypothesis of continued process of hybridisation and backcrossing. Even the natural hybrid *V. × heilbornii*, mostly propagated by cutting, shows a wide variability, indicating the species originates sporadically from seeds under natural conditions. This variability offers good opportunities to select accessions with low seed content but with smaller fruit size. This could lead to a diversification of *Vasconcellea* cultivation, which is at this moment mainly focused on babaco, and possible future export opportunities. The large size of present day babaco fruits, around 1 kilogram, was one of the reasons for the failure of babaco introduction in other countries. A cluster analysis and principal component analysis based on morphological characteristics could clearly not differentiate the *Vasconcellea* species in well-defined groups. Especially *V. stipulata* and as yet unclassified varieties of *V. × heilbornii* could not be distinguished amongst themselves. This can be due to wrong identification, results of poor identification keys, or to the continued process of hybridisation and backcrossing. A thorough taxonomic revision, on morphological as on DNA level, is therefore recommended.

In order to preserve *Vasconcellea* seeds, the mucilaginous sarcotesta must be removed. This is best realised using 10 % sodium carbonate (Na_2CO_3) for 24 h. Highland papaya species show erratic germination. Especially germination of *V. stipulata* and *V. × heilbornii* do often not surpass 10 %, even after pre-application of gibberellic acid (GA_3). A pre-application of seeds for 24 h in a solution of 1,000 ppm GA_3 showed, however, a significant increase of germination in most cases. Despite low germination of *V. × heilbornii*, interesting perspectives for the development of new varieties are offered, as the obtained seedlings, due to the absence of male *V. × heilbornii* plants, probably will be the result of natural hybridisation with other *Vasconcellea* species.

Papain, a general term used for a complex of proteolytic enzymes, is currently extracted from latex of green *Carica papaya* fruits. A preliminary study on proteolytic activity of *Vasconcellea* species showed that these species, especially *V. stipulata* and a number of as yet unclassified varieties of *V. × heilbornii*, contain latex that shows a proteolytic activity up to 17 times higher than that of the reference *Carica papaya*.

It is clear that highland papayas offer an unexploited potential at local and international level. The existing variability can be used to select excellent accessions whereas mass selection of *V. × heilbornii* progeny could widen possibilities even more. The actual situation, however, shows a marked shift towards babaco cultivation, even in Loja Province, centre of diversification and hybridisation of highland papayas. Again, an effort is necessary to fully exploit the unused potential. Local accessions, selected for their fruit quality or high papain content, should be introduced with local farmers, accompanied by the extension of adequate cultivation practices.

The presented study, covering only a few of the 334 native edible fruit species present in southern Ecuador, clearly shows the cultivation potential of cherimoya and highland papayas. As results are often preliminary, continuation of research to confirm and elaborate on these results is essential. At this moment several spin-off projects, based on the presented results are being undertaken. In order to safeguard and optimally use the existing potential, farmer participation is crucial. It is the role of governmental or non-governmental organisations to promote and to continue the research on local fruit potential. The optimal use of this fruit potential could be one way to finally improve the economic situation of local farmers.

SAMENVATTING

Ecuador staat bij wetenschappers bekend om zijn biologische rijkdom. Door de grote variabiliteit aan klimaten, van polair tot tropisch, kunnen verscheidene ecologische zones, van woestijn tot regenwoud, aangetroffen worden. Op wereldschaal behoort Ecuador tot de landen met de hoogste biodiversiteit per oppervlakte-éénheid. Aangezien ook verschillende landbouwgewassen, of verwanten ervan, in Ecuador in het wild voorkomen, biedt deze omvangrijke biodiversiteit unieke mogelijkheden om gekende of nieuwe gewassen te bestuderen in hun natuurlijke omgeving.

Ondanks deze biologische rijkdom, lijdt Ecuador onder zware economische problemen, die vooral te wijten zijn aan de negatieve lokale en regionale situatie en fiscaal wanbeheer, maar verergerd worden door natuurlijke rampen als het 'El Niño'-fenomeen. Schattingen wijzen uit dat in het jaar 2000, 88 % van de landelijke bevolking onder de armoedegrens leefde. Landbouw blijft een belangrijke bron van inkomsten voor het land. Ecuador is bijvoorbeeld de grootste exporteur van bananen ter wereld. Het grootste deel van dit inkomen wordt echter voortgebracht door de zogenaamde agri-business terwijl kleine boeren dikwijls op het niveau van zelfvoorzieningslandbouw blijven.

Deze studie stelt de resultaten voor van het onderzoeks- en ontwikkelingsproject '*Conocimientos y prácticas culturales sobre los recursos fitogenéticos nativos en el austro Ecuatoriano*' (Kennis over en culturele praktijken gerelateerd met de inheemse plantenrijkdom in Zuid-Ecuador). Het project had als objectief het potentieel van lokale plantensoorten aan te tonen en hun teelt voor te bereiden. De grote draagwijdte van dit project vroeg om een langetermijnonderzoek, typisch voor meerjarige gewassen. Daarom moeten de voorgestelde resultaten, bereikt na slechts vier jaar onderzoek, als preliminair beschouwd worden. Ze zijn er vooral op gericht om de interesse van lokale en internationale onderzoekers en gouvernementele en niet-gouvernementele organisaties op te wekken om het onderzoek verder te zetten. Het uiteindelijke doel van deze studie is het creëren van een betere economische situatie voor de lokale landbouwers en dit door de teelt van de geselecteerde plantensoorten, gebruik makend van aangepaste teelttechnieken.

Een etnobotanische studie in Zuid-Ecuador toonde de aanwezigheid aan van 334 inheemse eetbare plantensoorten. Gebaseerd op abundantie, lokale appreciatie en vermarktingsmogelijkheden, werden cherimoya (*Annona cherimola* Mill.) en het complex van hooglandpapaja's (*Vasconcellea* spp.) geselecteerd voor een meer gedetailleerde studie rond hun teeltpotentieel. Op internationaal niveau worden deze vruchten beschouwd als ongeëxploiteerde Andijnse gewassen waarop verder onderzoek vereist is. Dit onderzoek was de eerste uitgebreide research rond de geselecteerde soorten in hun oorsprongsgebied.

Cherimoya (familie Annonaceae) kent zijn oorsprong in Zuid-Ecuador en Noord-Peru, waar de soort in het wild aangetroffen wordt in bossen of in velden, waar de planten getolereerd worden zonder interventie van de boeren. In Zuid-Ecuador wordt cherimoya niet op commerciële schaal geteeld. Wereldwijd echter is Spanje de belangrijkste producent, terwijl Peru, Chili, Bolivia en Australië andere belangrijke cherimoyaproducerende landen zijn.

Hooglandpapaja's (familie Caricaceae) is een algemeen gebruikte term voor *Vasconcellea*-soorten die voorkomen in tropische regio's, vooral in Zuid-Amerika, op hoogtes boven 1.000 meter. Vijftien van de 21 beschreven *Vasconcellea*-soorten komen voor in Ecuador. Negen soorten kunnen gevonden worden in Zuid-Ecuador. Tot nu toe werd deze regio echter weinig bestudeerd. Dit wordt geïllustreerd door de recente beschrijving van een nieuwe soort, *V. palandensis* (V.M. Badillo *et al.*) V.M. Badillo, die er gevonden werd. Niettemin is de regio het oorsprongsgebied van twee van de meest belangrijke taxa, *V. stipulata* (V.M. Badillo) V.M.

Badillo en *V. × heilbornii* (V.M. Badillo) V.M. Badillo. In de provincie Loja werden tot nu toe zeven *Vasconcellea*-soorten beschreven. De hier voorgestelde resultaten behandelen enkel de meest belangrijke en meest voorkomende taxa: *V. stipulata* (V.M. Badillo) V.M. Badillo, *V. cundinamarcensis* V.M. Badillo en de natuurlijke hybride tussen deze twee soorten *V. × heilbornii* (V.M. Badillo) V.M. Badillo. Op wereldschaal wordt *V. cundinamarcensis* enkel in Chili op kleine schaal geteeld en geëxporteerd. Eén bepaalde *V. × heilbornii*-variëteit, gekend als babaco (*V. × heilbornii* 'Babacó'), werd geïntroduceerd in verscheidene landen zoals Nieuw-Zeeland, Spanje en Italië en wordt er momenteel geteeld. Tot nu toe was de aanvaarding van de vrucht bij de consumenten laag, vooral door zijn onbekendheid en grote omvang van zijn vruchten.

Voor zowel cherimoya als hooglandpapaja's werden er in dit project multi-disciplinaire studies uitgevoerd. Vooreerst beschrijft een etnobotanische studie de actuele situatie, de lokale kennis, teeltpraktijken en commercialisering van de geselecteerde fruitsoorten. Daarnaast werd via een ecologische studie de voorkeur van bodem en klimaat bepaald, meestal gebruik makend van data verkregen op plaatsen met wilde of getolereerde planten. Deze voorkeuren werden via een GIS gecombineerd om een idee te krijgen over de zones die in de provincie Loja het best geschikt zijn voor toekomstige teelt. Verder wijst een collectie en *in situ* karakterisatie van lokaal germoplasma op de grote morfologische variabiliteit die gevonden kan worden in het oorsprongsgebied. Dit opent heel wat selectiemogelijkheden en kan uiteindelijk leiden tot uitstekend materiaal voor een toekomstige teelt. Resultaten van een studie rond generatieve vermeerdering moet leiden tot een beter begrip van het kiemingsproces wat essentieel is voor toekomstige zaadbewaring en –management. Tot slot werd voor hooglandpapaja's het gebruik van de latex als een bron van papaine getest, om het potentieel van de vruchten verder te verruimen.

De etnobotanische studie toont aan dat cherimoya inderdaad een algemene fruitsoort is, die echter zelden actief vermeerderd wordt door lokale boeren. De meeste planten (83 %) die men in de natuur vindt, hoewel bezit van de boeren, zijn er gekomen via natuurlijke zaadverspreiding. Gezien cherimoya een kruisbestuiver is, leidt deze passieve vermeerderingswijze tot zeer heterogene bestanden met sterk verschillende individuen wat zorgt voor een oogst van wisselende, dikwijls inferieure, kwaliteit. Het gebruik van meststoffen, vooral organische, is zo goed als onbestaande in de regio, terwijl toepassing van pesticiden zelfs compleet afwezig is. Andere teeltpraktijken zoals irrigatie en snoei zijn niet rechtstreeks op de cherimoyabomen gericht, hoewel deze leiden tot een aanzienlijk beter rendement. Ze maken deel uit van een algemeen onderhoud van de tuintjes. Iets meer dan de helft van de ondervraagde boeren verkoopt een deel van zijn cherimoyaoogst. De verkregen prijzen zijn laag, zowel absoluut als in vergelijking met andere landbouwproducten, en bedragen ongeveer 1 – 5 euro voor 100 vruchten (prijsniveau jaar 2000). Prijzen in de handel zijn tot 10 keer hoger, een aanwijzing dat de verkoopkanalen vooral tussenhandelaars en handelaars bevoordelen. Cherimoya vormt geen belangrijke bron van inkomsten voor de lokale boeren. Fytopathologische problemen, vooral de fruitvlieg (*Anastrepha* spp.), vormen, naast de lage prijzen, de voornaamste teeltbelemmeringen. Deze belemmeringen leiden tot een beperkte interesse voor de vrucht bij lokale boeren en een groot risico op genetische erosie, daar de boeren geen voordeel zien in het behoud van de variabiliteit.

De overvloed aan cherimoyabossen in de provincie Loja geeft unieke mogelijkheden om de natuurlijke groeivoorwaarden van deze soort te bestuderen. De meeste bomen komen voor tussen 1.500 en 2.000 meter hoogte, wat overeenkomt met een gemiddelde jaartemperatuur tussen 14 en 24 °C. Temperatuur is de belangrijkste klimaatsfactor voor cherimoya en de optimale gemiddelde jaartemperatuur ligt tussen 16 en 20 °C. Qua neerslag prefereren cherimoyabomen een jaarlijkse neerslag tussen 800 – 1.200 mm. Ze verkiezen een standplaats met een lichte (USDA: sandy loam, loam or sandy clay loam), goed gedraineerde bodem, die licht zuur (pH 5.0 – 6.5) is met een matig gehalte aan organisch

materiaal. Nagenoeg 27 % de provincie Loja beschikt over deze edafoklimatologische condities die dus als geschikt kan beschouwd worden voor een toekomstige teelt.

De aanwezigheid van deze cherimoyabossen gecombineerd met de passieve vermeerdering resulteert in een enorme morfologische variabiliteit. Waarden van belangrijke pomologische kenmerken als vruchtgewicht en zaadindex (aantal zaden per 100 g vrucht) tonen een aanzienlijke variabiliteit. Ondanks het lokaal traditioneel gezegde dat het aantal zaden kan afgeleid worden van de structuur van de schil, werd geen significant verband gevonden tussen beide vruchtkenmerken. De grote pomologische variabiliteit opent unieke mogelijkheden om excellente accessies te selecteren voor toekomstige teelt en voor veredeling. Een preliminaire selectie, gebaseerd op *in situ* karakterisatie toont aan dat sommige lokale accessies gemakkelijk kunnen concurreren met cultivars uit cherimoya-exporterende landen.

Cherimoya bezit een heel onregelmatige kieming die kan doorgaan tot 900 dagen na de zaai. Een voorbehandeling met gibberellinezuur (GA_3) versnelt en homogeniseert deze kieming echter aanzienlijk. Het weken van zaden gedurende 24 h in 1.000 ppm GA_3 werd geëvalueerd als de beste voorbehandeling, hoewel 72 h weken in water de kieming ook al significant verbeterde.

Hoewel het zich in zijn oorsprongsgebied bevindt, wordt cherimoya in de provincie Loja niet als een belangrijk gewas beschouwd. In tegenstelling tot in andere landen, als Spanje, zorgt cherimoya niet voor een belangrijk inkomen voor de lokale boeren. Niettemin zou de cherimoyateelt, gezien de aanwezigheid van excellent plantenmateriaal en ideale edafoklimatologische omstandigheden, een belangrijke bron van inkomsten kunnen vormen. Een zorgvuldige selectie van de lokatie en het plantenmateriaal gecombineerd met een vegetatieve vermeerdering (enten) en de toepassing van eenvoudige algemene teelttechnieken zoals bemesting, snoei en gericht gebruik van pesticiden zou de kwaliteit aanzienlijk homogeniseren en de kwantiteit (opbrengst) doen stijgen, wat uiteindelijk moet leiden tot hogere prijzen. Gezien kleine boeren niet de economische draagkracht bezitten om dit teeltsysteem te adapteren, is het de taak van governementele of niet-governementele organisaties om initiatief te nemen en de lokale boeren te begeleiden in een beter gebruik van het aanwezige cherimoyapotentieel, onder andere door een beter gebruik van lokaal plantenmateriaal en een geoptimaliseerde marketing en organisatie.

De huidige situatie van hooglandpapaja's in Zuid-Ecuador is gelijkaardig aan die van cherimoya. Kennis en naamgeving blijken erg plaatsgebonden te zijn. In één regio krijgen verschillende soorten dikwijls dezelfde vernaculaire naam, terwijl éénzelfde soort een verschillende naam krijgt naargelang de regio. De etnobotanische studie toonde een verminderde kennis aan bij de jongere bevolking wat hooglandpapaja's betreft en een verenging in de richting van babaco. In regio's waar *V. stipulata* algemeen is, wordt hij sterk geapprecieerd, dikwijls meer dan de gekende commerciële babaco (*V. × heilbornii* 'Babacó'). Door hun laag suikergehalte moeten *Vasconcellea*-soorten verwerkt worden vooraleer ze kunnen geconsumeerd worden. Dit gebeurt meestal door toevoeging van suiker om sap, jam of geconfijt fruit te bekomen. Aan de teelt van *V. cundinamarzensis* en *V. stipulata* komen weinig teelttechnieken te pas en hun aanwezigheid is dikwijls het gevolg van natuurlijke vermeerdering via zaden. *V. × heilbornii*, zaadloos of met een laag zaadgehalte, wordt vegetatief vermeerderd en de teelt gaat gepaard met uitgebreidere teelttechnieken, zoals organische bemesting en irrigatie. Een speciaal geval betreft babaco, die vaak op commerciële schaal geteeld wordt met een hoge input van meststoffen en fungiciden. De meeste hooglandpapaja's worden enkel lokaal verhandeld of zelfs totaal niet. Een uitzondering is opnieuw babaco, commercieel goed ontwikkeld met relatief hoge verkoopprijzen (0,36 euro/vrucht: prijsniveau jaar 2000). Een duidelijke trend in de richting van investering in de rendabele babacoteelt kon worden vastgesteld. Dit houdt echter een groot risico in op het verlies van de grote variabiliteit aan hooglandpapaja's. Enkel in

afgelegen gebieden met nog een groot aanbod aan wilde hooglandpapaja's en minder dominantie van babaco, maken de consumenten nog uitvoerig gebruik van deze andere soorten of types.

De ecologie van hooglandpapaja's is voor de verschillende *Vasconcellea*-soorten vrij gelijklopend. *V. cundinamarcensis* vertoont met een optimale gemiddelde jaartemperatuur tussen 12 en 14 °C meer beperkte voorkeuren betreffende temperatuur en dus hoogte. *V. stipulata* past zich beter aan aan variërende hoogte en kan gevonden worden tussen 1.000 en 2.900 m hoogte, met een optimale gemiddelde jaartemperatuur tussen 14 en 18 °C. *V. × heilbornii* prefereert klimaatcondities die intermediair zijn tussen die van zijn twee veronderstelde voorouders. De bodemkenmerken van de plaatsen waar hooglandpapaja's aanwezig zijn zeer variabel en lijken, met uitzondering van *V. cundinamarcensis*, geen belangrijke rol te spelen. Ongeveer 20 % van de provincie Loja bezit edafoklimatologische kenmerken geassocieerd met een frequente aanwezigheid van *V. × heilbornii* en *V. stipulata*. Voor *V. cundinamarcensis* is dit slechts het geval voor 6 % van de provincie. Opmerkelijk is het ontbreken van een duidelijke overlapping tussen het verspreidingsgebied van *V. cundinamarcensis* en *V. stipulata*, de vermoedelijke voorouders van *V. × heilbornii*.

Karakterisatie van germoplasma toont opnieuw de grote morfologische variabiliteit aan, wat kan leiden tot identificatiemoeilijkheden en tot de hypothese van een continu proces van hybridisatie en terugkruising. Zelfs de natuurlijke hybride *V. × heilbornii*, vooral vermeerderd via stekken, toont een grote variabiliteit. Dit zou een aanwijzing kunnen zijn dat deze plant onder natuurlijke omstandigheden sporadisch uit zaden ontstaat. De variabiliteit biedt goede perspectieven om op termijn plantenmateriaal met een laag zaadgehalte en kleiner vruchtgewicht te selecteren. Dit zou kunnen leiden tot een diversificatie van de *Vasconcellea*-teelt, die op dit moment vooral gericht is op babaco, en tot mogelijke toekomstige exportmogelijkheden. Het hoge gewicht van de huidige babacovruchten die momenteel op de markt te krijgen zijn, gemiddeld 1 kilogram, was één van de redenen voor het falen van de introductie in andere landen. Een clusteranalyse en een principale componentenanalyse gebaseerd op morfologische kenmerken, kon geen duidelijk gedifferentieerde groepen aantonen tussen de *Vasconcellea*-soorten. Vooral *V. stipulata* en voorlopig ongekende variëtiëten van *V. × heilbornii* konden moeilijk van elkaar onderscheiden worden. Dit kan te wijten zijn aan een verkeerde identificatie van het uitgangsmateriaal, als gevolg van slechte identificatiesleutels, of aan het continu proces van hybridisatie en terugkruising. Een grondige taxonomische revisie, zowel op morfologisch als op DNA-niveau, is daarom aangewezen.

Om *Vasconcellea*-zaden te bewaren moet de slijmerige sarcotesta worden verwijderd. Dit wordt het best gerealiseerd door het gebruik van 10 % natriumcarbonaat (Na_2CO_3) gedurende 24 h. Hooglandpapaja's vertonen een onregelmatige kieming. Vooral de kieming van *V. stipulata* en *V. × heilbornii* haalt nauwelijks 10 %, zelfs na een voorbehandeling met gibberellinezuur (GA_3). Een voorbehandeling van de zaden gedurende 24 h in een oplossing van 1.000 ppm GA_3 beïnvloedt de kieming echter wel significant ook al blijven absolute kiemingscijfers dikwijls eerder beperkt. Ondanks de gebrekkige kieming van *V. × heilbornii* worden interessante perspectieven geopend, gezien de zaailingen, door de afwezigheid van mannelijke *V. × heilbornii*, hoogstwaarschijnlijk het resultaat zullen zijn van natuurlijke hybridisatie met andere *Vasconcellea*-soorten.

Papaine, een algemene term gebruikt voor een complex van eiwitafbrekende enzymen, wordt momenteel gewonnen uit de latex van onrijpe *Carica papaya*-vruchten. Een preliminaire studie rond de proteolytische activiteit van *Vasconcellea*-soorten toont aan dat deze soorten, vooral *V. stipulata* en een aantal voorlopig ongeclassificeerde variëteiten van *V. × heilbornii*, latex bevatten die een tot 17 maal hogere eiwitafbrekende activiteit bezit dan de referentie, *Carica papaya*.

Het is duidelijk dat hooglandpapaja's een ongeëxploiteerd potentieel, zowel op nationaal als internationaal niveau, bezitten. De bestaande variabiliteit kan gebruikt worden om uitzonderlijke accessies te selecteren. Bovendien kan massale selectie van *V. × heilbornii*-nakomelingen de mogelijkheden nog uitbreiden. De actuele situatie wijst echter op een duidelijke verschuiving naar de babacoteelt, zelfs in de provincie Loja, een gebied met grote diversiteit en waar hooglandpapaja's volop hybridiseren op natuurlijke wijze. Ook hier is er een inspanning nodig om dit ongebruikt potentieel volledig te exploiteren. Lokale accessies, geselecteerd voor hun vruchtkwaliteit of hoog papaïnegehalte, moeten geïntroduceerd worden bij lokale boeren en dit in combinatie met de geschikte teelttechnieken.

De voorgestelde studie, hoewel die slechts over enkele van de 334 eetbare inheemse plantensoorten aanwezig in Zuid-Ecuador handelt, toont duidelijk het teeltpotentieel aan van cherimoya en hooglandpapaja's. Aangezien de resultaten slechts preliminair zijn, is het essentieel het onderzoek verder te zetten en de resultaten uit te diepen. Momenteel worden verschillende spin-off projecten, gebaseerd op de voorgestelde resultaten, uitgevoerd. Om het bestaande potentieel te beschermen en optimaal te gebruiken, is de deelname van lokale boeren cruciaal. Het is de rol van gouvernementele en niet-gouvernementele organisaties om het onderzoek naar het lokaal fruitpotentieel te promoten en verder te zetten. Het optimaal benutten van dit potentieel zou een mogelijkheid zijn om uiteindelijk ook de economische situatie van lokale boeren te verbeteren.

RESUMEN

Entre científicos, Ecuador es conocido por su riqueza biológica. Debido a una amplia variabilidad de climas, extendiéndose de polar a tropical, diferentes zonas ecológicas, de desierto hasta selva tropical, están presentes. De un punto de vista de diversidad biológica por unidad de superficie, Ecuador alinea entre los más altos del mundo. La amplia biodiversidad de varios cultivos agrícolas, o sus parientes, encontrados en forma silvestre en Ecuador, ofrece posibilidades únicas para estudiar cultivos conocidos o nuevos en su ambiente natural.

A pesar de esta riqueza biológica, Ecuador sufre grandes problemas económicos, principalmente debido a la situación económica negativa a nivel local y regional y la mala gestión fiscal, pero agravado por catástrofes naturales como el fenómeno del Niño. En el año 2000, se estimaba que el 88 % de la población rural vivía en pobreza. La agricultura permanece una fuente importante de ingresos para el país, p.ej. Ecuador es el líder mundial de exportación de plátanos. Sin embargo, notemos que la mayor parte de estos ingresos genera la agroindustria, mientras que los campesinos de bajos recursos permanecen en un nivel de agricultura de subsistencia.

Este estudio presenta los resultados del proyecto de investigación y desarrollo 'Conocimientos prácticas culturales sobre los recursos fitogenéticos nativos en el austro Ecuatoriano'. El proyecto tuvo como objetivo demostrar el potencial de las plantas nativas y preparar su cultivo. El alcance amplio del proyecto pidió una investigación a largo plazo cual es típica para cultivos perennes. Por lo tanto, los resultados presentados, obtenidos después de sólo cuatro años de investigación, deben ser considerados como preliminares. Aspiran despertar interés en continuar la investigación, dentro los científicos locales e internacionales y las organizaciones gubernamentales y no gubernamentales. El objetivo final de este estudio es ayudar a crear una mejor situación económica para los campesinos locales a través del cultivo de especies nativas seleccionadas, utilizando prácticas de cultivo localmente adaptadas.

Un estudio etnobotánico en el austro Ecuatoriano reveló la presencia de 334 especies de plantas nativas comestibles. Basándose en la abundancia, su aceptación local y las posibilidades de mercado, la chirimoya (*Annona cherimola* Mill.) y el complejo de las papayas de montaña (*Vasconcellea* spp.) fueron seleccionados para un estudio más detallado de su potencial de cultivación. También a nivel internacional estas especies son consideradas como cultivos andinos poco utilizados que necesiten más investigación. Esta investigación fue la primera extensa sobre las especies seleccionadas en su centro del origen.

La chirimoya (familia Annonaceae) origina del sur del Ecuador y del norte del Perú, en donde puede ser encontrada silvestre en bosques o tolerada, sin la intervención de los campesinos. En el sur del Ecuador, la chirimoya no se explota comercialmente. Sin embargo, a nivel mundial, España es el productor principal con Perú, Chile, Bolivia y Australia siendo otros países importantes del cultivo de chirimoya.

Papaya de montaña (familia Caricaceae) es un término general usado para las especies de *Vasconcellea* que se pueden encontrar en regiones tropicales, principalmente en América del sur, en altitudes encima de 1,000 msnm. Ecuador posee 15 de las 21 especies de *Vasconcellea* descritas. Nueve especies se puede encontrar en el sur del Ecuador. Hasta ahora no se han realizado muchos estudios en esta región lo que fue ilustrado por la descripción de una nueva especie *V. palandensis* (V.M. Badillo *et al.*) V.M. Badillo, recién encontrado en la región. Sin embargo, la región es el centro del origen de dos de las

especies más importantes, *V. stipulata* (V.M. Badillo) V.M. Badillo y *V. × heilbornii* (V.M. Badillo) V.M. Badillo. En la provincia de Loja, siete especies de *Vasconcellea* han sido descritos hasta este momento.

Los resultados presentados sólo tratan las especies más importantes y más comunes: *V. stipulata* (V.M. Badillo) V.M. Badillo, *V. cundinamarcensis* V.M. Badillo y el híbrido natural entre estas dos especies *V. × heilbornii* (V.M. Badillo) V.M. Badillo. A nivel mundial, se cultiva y se exporta desde Chile, *V. cundinamarcensis* en una pequeña escala, mientras que una variedad de *V. × heilbornii*, conocida como babaco (*V. × heilbornii* 'Babacó'), fue introducida en diversos países tales como Nueva Zelanda, España e Italia. Hasta ahora la aceptación del babaco por el consumidor ha sido baja, principalmente debido a un desconocimiento de la fruta y el tamaño grande de sus frutas.

Para la chirimoya tal cual para las papayas de montaña, estudios multi-disciplinarios fueron realizados. En primer lugar, un estudio etnobotánico describió el estado actual, el conocimiento local, y las prácticas de cultivo y de comercialización regionales. En segundo lugar, un estudio de la ecología de la especie evaluó las preferencias de suelo y de clima, sirviéndose principalmente de datos obtenidos en sitios con plantas silvestres o toleradas, así evidenciando las condiciones naturales de crecimiento. Una zonificación, combinando las preferencias obtenidas en un SIG, dió una idea preliminar sobre las mejores zonas de cultivo para la Provincia Loja. En tercer lugar, una recolección y in situ caracterización de germoplasma evidenció la amplia variabilidad morfológica encontrada en el centro de origen. Eso debería ofrecer buenas posibilidades de selección para finalmente llegar a accesos excepcionales para un cultivo futuro. Resultados de un estudio sobre la propagación generativa deberían llegar a un mejor entendimiento del proceso de germinación, esencial para un futuro conservación y manejo de semillas. Finalmente, para papayas de montaña, el uso potencial del látex como fuente de papaina fue examinada para ensanchar todavía más las posibilidades del cultivo.

El estudio etnobotánico muestra que la chirimoya es de verdad una especie que ocurre comúnmente en el área, pero que casi nunca los campesinos locales la propagan. La mayor parte de las plantas (83 %), aunque poseído por los campesinos, se establecieron como consecuencia de una dispersión natural de semillas. Como la chirimoya es una especie alogama, esta propagación pasiva conduce a chirimoyales muy heterogéneos y así una cosecha de calidad muy heterogénea. El uso local de fertilizantes, sobre todo orgánicos, es prácticamente inexistente mientras que el uso de pesticidas es aún completamente ausente. Otras prácticas de cultivo como el riego o la poda, que resulten en una mejor producción, no son directamente apuntadas a las plantas de chirimoya. Son parte de un mantenimiento general del huerto casero.

Un poco más que la mitad de los campesinos encuestados vende una parte de sus frutas de chirimoya. Los precios recibidos son muy bajos, en absoluto y/o comparado con otros productos agrícolas, aproximadamente 1 - 5 euro por 100 frutas (nivel de precio 2000). Los precios a nivel del comercio minorista son 10 veces más alto, indicando la existencia de canales de comercialización que favorecen a intermediarios y vendedores al por menor. Chirimoya no forma una fuente de ingresos importante para los campesinos locales. Problemas fitopatológicos, principalmente mosca de la fruta (*Anastrepha* spp.), forman, fuero de los precios bajos, las coacciones de producción principales para campesinos locales. Todas estas coacciones conducen a una falta de interés de parte de los campesinos locales para cultivar chirimoya y a un alto riesgo de erosión genética porque los agricultores no encuentran ningún interés en mantener germoplasma que les parece inútil o menos interesante.

La abundancia de chirimoyales silvestres en la provincia de Loja ofrece posibilidades únicas para estudiar las preferencias naturales de esta especie. La mayoría de los árboles se

encuentran entre el 1,500 y 2,000 msnm, lo cual corresponde con una temperatura promedio anual entre 14 y 24 °C. Esta temperatura es el factor climático más importante para la chirimoya y su óptima se sitúa entre 16 y 20 °C. Los árboles prefieren una precipitación anual entre 800 - 1,200 mm. Árboles silvestres de chirimoya prefieren un suelo liviano (franco arenoso, franco o franco-arcilloso-arenoso), bien drenado, levemente ácido (pH 5.0 - 6.5) con un contenido moderado de materia orgánica. Un total de 27 % de la provincia de Loja posee estas condiciones edafoclimáticas y puede ser considerado como apto para un cultivo futuro.

La presencia de matas silvestres de chirimoya combinado con los métodos pasivos de propagación de los campesinos locales resulta en una variabilidad morfológica amplia. Los valores de las características pomológicas importantes como el peso de la fruta y el índice de la semilla (número de semillas por 100 g de fruto) muestran rangos muy amplios. A pesar de una máxima tradicional local que nos diga que el contenido de semillas se puede derivar del tipo de piel de la fruta, ningún lazo significativo sale entre ambas características. La variabilidad pomológica enorme ofrece posibilidades únicas para seleccionar accesiones excepcionales para el cultivo y el mejoramiento futuro. La selección preliminar basada en la caracterización insitu mostró que algunas accesiones locales pueden competir fácilmente con los cultivares de países de exportación de chirimoya.

La chirimoya muestra una germinación muy errática, lo cual continúa hasta 900 días después de la siembra. Una pre-aplicación de ácido giberélico (GA_3) acelera y homogeniza considerablemente esta germinación. Remojar las semillas para 24 h en una solución de 1,000 ppm GA_3 fue el mejor tratamiento de pre-aplicación, aunque remojar en agua durante 72 h también mostró un efecto positivo significativo sobre la germinación.

Aunque localizada en su centro de origen, la chirimoya no se considera como un frutal importante en la provincia de Loja. Al contrario de otros países, como España, la chirimoya no genera ingresos importantes para los campesinos locales. Sin embargo, dado la presencia de accesiones excepcionales y de condiciones edafoclimáticas óptimas, el cultivo de chirimoya podría formar una buena fuente de ingresos. Una selección cuidadosa de los sitios y de las accesiones cultivadas combinado con una propagación vegetativa (injerto) y una aplicación de algunas prácticas de cultivo sencillos como la fertilización, la poda y la aplicación bien-apuntada de insecticidas debería homogeneizar la calidad y aumentar la cantidad (producción) considerablemente y finalmente conducir a precios más elevados. Como los campesinos de bajos recursos no tienen la capacidad económica de adaptar este sistema de cultivo, es el papel de las instituciones gubernamentales o no gubernamentales de tomar la iniciativa y de dirigir a los campesinos a un uso mejor del potencial de chirimoya, entre otros a través de una explotación mejor de los recursos locales y una mejor comercialización y organización.

La situación actual de las papayas de montaña en el sur del Ecuador es similar a la de chirimoya. El conocimiento y la nomenclatura vernácula parece ser determinado muy localmente. En una región dan a menudo el mismo nombre a diferentes especies, mientras que la misma especie puede recibir un diferente nombre según la región. El estudio etnobotánico mostró una disminución del conocimiento de las papayas de montaña por la parte más joven de la población y un estrechamiento hacia el babaco. En regiones donde *V. stipulata* está presente abundantemente siempre se lo aprevie y además mucho más que el babaco (*V. × heilbornii* 'Babacó'), que es una especie comercialmente bien desarrollada. Debido a su bajo contenido de azúcar, las especies de *Vasconcellea* necesitan ser procesadas antes del consumo, principalmente agregando azúcar para preparar jugos, conservas o mermeladas. *V. cundinamarcensis* y *V. stipulata* no reciben muchas prácticas de cultivo y son a menudo el resultado de una multiplicación natural por semillas. *V. × heilbornii*, sin o con bajo contenido de semillas, es propagado vegetativo por estacas y recibe prácticas agrícolas tales como fertilización orgánica y riego. Un caso especial es el

babaco, que se cultiva muchas veces a un nivel comercial con un alto aporte de fertilizantes y de fungicidas. La mayoría de las papayas de montaña son comercializadas solamente localmente. La excepción es el babaco que está comercialmente bien desarrollado con precios de venta relativamente altos a 0.36 euro/fruta (nivel de precio 2000). Se podía observar una tendencia clara hacia la inversión en el cultivo rentable del babaco, implicando un alto riesgo de perder la variabilidad amplia de las papayas de montaña. Solamente en áreas más remotas con todavía una presencia significativa de papayas montañosas silvestres y menos predominio de babaco, consumidores tienden a agarrarse a otras especies o tipos.

La ecología de las papayas de montaña es bastante similar para las diferentes especies de *Vasconcellea*. *V. cundinamarcensis* muestra preferencias más restringidas para la temperatura y como consecuencia la altitud, con una óptima temperatura promedia anual entre 12 y 14 °C, mientras que *V. stipulata* se adapta mejor a niveles diferentes de altitud. Puede ser encontrado entre 1,000 y 2,900 msnm, con una óptima temperatura promedia anual comprendida entre 14 y 18 °C. *V. × heilbornii* prefiere condiciones climáticas intermedias entre sus dos progenitores. Las características edáficas en los sitios con presencia de papayas de montaña son muy variables y parecen, a excepción de *V. cundinamarcensis*, no desempeñar un papel importante. Cerca de 20 % de la provincia de Loja muestra características edafoclimatológicas asociadas a una frecuente presencia de *V. × heilbornii* y de *V. stipulata*. Éste es solamente el caso para 6 % de la provincia para *V. cundinamarcensis*. Sorprendente es la falta en traslapeo en la distribución de *V. cundinamarcensis* y de *V. stipulata*, los progenitores supuestos de *V. × heilbornii*.

Caracterización de germoplasma revela, otra vez, una enorme variabilidad morfológica, conduciendo a dificultades en la identificación y a la hipótesis de un proceso continuo de hibridación y cruzamiento. Incluso el híbrido natural *V. × heilbornii*, propagado sobre todo a través de estacas, muestra una variabilidad amplia, indicando que bajo condiciones naturales la especie origina esporádico de semillas. Esta variabilidad ofrece buenas oportunidades de seleccionar accesiones con bajo contenido de semillas pero con un tamaño de fruta pequeña. Eso podría conducir a una diversificación del cultivo de *Vasconcellea*, en este momento concentrado principalmente en babaco, y a oportunidades futuras de exportación. El tamaño grande de las actuales frutas de babaco, al rededor de 1 kilogramo, fue una de las razones de no introducir el babaco en otros países. El análisis cluster y el análisis de componentes principales, basado en características morfológicas, no podían distinguir claramente las especies de *Vasconcellea* en grupos bien definidos. Especialmente *V. stipulata* y las variedades aún no clasificadas de *V. × heilbornii* no podían ser distinguidos entre ellos. Eso puede ser debido a una identificación incorrecta, resultado de claves de identificación malas, o al proceso continuo de hibridación y cruzamiento. Por lo tanto se recomienda una revisión taxonómica completa, al nivel morfológico como al nivel de ADN.

Para preservar las semillas de *Vasconcellea*, la sarcotesta mucilaginosa debe ser quitada. La mejor manera para realizarlo es a través del uso de una solución de 10 % de carbonato de sodio (Na_2CO_3) durante 24 h. Las especies de papaya de montaña muestran una germinación errática. Especialmente la germinación de *V. × heilbornii* y de *V. stipulata* no sobrepasa a menudo el 10 %, incluso después de una pre-aplicación de ácido giberélico (GA_3). Una pre-aplicación de las semillas que existió en un remojo durante 24 h en una solución de 1,000 ppm GA_3 mostró, sin embargo, un aumento significativo de la germinación en la mayoría de los casos. A pesar de la germinación baja de *V. × heilbornii*, se ofrecen perspectivas interesantes para el desarrollo de nuevas variedades, como las plantas obtenidas por semilla, debido a la ausencia de las plantas masculinas de *V. × heilbornii*, serán probablemente el resultado de una hibridación natural con otras especies de *Vasconcellea*.

La papaína, un término general usado por un complejo de enzimas proteolíticas, es extraída actualmente del látex de frutas verdes de *Carica papaya*. Un estudio preliminar de la actividad proteolítica de algunas especies de *Vasconcellea* mostró que estas especies, especialmente *V. stipulata* y un número de variedades aún no clasificadas de *V. × heilbornii*, contienen un látex que posee una actividad proteolítica hasta 17 veces más alta que la referencia *Carica papaya*.

Es evidente que las papayas de montaña ofrecen un potencial inexplorado a nivel local e internacional. La variabilidad existente se puede utilizar para seleccionar accesiones excelentes mientras que una selección masiva de la progenie de *V. × heilbornii* podría ensanchar estas posibilidades aún más. La situación actual, sin embargo, muestra una conversión marcada hacia el cultivo de babaco, aún en la provincia de Loja, el centro de diversificación y hibridación de las papayas de montaña. Una vez más un esfuerzo es necesario para explotar completamente el potencial inaprovechado. Accesiones locales, seleccionadas por su calidad de fruta o su alto contenido de papaína, deben ser introducidas dentro de la comunidad campesina local, acompañadas de una extensión de las prácticas de cultivo adecuadas.

El actual estudio, cubriendo solamente algunas de las 334 especies frutales nativas comestibles presentes en el sur del Ecuador, muestra claramente el potencial de cultivo de la chirimoya y de las papayas de montaña. Como los resultados obtenidos son muchas veces preliminares, la continuación de la investigación para confirmar y para profundizar estos resultados es indispensable. En este momento varios proyectos derivados, basándose en los actuales resultados se están emprendiendo. Para salvaguardar y utilizar óptimamente el potencial existente, la participación de los campesinos es crucial. Es el papel de las organizaciones gubernamentales o no gubernamentales para promover y para continuar la investigación sobre el potencial frutal local. El uso óptimo de este potencial frutal podría ser una posibilidad para finalmente mejorar la situación económica de los campesinos locales.

1. INTRODUCTION

Plant genetic resources and agricultural biodiversity have recently gained importance in both the scientific community and the development cooperation. Since the Conference on Biodiversity in Rio de Janeiro in 1992, special attention goes to the use, management and conservation of plant genetic resources in countries with high biodiversity, ironically often developing countries. These countries, which since 1992 are not only owner but also responsible for their own genetic resources, offer an often unknown potential but often lack the means to study this potential thoroughly.

Due to its unique position between the Sechura desert of northern Peru and the humid Amazon Basin and influenced by typical Andean relief forms, southern Ecuador shows a wide variety of ecological zones. Short distance differences in climate and soil result in a unique biological composition and a huge plant biodiversity, e.g. a total of 334 native edible plant species, belonging to 63 different plant families have been recorded in southern Ecuador (Van den Eynden *et al.*, 1999).

Despite the availability of a wide range of plant genetic resources, the economic situation in southern Ecuador is depressed. Especially peasants, the main part of the population, cannot exceed the level of subsistence farming and are very dependent on the local climatic situation. A remarkable feature of the agricultural system is the low proportion of native species in the total of cultivated crops, especially in fruit crops. Where banana, one of Ecuador's main export products, is occupying considerable areas, little attention is given to native fruit crops.

The project '*Conocimientos y prácticas culturales sobre los recursos fitogenéticos nativos en el austro Ecuatoriano*' aimed at broadening knowledge on native plant genetic resources in southern Ecuador in order to prepare future cultivation of some of these local species with good cropping potential. The project was implemented as a research and development programme in a cooperation between the 'Universidad Nacional de Loja' and Ghent University.

After an initial phase in which the ethnobotany of southern Ecuador was studied, the project targeted cherimoya (*Annona cherimola* Mill.) and highland papayas (*Vasconcellea* spp.) for a more detailed study. The aim was to show cultivation potential and possibilities, and prepare future cultivation. One of the main objectives was to share the obtained information on the existing potential and cultivation possibilities of local genetic resources among farmers, scientists and policy makers so that this could eventually lead to concrete opportunities for a successful economic exploitation of these species. Long-term objectives aimed at improving the local economic situation of the farmers through introducing adequate cultivation of some native fruit species in the existing farming systems. Taking into account the absence of previous extensive studies on these fruit species in their centre of origin and the different research fields that need to be tackled in a general study on crop potential and its cultivation preparation, the obtained preliminary results aim at forming a starting point for further more detailed further studies and a basis for local and/or

international policy makers. The results must therefore not be considered as final scientific results.

Dealing with relatively unknown fruit species, an extended literature review was necessary to collect existing information and to get an idea about current scientific advances on the targeted fruit crops. Most literature was found to deal with commercial production in developed countries and did not apply to field conditions in Ecuador.

The present study, part of a broader research and development programme, tries to focus on local cropping conditions and on widening the knowledge of local fruit potential thus demonstrating the existing crop potential. A complete study of the crop potential in a region must consider a complex set of factors:

- (i) physical factors as soil and climate conditions determine the cropping possibilities and play an important role in ultimate potential yield;
- (ii) biological factors as the crop's genetic diversity (determining partly important traits as pomological characteristics, disease resistance and yield), the existing pests and diseases and the crop's pollination and propagation mechanisms, are some characteristics that need to be taken into account;
- (iii) economic factors as local commercialisation systems, market situation and economic condition of the local population need to be contemplated;
- (iv) human factors as the farmers' interests and preferences and agronomical practices must be included to obtain a successful crop introduction; and
- (v) social factors as local structures and organisations may form important organisms in a successful introduction of potential crops.

This research focuses mainly on physical and biological factors. Research was conducted simultaneously on different fields. A first study on local status of the targeted fruit species analyses the actual situation and the interest of the local population in these wild species to assess the actual local status. Secondly, a study of the preferred climate and soil conditions aims at giving an insight in the environmental conditions in which the species locally prevail. A combination of these preferences with the limited set of thematic geographically data for southern Ecuador can result in preliminary suitability maps and can give a first indication of potential cultivation zones. Thirdly, a germplasm collection and *in situ* characterisation analyses the existing variability and potential. Fourthly, generative propagation, as one of the possible propagation methods, is studied more in detail. This study is considered necessary to manage properly existing germplasm and to understand better the germination process and techniques to improve it. Finally, a study on alternative uses of highland papayas, analysing the proteolytic activity of the fruit latex, aims at widening the possibilities of local native fruit species. The obtained results should raise interest at level of local and international scientists in the existing, yet under-used and threatened, potential of a number of local fruit resources.

2. LITERATURE REVIEW

2.1 STUDY AREA

2.1.1 ECUADOR

2.1.1.1 GENERAL CHARACTERISTICS

The Republic of Ecuador (276,840 km²) is located on the northwest coast of South America, right on the equator, bordering Colombia in the north and Peru in the east and south (Figure 1.1). Geographically, the Andean ridge divides the country in three different and ecologically diverse zones: the 'Costa' (western coastal plain), the 'Sierra' (central Andean highlands) and the 'Oriente' (eastern Amazon basin). The barren isolated Galapagos-archipelago forms a fourth distinct zone. The population (13,183,978 habitants) of Ecuador is ethnically divided in mestizo (55 %), Amerindian, mostly Quechua (25 %), Spanish (7 %) and black (3 %) (CIA, 2002).

A typical feature of the Ecuadorian climate, and equatorial climates in general, is the solar radiation that is received in a perpendicular way due to the astronomical position of earth and sun. This has some important consequences for the climate: the presence of a neutral day length (12 h day – 12 h night), the absence of the four meteorological seasons occurring at higher latitudes, isothermal temperatures throughout the year, low atmospheric pressure and stable wind currents (Maldonado Astudillo, 1985).

The Ecuadorian climate shows a huge spatial variation but has, with exception of precipitation, little seasonal variation. This is illustrated by the amplitude of daily temperature variation being superior to the variation in monthly average temperature (Huttel *et al.*, 1999). Apart from the temperature determining relief (annual average temperature descends 1 °C with every 200 m of altitudinal ascend), main factors influencing the climate are on the one hand the oscillating Intertropical Convergence Zone (low pressure belt), inducing seasonal rains, especially in the Andes, and on the other hand the influence of the moving sea currents. The southern, cold and arid Humboldt Sea Current, originating in Antarctica, and the warm humid equatorial El Niño Sea Current, coming from the Gulf of Panama, meet each other at the Equator (Huttel, 1997). The El Niño Sea Current induces rains, especially at the coast, while the Humboldt Sea Current induces aridity along Ecuador's southern coast, resulting in considerable desertification problems.

Ecuador, sometimes called the museum of world climates (Nelson Gómez, 1989), can be divided in numerous variable climatic zones with mean annual temperature ranging 5.6 °C (Rio Pita) up to 25.8 °C (Esmeraldas) and annual precipitation varying between 180 mm/year (Manta) and 4,410 mm/year (Puyo).

Two thirds of the Ecuadorian territory consists of soils developed on old substrates (metamorphic, eruptive or sedimentary rocks) while the northern and central part of the Andes is composed of soils developed on young volcanic material (ashes and

lapilli). Small parts along the coast and rivers consist of alluvial soils (Zebrowski & Sourdat, 1997).



Figure 1.1. Map of Ecuador (Texas A&M University Library, 2002)

The three major ecological zones, 'Costa', 'Sierra' and 'Oriente', are not only different at edaphoclimatic level, but also at ethnographic, economic and social level, dividing the country thoroughly and inducing political regionalism. The Andean highlands with the capital Quito have a large population of mestizos and Amerindians, and economic activity is dominated by small-scale farming and services. The coastal area

and its major port of Guayaquil thrives on plantations, commerce and export. Finally, the sparsely populated Amazon region contains most of the country's main natural resource, petroleum (World Bank, 2002).

2.1.1.2 RESOURCES

The Ecuadorian economy is organised along two major clusters: oil and agribusiness, a third growing cluster being tourism (CORPEI, 2002). Because the country exports primary products such as oil, bananas and shrimps, fluctuations in world market prices can have a substantial domestic impact. In recent years, growth has been uneven due to ill-conceived fiscal stabilisation measures, mismanagement, El Niño damage to key export sectors (agriculture) and infrastructure, and low world commodity prices in the wake of the Asian financial crisis. In 1999, 50 % of the population was reported to live under the poverty line (CIA, 2002). Recent political crises and subsequent economical uncertainty resulted in a deterioration of the economy, clearly illustrated by some important macro-economic indicators (Table 1.1). Recent data confirm the downward trend in the macro-economic situation. About 88 % of the rural population is now living in poverty, compared with 69 % in 1999 and 54 % in 1995. A positive sound comes from the average growth of the GDP which changed from – 7.3 % in 1999 to 2.3 % in 2000 (World Bank, 2002).

Table 1.1. Annual economic indicators of Ecuador (Cornell University, 2002)

| | 1997 | 1999 |
|--|-------|--------|
| Total exports FOB (US\$ millions) | 5,264 | 4,662 |
| Total imports CIF (US\$ millions) | 4,666 | 2,596 |
| Inflation | 30.7 | 60.7 |
| Exchange rate US Dollar – Ecuadorian Sucre | 4,428 | 20,243 |
| Devaluation | 21.8 | 196.6 |

Agriculture is still an important source of income for the country (10 % of GDP) with 30 % of the labour force being employed in agriculture. Still, only 6 % of the total land surface is currently used for agricultural purposes (CIA, 2002; World Bank, 2002). In 1998, export of bananas was responsible for more than a quarter (26 %) of the export incomes, making it in 1998 the main export product of Ecuador, more important than petroleum. Recently however, petroleum regained its role as Ecuador's main export product (World Bank, 2002). Ecuador is currently the largest exporter of bananas at world level and the world's third producer (CORPEI, 2002). Other important agricultural export products of Ecuador include cut flowers, coffee, cocoa, vegetables (asparagus, broccoli and palm hearts) and fruits (passion fruit, pineapple, melon, strawberry and mango) (CORPEI, 2002).

Following the increase in demand of 'exotic' vegetables and fruits in developed countries, typical non-traditional agricultural products are gaining more importance. Given the importance and growth of these non-traditional crops in the Ecuadorian

economy and the potential in the world at large, it is essential to look for new crops to satisfy the ever-growing demand for these new fruits and vegetables.

2.1.1.3 BIODIVERSITY

The wide variety of climates gives Ecuador a broad range of ecological zones resulting in a lot of biodiversity. As much as 25 of the 38 life zones described by Holdridge can be found in Ecuador (INIAP, 1996). Ecuador possesses 16,087 different vascular plant species in its territory (Jørgensen & León-Yáñez, 1999), more species than the entire North American subcontinent, with exclusion of Mexico. This gives Ecuador the highest number of species per unit area of South America. A total of 25 % of this species is endemic (Valencia *et al.*, 2000). Due to the huge variety of ecosystems and species, Ecuador is one of the few countries on earth labelled as 'megadiverse' (Mittermeier *et al.*, 1997). Nevertheless Ecuador is the country with the highest deforestation and population growth of South America, raising urgent questions on conservation of its biodiversity (INIAP, 1996).

The Andean region with its striking geographical contrasts is an important centre for plant domestication. This diverse ecological mosaic creates countless microclimates, among the driest and wettest, coldest and hottest, found anywhere in the world (Hawkes, 1983). The Russian plant collector Vavilov (1950) proposed the Andean centre as one of the eight centres of plant genetic diversity. Harlan (1971) later narrowed the concept of Vavilov down to three centres and three non-centres and proposed the Andean region, including the Pacific lowlands and the slopes to the Amazon rain forests, as a non-centre of the Mesoamerican centre. The Andean region holds some important crop species that, due to 'agro-botanical colonialism', have been neglected and replaced by crops from Asia, Mexico and Europe (National Research Council, 1989). These so-called 'lost crops of the Incas' consist of three grains (e.g. *Chenopodium quinoa*, quinoa), a dozen root crops (e.g. *Canna edulis*, achira), three legumes (e.g. *Lupinus mutabilis*, tarwi) and a dozen fruits (Table 1.2) (National Research Council, 1989).

Table 1.2. Typical native fruit crops growing in the Andes (National Research Council, 1989)

| Scientific name | Common name | English name |
|--|-------------------------|------------------------|
| <i>Annona cherimola</i> (Annonaceae) | Chirimoya | Cherimoya |
| <i>Carica</i> spp. (Caricaceae) | Toronche, Chamburo, ... | Highland Papaya |
| <i>Cyphomandra betaceae</i> (Solanaceae) | Tomate de Árbol | Tamarillo, Tree Tomato |
| <i>Inga</i> spp. (Fabaceae) | Guaba | Pacay, Ice-cream Bean |
| <i>Myrtus ugni</i> (Myrtaceae) | Ugni | Myrtle Berry |
| <i>Passiflora</i> spp. (Passifloraceae) | Granadilla, Taxo, ... | Passion Fruit |
| <i>Physalis peruvianum</i> (Solanaceae) | Uvilla | Cape Gooseberry |
| <i>Pouteria lucuma</i> (Sapotaceae) | Lucuma | Lucuma, Lucmo |
| <i>Prunus salicifolia</i> (Rosaceae) | Capuli | Capuli Cherry |
| <i>Solanum muricatum</i> (Solanaceae) | Pepino dulce | Pepino |
| <i>Solanum quitoense</i> (Solanaceae) | Naranjilla | Naranjilla, Lulo |
| <i>Rubus</i> spp. (Rosaceae) | Mora | Blackberry |

Many of these fruits still occur exactly as they were at the time of discovery by the Spanish. Most are dooryard plants whose cultivation is primitive by modern standards: varieties are unselected, soil requirements are unknown and propagation techniques have not been perfected. Despite this, these native Andean fruits are not inferior to those of other areas. They have limited use only because of lack of awareness of their potential not because they taste bad. Samples of any undeveloped fruit tend to vary greatly in taste, size appearance and texture, but careful selection, clonal propagation and appropriate horticultural practices can bring huge improvements almost overnight. One of the most vital and rewarding activities is to collect and sort through such varieties, seeking the individual specimen with outstanding qualities (National Research Council, 1989). Basic research in genetics, reproductive biology, future uses and adaptation to other regions needs to be initiated (Castillo, 1995).

2.1.2 LOJA PROVINCE

2.1.2.1 GENERAL CHARACTERISTICS

Loja Province is the southernmost province of the Ecuadorian Sierra situated between 3°19' S and 4°45' S latitude and between 79°05' W and 80°29' W longitude (Figure 1.2). The total area covers approximately 10,790 km², equivalent to 4 % of the Ecuadorian land area (Organización de los Estados Americanos, 1994).



Figure 1.2. Map of Loja Province (Microsoft Encarta, 1999)

Due to its position between the humid Amazon basin and the coastal Sechura Desert in Peru and marked by a characteristic relief, Loja province shows a large variety of microclimates, resulting in numerous ecological zones, each characterised by its typical vegetation.

2.1.2.2 GEOLOGY AND GEOMORPHOLOGY

Little is known about Ecuadorian geological history before the Mesozoicum, although comparison with data obtained from the Colombian and Peruvian Andes supposes important orogenic Paleozoic cycles (Caldonian, Hercynian). The Mesozoic is marked by an accretion of the oceanic crust by the attachment of volcanic magmatic insular arches to the mainland, combined with simultaneous sedimentation, magmatic and tectonic events, resulting in a puzzling chronology of events. In the Cenozoic, all elements that make up the actual situation were established, created by forces released by the subduction of the Nazca plate under the South American crust. The typical Andean mountain system is the result of global plate-tectonic forces that were active during the Cenozoic, building upon earlier geological activity. This era is also marked by typical periods of sedimentation and magmatism. The Quaternary experienced an intense magmatic activity with formation of the present volcanoes, while some glacial influences during the Pleistocene attributed to the final formation of the present landscape (Marrocco & Winter, 1997).

The geology in Loja Province shows the typical Andean geological formation, consisting mainly of Paleozoic metamorphic rocks, in the eastern part, and volcanic rocks and Cretaceous and Tertiary sediments in the rest of the area (PREDESUR, 1998). The well-studied geological history of Loja Province begins with the general uprising at the end of the Cretaceous that ended the marine environment and resulted in continental conditions becoming dominant. Erosion of Cretaceous and earlier rocks, leading to formation of valleys (e.g. Valley of Loja), was followed by a period of volcanism with extrusion of pyroclasts and lava, especially in the northern part of the Province (Saraguro Group). Clastic fine and coarse materials were deposited. At the end of the Tertiary (Miocene - Pliocene) another orogenic event raised the total area and made continental conditions permanent. Tertiary sediments were again faulted and folded in north-south axis by an east-west compression force (PREDESUR, 1998).

Geomorphologically, Loja province has a unique relief. In the northern part of the province, the typical Andean structure with its two distinct parallel mountain ranges (Cordillera Occidental and Cordillera Oriental) ends at the Loja knot, indicating the end of the Central Ecuadorian Sierra. The eastern Cordillera Oriental remains the only real mountain range, with altitudes still above 2,800 masl, but with its maximum peak of 3,791 masl being considerable lower than in other parts of the Andes where peaks above 5,000 masl are common. Lower mountain ranges descend in an

western direction from the main ridge, making the mountain range considerable broader than in the north of the country.

Orography is chaotic, showing ranges without clear orientation that are separated by valleys with abrupt changes in direction, resulting in a unique relief. Altitude in Loja Province varies between 150 and 3,761 masl (Figure 1.3). Atypical for the Andean development is the absence of any recent, post-Oligocene, volcanic activity, lacking the presence of superficial pyroclastic material and volcanic sedimentation that covers most of the rest of the Ecuadorian Andes (Winckel & Zebrowski, 1997).

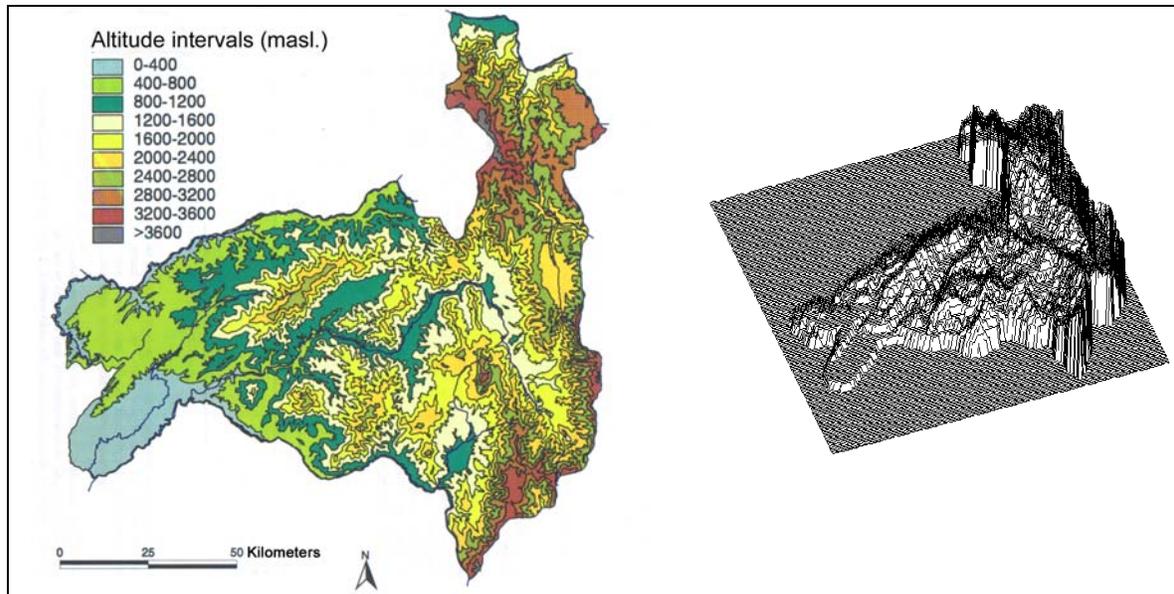


Figure 1.3. Altitude and relief of Loja Province (Instituto Geográfico Militar, 1989)

2.1.2.3 PEDOLOGY

The unique topography of Loja Province and its typical formation result in a huge soil diversity. In relation to the rest of the soils in the Ecuadorian Sierra, soils in southern Ecuador show an original composition, mainly caused by the absence of recent volcanic activity and their volcanic cover. Soils are generally formed by transformation of ancient substrates. Loja Province also shows more short-distance variability than the rest of Ecuador (Winckel & Zebrowski, 1997).

A soil survey executed in Loja Province by the Organisation of American States defined 13 distinct soil formations (Organización de los Estados Americanos, 1994). Table 1.3 shows the 13 different soil formations with their respective soil classification (USDA) and frequencies, while Figure 1.4 shows the soil map using the same classification. S and F soil formations account for more than 50 % of the province. These typical young Entisols, abundant in lower valleys, and the more developed Inceptisols are a typical feature for Loja Province.

The applied soil classification is a clear illustration of the soil variability found in Loja Province. Firstly, almost 15 % of the area could not be assigned to one particular soil formation and soil associations needed to be established. Secondly, due to the wide variability, even within one soil formation, the applied USDA classification system could not describe completely the soil formation and different USDA soil types needed to be combined in order to fully describe some soil formations.

2.1.2.4 CLIMATE

The same factors that influence the country's climate, are defining climate in Loja Province: the typical influence of the equatorial solar position; the atmospheric circulations with annual movements of the Intertropical Convergence Zone; the oceanic influence of the southern cold and dry Humboldt Sea Current and the northern warm and humid El Niño Sea Current and finally the regional orography, that is determining local temperature regimes, forming natural barriers and inducing orographic rains. Considerable differences over short distances, for example La Toma and Loja at only 30 km apart, illustrate the wide variability in climates that can be found in Loja Province (Figure 1.5).

The most peculiar aspect of the climate in Loja Province, in which it differs from the rest of the country, is on the one hand the abrupt and chaotic relief, with absence of the Cordillera Occidental (maritime winds can thus penetrate the mainland more easily) and the desertification phenomenon, advancing north from the Peruvian Sechura desert on the other hand. Gongard (1983) considers Loja Province as a 'Sahel' zone, a transition zone towards the Peruvian desert. Increasing human pressure on forests, mainly for agricultural purposes, is significantly altering natural vegetation and is even hastening this desertification process.

TEMPERATURE

Due to the wide range of altitudes that occur in the province, it is difficult to give a general idea about temperature regimes. Temperature is closely related to altitude and, due to the equatorial position, not very variable throughout the year (yearly fluctuation in monthly average temperature is comprised between 0.6 and 2.4 °C) (Maldonado Astudillo, 1985).

Only 13 stations register temperature in Loja Province, some of their climatograms being shown in Figure 1.5. Generally, months with high precipitation are also the warmest months. This is the result of the influence of the warm and humid El Niño current that influences the region from January till April (Organización de los Estados Americanos, 1994).

Table 1.3. Different soil formations in Loja Province (Organización de los Estados Americanos, 1994)

| Soil formation | Characteristics | Classification | Frequency (%) |
|--|--|---------------------------------|---------------|
| S | Young, shallow, eroded soils of variable texture. Found on different humidity regimes. | Orthent | 39.6 |
| F | Red to brownish-yellow soils with deep pedologic alterations. Caolinite clay types. Base saturation < 35 %. Humid regimes. | Tropepts | 18.4 |
| K | Red to brownish-yellow clay soils with little disruption in mother material. Base saturation > 50 %. All humidity regimes. | Udalf – Tropepts | 8.7 |
| G | Red to brownish-yellow soils mixed with rock fragments. Base saturation > 50 %. All humidity regimes. | Udalf – Ustalf – Tropept | 6.3 |
| R | Acid, red clay soils with presence of iron and aluminium oxides. | Orthox – Tropept – Ustalf | 4.1 |
| T | Alluvial soils with variable granulometry. | Fluvent - Tropept | 2.2 |
| V | Alkaline clay soils with more than 30 % montmorillonite clay. Dry areas. | Ustert – Torrert | 2.1 |
| A | Shallow paramo soils with very high organic matter content (> 30 %). | Aquept | 1.0 |
| I | Acid brownish-red clay soils with high montmorillonite clay content. | Ustalf –Tropept | 1.5 |
| N | Black sandy clay soils. Base saturation > 50 %. | Ustoll | 0.5 |
| L | Young yellow clay soils. Different humidity regimes. | Udalf – Ustalf Tropept – Orthid | 0.4 |
| E | Very shallow eroded soils of dry areas with abundant rock fragments | Orthent | 0.5 |
| Association of different soil formations | | | 14.9 |

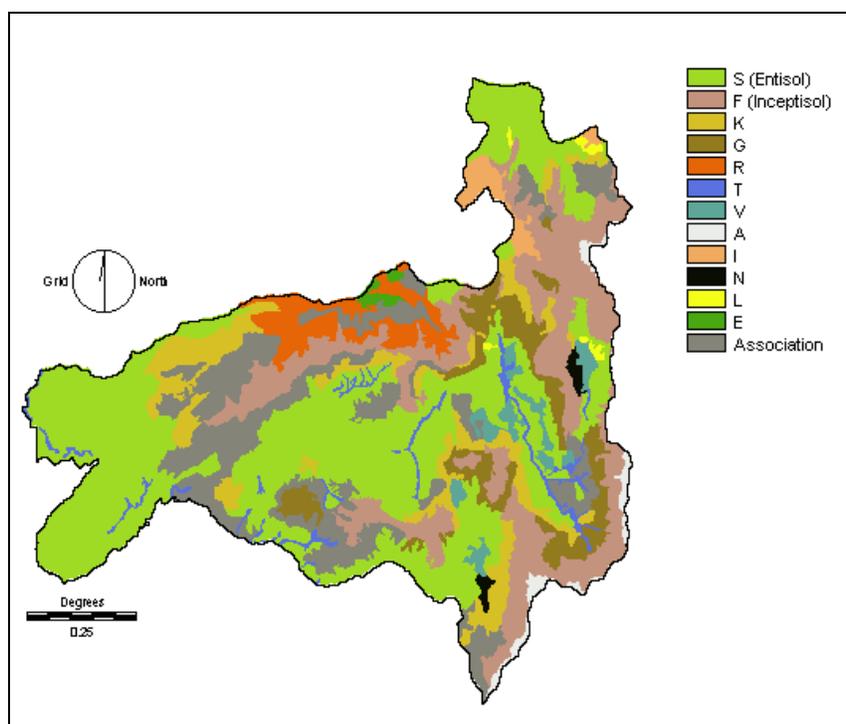


Figure 1.4. Soil map of Loja Province with its soil formations (Organización de los Estados Americanos, 1994)

Using the mean average annual temperature classification adapted by Cañadas (1983), the most common temperature regime is tropical (mean average temperature $> 22\text{ }^{\circ}\text{C}$), covering 35.6 % of the area and occurring in the lower western plains below 1,200 masl. Next are the subtropical (18 - 22 $^{\circ}\text{C}$) and temperate (12 - 18 $^{\circ}\text{C}$) regimes, which cover respectively 26.2 and 27.4 % of the area (Maldonado Astudillo, 1985). Cold temperature regime is rare and covers only 10.8 % of the area. Absolute maxima are 38.6 $^{\circ}\text{C}$ (Zapotillo) and 37.0 $^{\circ}\text{C}$ (Mácará), while absolute minima are 0.3 $^{\circ}\text{C}$ (Loja) and 2.0 $^{\circ}\text{C}$ (Saraguro), indicating that no frost has been observed by the climatic stations in Loja Province in the period 1971-1986 (Organización de los Estados Americanos, 1994).

PRECIPITATION

Loja Province shows the three pluviometric regimes that can be found in Ecuador: the Costa or Pacific regime with its unimodal precipitation (maximum at the beginning of the year); the Sierra regime with two maximums (at the end and at the beginning of the year) and the Oriente or Amazon regime with one maximum in the middle of the year (Maldonado Astudillo, 1985).

Precipitation in Loja Province is being registered by 33 stations, with mean annual precipitation ranging 383 mm (La Toma) – 1,448 mm (Lauro Guerrero). It is very variable in time. From January till April - May, the El Niño Current brings hot humid air, which produces convective rains, to the mainland. This phenomenon influences climate in the whole province, with exception of a small region around the town of Jimbilla, where climate is totally marked by Amazonian influences. Movement of the Intertropical Convergence Zone has little effect on the lower parts of the province (under 1,000 masl), where rains are unimodal, with a yearly maximum only determined by the El Niño Current. In mountainous regions pluviometric regime is more influenced by the movement of this Intertropical Convergence Zone, where six weeks after the equinox this low-pressure zone brings high precipitation in November - December. Another factor is the presence of trade winds, which with their prevailing south-eastern origin carry humid Amazon air to the eastern part of the Andes (e.g. city of Loja), inducing orographic rains. Loja Province forms a clear transition zone between the Andes, with strong Amazon influences, and the Peruvian Sechura desert. The pluviometric regime, unimodal in the lower western part, gradually homogenises moving eastwards.

Precipitation is also very variable in space. Semi-arid (< 500 mm annual precipitation) zones can be found in the eastern part of the province, in the north-eastern outermost part and around the city of La Toma, covering 2.6 % of the area. The most common precipitation regime is the semi-humid regime, which can be found in most of the province (61.2 %), especially in the central and western parts. The humid precipitation regime ($> 1,000$ mm annual precipitation) can be found in 36.2 % of the area mainly at the northern and eastern province limits.

Important annual differences in precipitation, influenced by changes in the still poorly understood El Niño Current, are also an important characteristic of the climate in Loja Province. This is illustrated by precipitation figures for March, the month with the highest precipitation, for the years 1968, 1972, 1982 and 1983, the latter being a notorious El Niño Year (Table 1.4) (PREDESUR, 1998). Differences between different years are clear and more pronounced in coastal areas.

Table 1.4. Examples of yearly differences in monthly precipitation in different zones of Loja Province: example for March (PREDESUR, 1998)

| | Zapotillo (Costa) | Loja (Sierra) |
|----------------------------|-------------------|---------------|
| Mean precipitation (mm) | 246 | 123 |
| Precipitation in 1968 (mm) | 0 | 215 |
| Precipitation in 1972 (mm) | 790 | 201 |
| Precipitation in 1982 (mm) | 0 | 143 |
| Precipitation in 1983 (mm) | 680 | 220 |

OTHER CLIMATE CHARACTERISTICS

Air humidity is generally high, with annual averages varying from 63 % (La Toma) to 88 % (Gonzanamá). Obviously relative humidity is highly correlated with precipitation, with highest values occurring from December till May. Relative humidity is also higher in temperate temperature regimes (70 - 85 %) than in tropical regimes (60 - 70 %).

Wind directions are very variable in Loja Province, as they are strongly influenced by the relief. Only in the eastern parts of the province, marine winds generally come from the west. Higher parts tend to receive stronger winds, especially in the months of July and August, due to considerable air pressure gradients induced by the large distance to the Intertropical Convergence Zone.

The combination of lower air humidity and stronger winds in the months of July and August result in high evaporation rates during these months, quickly depleting the soil humidity reserves.

2.1.2.5 BIOTIC ENVIRONMENT

The unique topography combined with desert and rain forest influences have given Loja Province a characteristic and unique biotic environment. Humboldt considered Loja Province as the botanical garden of Ecuador (Organización de los Estados Americanos, 1994).

Various classification schemes have been developed to reflect specific conditions of climate and vegetation. Köppen and Holdridge classifications have been applied for Loja Province (Organización de los Estados Americanos, 1994).

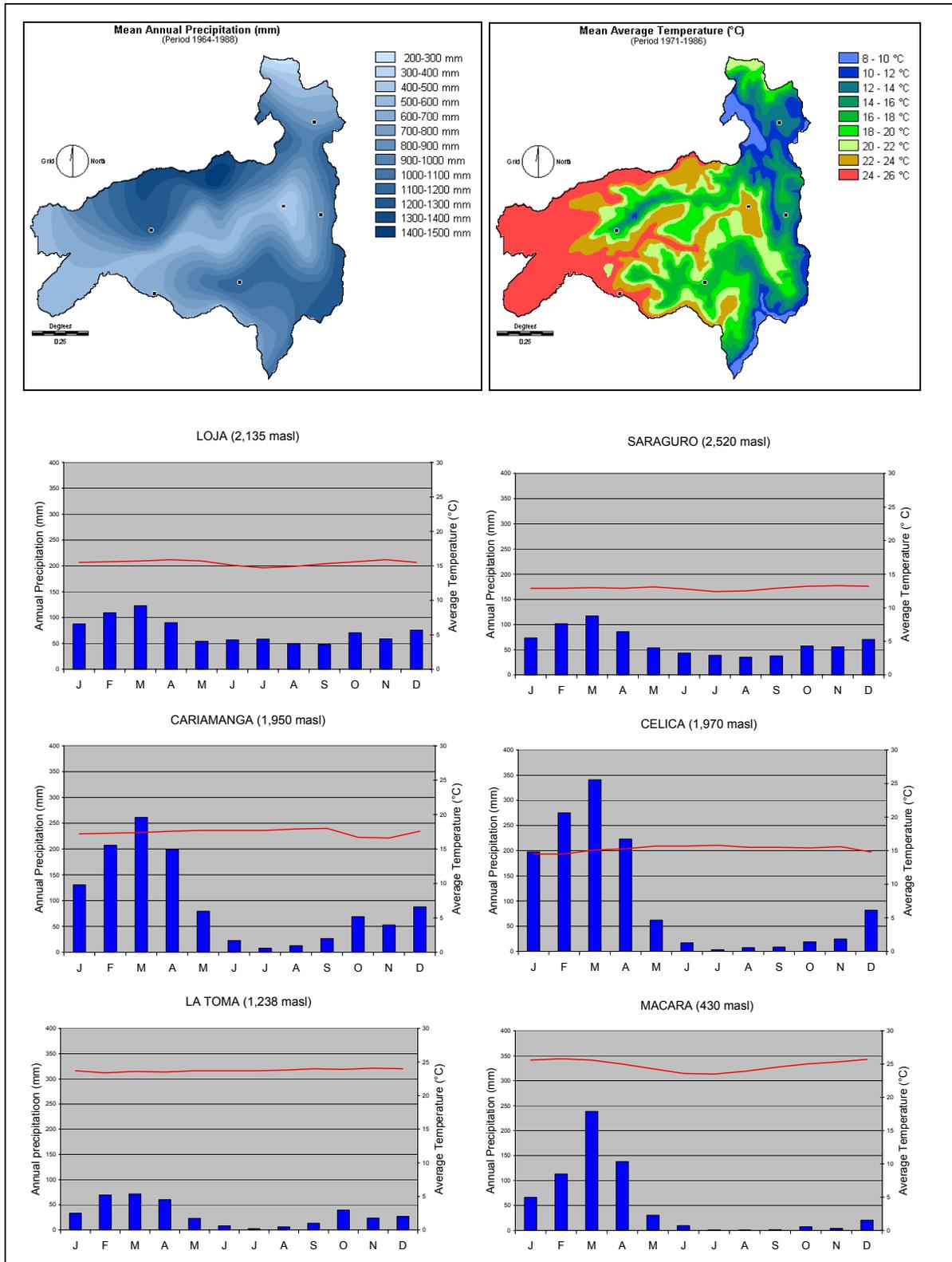


Figure 1.5. Isotherm and isohyete map of Loja Province and climatograms of six different climatic stations (Organización de los Estados Americanos, 1994)

The empirical climatic classification developed by Wladimir Köppen in 1930 is based on temperature and precipitation. It distinguishes Humid Tropical climate (A) with high temperatures and large amounts of precipitation, Arid and Semi-Arid climates (B) characterised by low precipitation, Humid Temperate (C) and Humid Cold (D) climates containing the maritime and continental temperate climates, and finally the Polar (E) climates. Loja Province (Figure 1.6) is divided in Tropical Savannah climate (Aw), covering 32.6 % of the area and characterised by an annual temperature above 18 °C and annual precipitation, occurring in summer, between 750 and 1,000 mm. This zone is characterised by grasses and dispersed trees (*Acacia*, *Bombax*, *Ceiba*, *Cavanillesia*, *Machura* and *Jacaranda*). The Semi-arid Steppe climate (Bw) covers 30.1 % of Loja province. These zones are all situated under 1,200 masl and can be considered as the extension of the Sechura desert occurring in Northern Peru. This zone has sparse vegetation with grasslands and some trees (*Tabebuia*, *Pithecellobium*, *Guazuma* and *Prosopis*). The Humid Temperate climates are divided by the presence (Cw) and absence of a dry winter (Cf). They cover 26 % of the area. The Cf climate has forest (*Podocarpus*, *Alnus*, *Pouteria* and *Juglans*) or grassland vegetation while *Cordia*, *Chyonanthus*, *Lafoensia* and *Annona* trees characterise Cw climates. Finally, the Humid Cold climate with dry winter (Dw) is covering 11.3 % of the area, mostly above 3,000 masl. In these zones, air humidity is very high resulting in a typical cloud forest with typical trees (*Myrcianthes*, *Weinmannia*, *Oreopanax*, *Cyathea* and *Chinchona*) and bromeliads. Higher zones have the typical paramo vegetation with the dominant *Stipaichu*-grasses (PREDESUR, 1998).

In 1947, Holdridge proposed a life zone classification more appropriate to the complexities of tropical vegetation. Holdridge defined the life zones according to biotemperature (not taking into account temperatures below 0 °C and above 30 °C), precipitation and evapotranspiration. A total of 38 different life zones were thus described. Loja Province alone accounts for 11 of these life zones (Figure 1.7) with Dry Forest, with its different altitudinal subdivisions, and Moist Forest accounting for respectively 41.1 % and 25.0 % of Loja Province (PREDESUR, 1998).

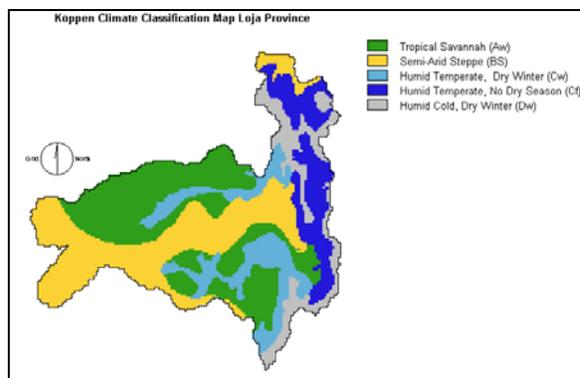


Figure 1.6. Köppen climate classification of Loja Province (Organización de los Estados Americanos, 1994)

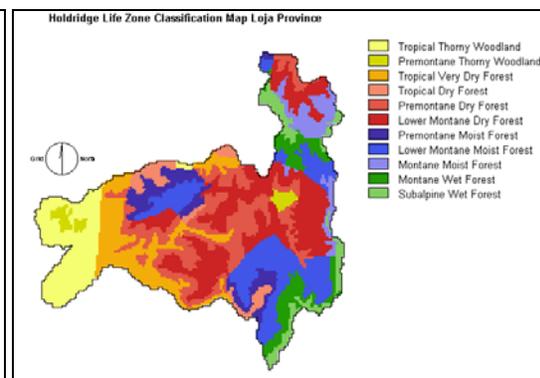


Figure 1.7. Holdridge life zone classification of Loja Province (Organización de los Estados Americanos, 1994)

2.1.2.6 ECONOMY AND AGRICULTURE

The economic structure of Loja Province is based on agricultural and livestock sectors, in which the population uses the natural resources to obtain plant and animal products. Loja Province holds no industry. Commercial activities, concentrated mainly around the cities, are the only other important economic activity.

In 1990, 56.3 % of the active population was working in agriculture and animal husbandry. Projections for 2000 indicate a fall to 49.4 %, but the sector remains clearly the most important for the province (Organización de los Estados Americanos, 1994).

Main agricultural products in Loja Province are in order of importance: maize (*Zea mays*), coffee (*Coffea arabica*), beans (*Phaseolus* spp.), sugar cane (*Saccharum officinarum*), banana (*Musa* sp.), cassava (*Manihot esculenta*), peanut (*Arachis hypogaea*) and rice (*Oryza sativa*). Husbandry consists mainly of the breeding of cattle and pigs with regional presence of goats, sheep and poultry.

Production systems consist mainly of small family-run farms that use traditional farming techniques with little inputs. The basic objective of these farms, predominantly with extensions covering less than 5 ha (70.8 %), is subsistence farming, often limited by the fragile, ecological conditions, resulting in very low yields.

Only 4.3 % of the area of Loja Province is considered well suited for crop cultivation and this area is already almost entirely under use. Some 37.7 % of the province is considered appropriate for grassland but consists actually still of vast areas of natural vegetation (PREDESUR, 1998).

Looking in detail at fruit production, it is striking that main fruit production consists of introduced species while native fruit production is of little significance, at least when considering officially registered fruit production figures (Table 1.5).

Table 1.5. Annual fruit production in Loja Province, figures for 1995 (PREDESUR, 1998)

| Fruit | Annual production (ton) |
|---|--------------------------------|
| Banana (<i>Musa</i> spp.) | 45,197 |
| Pineapple (<i>Ananas comosus</i>) | 5,191 |
| Coconut (<i>Cocos nucifera</i>) | 2,988 |
| Orange (<i>Citrus sinensis</i>) | 1,094 |
| Papaya (<i>Carica papaya</i>) | 555 |
| Mexican Lime (<i>Citrus aurantifolia</i>) | 377 |
| Avocado (<i>Persea americana</i>) | 296 |
| Ice-cream bean* (<i>Inga</i> spp.) | 108 |
| Sweet Lime (<i>Citrus limetoides</i>) | 103 |
| Cherimoya* (<i>Annona cherimola</i>) | 64 |
| Babaco* (<i>Vasconcellea</i> × <i>heilbornii</i> 'Babacó') | 3 |

* Native fruit species

Recently, Loja Province developed some new non-traditional products for exportation. In 1997, new agricultural export products were flowers (33,550 US\$ FOB) and frogs (35,445 US\$ FOB) (PREDESUR, 1998).

2.2 CHERIMOYA (*ANNONA CHERIMOLA* MILL.)

2.2.1 BOTANY

2.2.1.1 FAMILY ANNONACEAE

Cherimoya (*Annona cherimola* Mill.) belongs to the family Annonaceae, evolutionary, ecologically as well as economically an important plant family. Annonaceae are, with about 2,500 species in 140 genera, the biggest family (Mabberley, 1990) within the order of Magnoliales (Cronquist, 1981), an order of rather primitive angiosperms. Annonaceae are distributed (Figure 1.8) throughout the tropical areas of America (900 species), Africa (450 species) and Australasia (1,200 species). Two clear subfamilies are distinguished: Annonoideae with free spirally arranged carpels and Monodoroideae with united cyclically arranged carpels. Genus *Annona* (ca 150 spp.) is, together with *Guatteria* (265 spp.) and *Duguetia* (100 spp.), one of the largest genera of the family and has its main distribution area in the Neotropics, but occurs in tropical Africa as well (Chatrou, 1999). Commercially, the most significant Annonaceae are genera *Annona* and *Rollinia* (Sanewski, 1991).

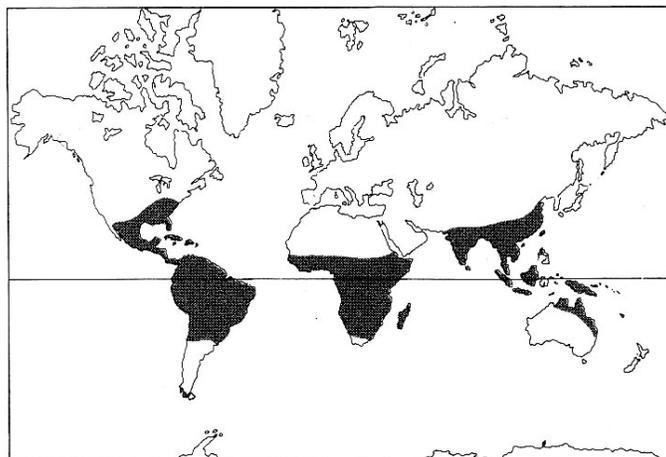


Figure 1.8. Worldwide distribution of Annonaceae (Heywood, 1985)

The large number of species and taxa in Annonaceae, and their subsequent classification problems, cannot be detached from the huge variety in, amongst others, flower, fruit and pollen morphology, habit, habitat preferences and pollination syndromes (Chatrou, 1999). This variety might explain their apparent ability to occupy a range of ecological niches. Recent studies in the upper Amazon Basin revealed that Annonaceae usually are among the five most important plant families in terms of species diversity and abundance (Valencia *et al.*, 1994).

Annonaceae (Figure 1.9) are usually evergreen trees, shrubs or lianas, with resin canals and septate pith in the stems. The entire leaves, typically distichous and without stipules, are often recognizable in the field by their glaucous or metallic sheen. The fragrant flowers, often nodding, frequently open before all floral parts are

fully developed. They are regular and bisexual (rarely unisexual). The perianth consists of three whorls of three tepals. The stamens are usually numerous and spirally arranged. Protogyny is widespread in Annonaceae flowers and is possibly related to a preponderance of crosspollination (Kessler, 1993). The carpels are usually separate, resulting in the fruit being an aggregate of berries (apocarp). In fruits of *Annona* species the berries coalesce with a receptacle to form an aggregate (syncarp). The seeds have ruminant endosperm and are of complicated construction. In many species, the flower acts as a trap for pollinating beetles belonging to Nitidulidae, Staphylinidae, Chrysomelidae, Curculionidae or Scarabaeidae family (Heywood, 1985; Chatrou, 1999).

South American species of Annonaceae were quickly introduced to the Old World for their fruits (notably soursop and sweetsop). The aromatic oils of *Cananga odorata* flowers give the precious ylang-ylang oil, highly rated in the perfume industry. Locally, the perfume of *Mkilua fragrans* is used by Arab and Swahili women. The spicy fruits of the West African *Xylopiya aethiopica* are the so-called 'Negro pepper', used as a condiment, and those of *Monodora myristica* of the same area are used as a nutmeg substitute. Although some *Xylopiya* woods are used, Annonaceae timber is of little importance, except for pliable lancewoods (Heywood, 1985).



Figure 1.9. Examples of Annonaceae species: 1. *Annona squamosa* (a) shoot with leaves in two ranks and axillary flower; (b) young fruit; (c) vertical section of fruit comprising an aggregate of numerous berries with the fleshy receptacle; 2. *Monodora myristica* (a) leafy shoot and flower; (b) androecium of numerous short-stalked stamens and gynoecium comprising united carpels; (c) vertical section of fruit with many seeds; 3. *Asimina triloba* (a) flowering shoot; (b) gynoecium of five free carpels; (c) stamen with anther surmounted by swollen extension of connective; (d) young fruit (Heywood, 1985)

2.2.1.2 GENUS *ANNONA*

The genus *Annona* contains approximately 100 species and can be found in tropical America and Africa (Mabberley, 1990). The name of the genus is derived from the Latin '*annona*' (literally 'yearly produce') and indicates their typical annual producing cycle. The characteristic feature of the genus *Annona* is their fruit that is a syncarpium, formed by amalgamation of many pistils and the fleshy receptacle. *Annona* trees are small trees to 7 m tall, with simple, entire and alternate leaves. The hermaphrodite flowers are yellowish with 3 to 6 tepals and numerous stamens and pistils.

The genus exhibits a large genetic diversity, reflecting the different centres of origin of many of the species. Most of them are believed to have originated in the hot humid lowlands of Central America and northern South America. An exception to this is the cherimoya (*A. cherimola*) which originated in the subtropical Andean highlands (Popenoe, 1970). The chromosome number for most *Annona* species is $2n = 2x = 14$ or 16, with the exception of *A. glabra* which is tetraploid (Kessler, 1993). Many of the species are apparently sexually compatible, e.g. the atemoya is a natural hybrid between *A. cherimola* and *A. squamosa* (Jordán & Botti, 1992).

Numerous species produce edible fruits with a taste that is highly appreciated. Due to the vulnerability of the fruit skin and the short shelf life, *Annona* species do not occupy yet an important niche in the ever-increasing commercial tropical fruit market. Figure 1.10 and Table 1.6 give an overview of the most important edible *Annona* species, with some common vernacular names and their origin.

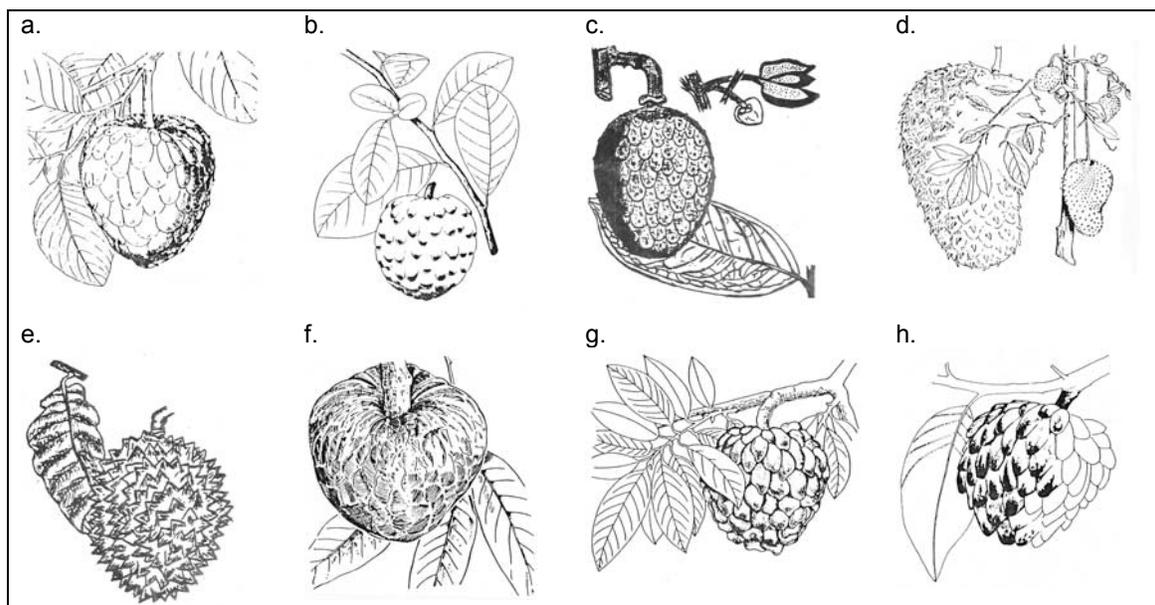


Figure 1.10. Examples of edible *Annona* fruits: a : *Annona cherimola* Mill.; b : *Annona diversifolia* Saff.; c : *Annona montana* Macfad.; d : *Annona muricata* L.; e : *Annona purpurea* Moc. et Sessé; f : *Annona reticulata* L.; g : *A. squamosa* L.; h : *A. squamosa* × *A. cherimola* (León, 1987; Calzada Benza, 1993)

Table 1.6. Overview of the most important edible *Annona* species, their vernacular names and origin (Fouqué, 1972; León, 1987; Morton, 1987; Mabberley, 1990; Teubner, 1990; Ochse *et al.*, 1991; FAO, 1992)

| <i>Annona</i> Species | Vernacular Name | Origin |
|--|---|--------------------------------|
| <i>A. cherimola</i> Mill. | Eng. : cherimoya, custard apple* Spa. : chirimoya Fre. : chérimolier, chérimole Dut. : cherimoya Por. : cherimólia | Andes of Peru and Ecuador |
| <i>A. diversifolia</i> Saff. | Eng.: ilama Spa.: ilama, anona blanca Fre.: ilama | Central America |
| <i>A. glabra</i> L. | Eng.: pond apple, alligator apple Spa.: cayur, corcho Fre.: anone des marais, mamin Por. : araticú do brejo | Tropical America & West Africa |
| <i>A. montana</i> Macfad. | Eng.: mountain soursop, wild soursop Spa.: guanábana cimarrona Fre.: corossolier bâtard Dut. : boszuurzak Por. : araticum apé | Central America |
| <i>A. muricata</i> L. | Eng.: soursop Spa.: guanábana Fre.: corossolier Dut.: zuurzak Por. : graviola | Tropical America |
| <i>A. purpurea</i> Moc.et Sessé | Eng.: soncoya, negro head Spa.: soncoya, cabeza de negro Fre.: atier Por. : cabeça de Negro | Central America |
| <i>A. reticulata</i> L. | Eng.: bullock's heart, custard apple* Spa.: corazón, anón, mamón Fre.: coeur de boeuf, cachiman Dut.: custardappel, ossehart Por. : coração de boi | Tropical America |
| <i>A. scleroderma</i> Saff. | Eng.: poshté, cawesh Spa. : anona del monte | Central America |
| <i>A. senegalensis</i> Pers. | Eng.: wild custard apple | West Africa |
| <i>A. squamosa</i> L. | Eng.: sugar apple, sweetsop, custard apple* Spa.: anón, anona blanca, saramuyo Fre.: pomme cannelle, attier Dut.: suikerappel, kaneelappel Por : ata, fruta do conde, pinha | Tropical America |
| <i>A. squamosa</i> × <i>A. cherimola</i> | Eng.: atemoya, custard apple* | Artificial hybrid |

* Custard apple is a general name applied for some *Annona* spp., but should strictly only be applied for *A. reticulata*

2.2.1.3 ANNONA CHERIMOLA MILLER

Cherimoya (*Annona cherimola* Mill.; Syn. *Annona tripetala* Ait.) is considered by

many, from a quality point of view, as the best fruit of the genus *Annona*, and even as one of the best subtropical fruits, hence its name 'queen of subtropical fruits' (National Research Council, 1989; Gardiazabal & Rosenberg, 1993).

The cherimoya (Figure 1.11) tree habit is erect but low branched and somewhat shrubby or spreading. Its height is ranging from 5 to 9 m. The leaves are briefly deciduous to semi-deciduous (just before spring flowering) due to the mitriform petiole concealing the bud. They are alternate, 2-ranked, with minutely hairy petioles; ovate to elliptic, short blunt pointed at the apex; slightly hairy on the upper surface, velvety on the underside (Morton, 1987).

Fragrant flowers, solitary or in groups of 2 or 3, on short hairy stalks, have 3 outer, greenish, fleshy, oblong petal-like tepals and 3 smaller inner tepals. The syncarp fruit, formed by amalgamation of pistils and receptacle, is conical or somewhat heart-shaped, 10 to 20 cm long and up to 10 cm in width, weighing on average 150 - 500 g, but extra large specimens of 2.7 kg or more have been reported (Farré Massip, 1999). The skin may be smooth with fingerprint-like markings or covered with conical or rounded protuberances. The fruit is easily broken or cut open, exposing the snow-white, juicy flesh of pleasing aroma and delicious, subacid flavour; and is containing numerous hard, brown or black beanlike, glossy seeds, 1.25 to 2 cm long (Morton, 1987). The fruit is composed of an exocarp (fruit skin), occupying between 15 and 25 % on a weight basis, an edible mesocarp (pulp and thalamus), varying between 65 and 80 %, and seeds, fluctuating ranging 3 - 10 % (Gardiazabal & Rosenberg, 1993). The diploid chromosome number of cherimoya is $2n=14$ (Ronning *et al.*, 1995) although $2n = 16$, has also been reported (Pascual *et al.*, 1993).

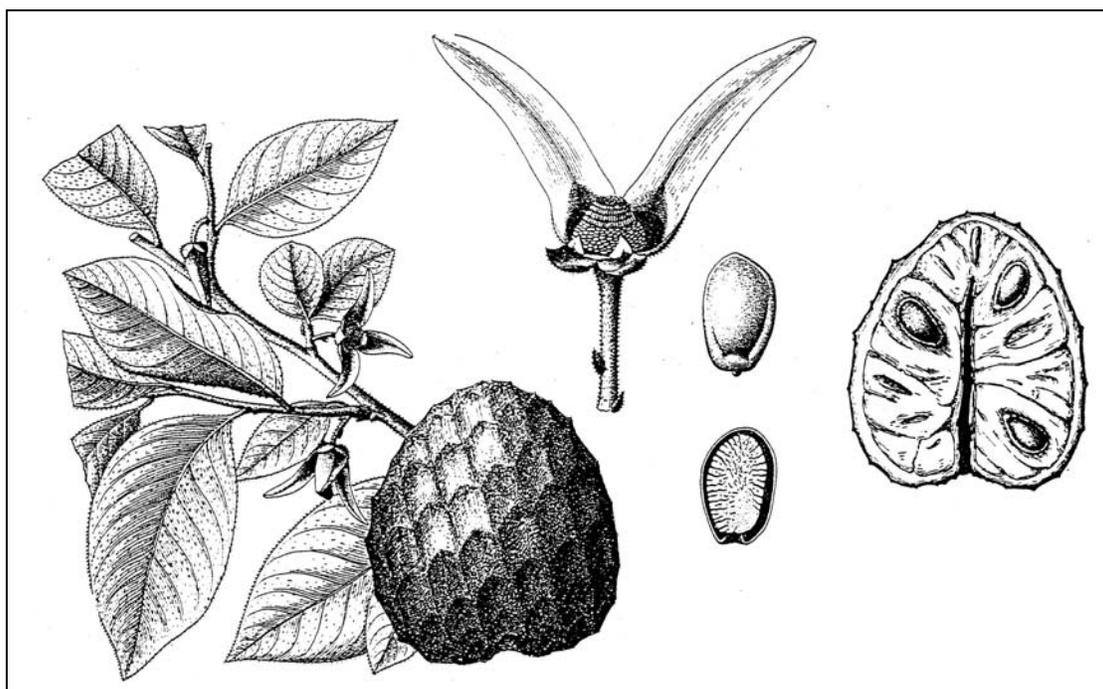


Figure 1.11. *Annona cherimola* Mill.: leaf, flower, fruit and seed (Van Damme, 1992)

2.2.2 ORIGIN

Although there is still no definite agreement about the exact location of the centre of origin of cherimoya, there is little doubt that Loja Province is situated in an important centre of biodiversity of this Andean fruit species.

Most early chroniclers agree about the Andean region and more specifically the Loja region as being the centre of origin of cherimoya trees, even though some of them put Central America as the cherimoya's centre of origin. In an ethnogeographic study done by Estrella (1988), Cobo, an early Spanish chronicler, is indeed cited to report cherimoya to be a 'new' fruit for the Andean region, but Farré Massip & Hermoso González (1995) explained this confusion by stating that some early chroniclers may have confused the cherimoya with other Annonaceae (*A. reticulata* or *A. squamosa*) which undeniably have a Central-American origin. In support of this hypothesis, Popenoe (1974) argued that the common name that the fruit bears, even in Mexico, is of Quechua origin, and moreover that terra cotta vases modelled from cherimoya fruits have been dug up repeatedly from prehistoric graves in Peru, showing their longstanding history in the area. Wolters (1999) describes ceramic cherimoya-formed vases found in the Ecuadorian Valdivia culture (3,500 – 1,600 A.C.) and suggests that this early culture played an important role in crop plant exchange between Peru and Middle America. Pittmann (1956) suggested that very early on, humans and maybe some animals distributed cherimoya seeds into South Mexico and Central America where they established as relatively wild plants. Popenoe (1921) presented it as being native to the valley of Loja where it grows in the wild, forming dense forest stands. Guzman (1951) states that cherimoya probably originates from the inter-Andean slopes of the Marañón river basin, covering northern Peru and southern Ecuador, at elevations ranging 1,500 – 2,200 masl. Most authors consider the temperate, dry inter-Andean valleys of southern Ecuador and northern Peru as being the centre of origin of the cherimoya (Chandler, 1962; Purseglove, 1968; Zeven & Zhukovsky, 1975; George *et al.*, 1987; National Research Council, 1989; Richardson, 1990; Sanewski, 1991; PROSEA, 1992).

León (1987) on the other hand, argued that the biological evidence for the exact location of the centre of origin of the species is difficult to define and will probably remain dubious as Annonaceae species tend to naturalise easily. He hereby refers to what happened to *A. squamosa* in India. Recent studies with molecular markers, cited by Hermoso González *et al.* (1999), would suggest the possibility of Mesoamerica being a second centre of origin.

2.2.3 CULTIVATION

2.2.3.1 PROPAGATION

In natural state, cherimoya easily disperses by generative propagation. Seeds show

a hard testa and an immature embryo, rendering quick germination more difficult, but promoting a scattering of the seeds. Wild animals like birds, rodents, wild boars or straying livestock help spreading fruits, and thus seeds. Traditionally, cherimoya is propagated by seed, but the resulting plants vary widely in yield and fruit quality (Purohit, 1995). If conserved adequately (dry and at low temperature), seeds maintain their viability for several years (Popenoe, 1974; Garwood, 1995). Seed germination can be improved by soaking the seeds 24 to 48 hours in water or gibberellic acid (GA₃) (Francioso Tijero, 1992; Gardiazabal & Rosenberg, 1993). Some disagreement exists about the supposed presence of dormancy in *Annona* seeds. Sanewski (1991) claimed that no dormancy exists, whereas most authors indicated the presence of dormancy (Hayat, 1963; Ellis *et al.*, 1985b; Purohit, 1995; Garwood, 1995). Germination of cherimoya seeds also depends on temperature, taking from three to four weeks at temperatures between 28 - 32 °C and up to three to six months at lower (< 20 °C) temperatures (George *et al.*, 1987; Sanewski, 1991; Richardson & Anderson, 1993b). Conservation of cherimoya seeds is another point of disagreement. Sanewski (1991) and Purohit (1995) claimed that germinating power declines very rapidly upon harvest, recommending an immediate use of the seeds. Popenoe (1974) and Garwood (1995) stated that cherimoya seeds can be conserved for a long time (3 - 4 years) in a dry environment. Ellis *et al.* (1985b) described cherimoya seeds as orthodox, implicating that adequately dried seeds can be stored for a long time in a dry and cold environment.

Due to cross pollination, cherimoya shows a high degree of heterozygosis and seed is not true-to-type. Therefore commercial cherimoya cultivation uses vegetative propagation in order to homogenise crop quality. This is done by grafting a selected cultivar on a seedling rootstock (Sanewski, 1991). Propagation by cuttings is very uncertain. Some authors even consider it impossible, due to the low morphological potential of the species, especially at rooting level (George & Nissen, 1987; Tazzari *et al.*, 1990; López Encina *et al.*, 1999). A grafted plant can be obtained in 18 to 24 months depending on growing conditions. The rootstock influences a tree's vigour and its tolerance to adverse soil and climatic factors. Because rootstocks are produced from seed, there are considerable differences in performance among individual trees (Sanewski, 1991). Propagation of cultivars on their own roots or on clonal rootstocks would eliminate this variability. The use of superior rootstocks is therefore the first step to obtain adequate cherimoya plants. To avoid a twisted taproot, rootstock seedlings should be sown in large containers containing light potting mixtures. At 15 months, rootstocks are up to 1 m high and ready to be grafted or budded. This is by preference done when sap flow starts again after the period of dormancy. The grafting wood, carefully chosen from trees with known performance, should be selected from the previous season's hardened (browned-off) growth and should contain three to six buds. The seedlings should be grafted 10 to 15 cm above ground level, using whip, whip and tongue, or cleft graft (PROSEA, 1992). T- or chip budding is also common (Sanewski, 1991). Success rates of grafting and budding seem to be very variable and strongly correlated with the moment of grafting (PROSEA, 1992; Gardiazabal & Rosenberg, 1993)

Recently investigation on *in vitro* propagation of cherimoya has been reported, especially in Spain (López Encina *et al.*, 1999) and Chile (Jordán *et al.*, 1993; Cautín Morales *et al.*, 1999), where techniques and protocols on somatic embryogenesis, adventitious organogenesis and cellular cultures for cherimoya are being developed. The strong oxidative capacity, due to the high phenolic compounds in the plant material, complicate *in vitro* culture so it is advised to collect material in periods of low phenolic production, e.g. with high vegetative growth and low lignification (Cautín Morales *et al.*, 1999). *In vitro* propagation could be a good alternative to eliminate influences of rootstock on grafted plants (Rasai *et al.*, 1995), but due to high costs, no commercial *in vitro* plantations have been reported to date.

2.2.3.2 CULTIVATION PRACTICES AND CROPPING

Grafted cherimoya plants should be planted, best in dormant stage, on site at distances varying between 5 and 9 m with classical trellis systems (Morton, 1987, Franciosi Tijero, 1992; Gardiazabal & Rosenberg, 1993) resulting in a total of 125 to 400 plants per hectare. Scientists in Chile are currently evaluating the possibilities of high density plantations of 1 m x 4 m and 2 m x 4 m which lead to 1,250 to 2,500 plants per hectare, but strong tree training is needed (Gardiazabal & Rosenberg, 1993).

Cherimoya trees need adequate tree training to produce good quality yields, to ease harvesting and to avoid limb breakage (Sanewski, 1991). Tree training consists of formation pruning during the first four to five years and fructification pruning that has to be carried out yearly before the tree breaks dormancy. Common trellis systems for cherimoya are the open-goblet system and the central leader system (Sanewski, 1991; PROSEA, 1992), depending on ecological conditions and plant distances. After adequate tree form is obtained, tree height, up to 4 m, and shape should be maintained and fructification pruning should be applied in order to maximise fructification and minimise tree growth. Most flowers, and thus fruits, appear on one-year wood (Franciosi Tijero, 1992), a fruit set comparable to that of peach (*Prunus persica*). This fructification system has its implications for fructification or winter pruning, where two-year-old wood has, apart from tree formation, no productive function. Although most cherimoya growers apply a soft to medium pruning, which means removing most of the two-year-old wood, studies carried out in Spain showed that a total pruning of the two-year-old wood did not decrease total yield and increased fruit weight (Farré Massip *et al.*, 1999). Defoliation, manually or using urea and ethephon (Ethrel[®]) (George *et al.*, 1987), can be used to induce stronger, earlier and more uniform flowering and subsequent earlier harvest.

Compared to other deciduous fruit trees, cherimoya trees have very high nitrogen, potassium and magnesium requirements and low phosphorous and calcium demands. In normal conditions a yield of 14 t/ha extracts: 95 kg/ha of N, 4.6 kg/ha of P, 38 kg/ha of K, 9 kg/ha of Ca, and 27.5 kg/ha of Mg (Farré Massip *et al.*, 1999). There is also a need for iron to avoid iron chlorosis, especially in calcareous soils

(Farré Massip *et al.*, 1999), and manganese, zinc and boron. It is common to apply fertiliser through fertigation to reduce labour costs (Grossberger, 1999).

Water deficiency reduces fruit set and fruit growth. Water shortages, depending on monthly precipitation, tree spacing, tree size and soil type should be complemented using irrigation. Plant water use peaks during flowering and fruiting (Figure 1.12) (Sanewski, 1991), but generally a producing cherimoya plantation needs 8,000 to 10,000 m³ of water per hectare, equivalent to 800 - 1,000 mm rainfall/year (Franciosi Tijero, 1992). Farré Massip *et al.* (1999) and Gardiazabal & Rosenberg (1993) however, mention lower water requirements, respectively 5,500 – 6,500 m³/ha.year in Spain and 4,500 m³/ha.year in Chile. Commercial cherimoya plantations use sprinkler or drip irrigation although in less developed plantations, e.g. in Peru (Franciosi Tijero, 1992), gravity irrigation is also commonly used. It is important to avoid soil water surpluses, as cherimoya is very susceptible to flooding, which results in irreparable flower and fruit fall.

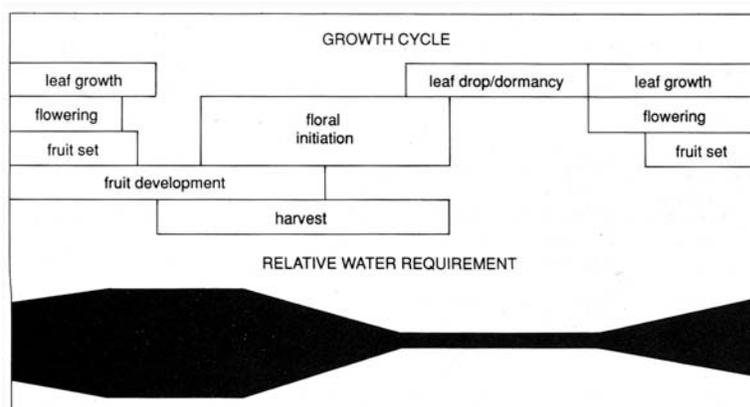


Figure 1.12. Relative water requirement of cherimoya during growth cycle (Sanewski, 1991)

Cherimoya fruit production starts two to four years after planting, reaching full production approximately 8 years after planting. Yearly, the crop follows a determined cycle, starting, after a period of dormancy, with leaf growth and flowering. The latter continues during a period of up to five months. Fruits are ready to be harvested five to eight months after flowering (Undurraga, 1989; Sanewski, 1991). Due to the long period of flowering, harvest period is also extensive, with weekly picking rounds during several months. Each tree bears, depending on plant distances, tree training and cultivar, between 10 and exceptionally 300 fruits/year (Morton, 1987) with an average of 30 fruits/year (IICA, 1989). Yields vary between 6 - 8 ton/hectare in Peru (Franciosi Tijero, 1992) and Mexico (Agustín, 1997), over 10 - 15 ton/hectare in most cherimoya cultivating countries (National Research Council, 1989; PROSEA, 1992; Farré Massip *et al.*, 1999) to 25 - 30 ton/hectare in highly technified plantations in Chile (Gardiazabal & Rosenberg, 1993). Individual fruit weight varies between 150 and 2,000 g (Ibar Albiñana, 1986) with an average weight between 300 and 400 g (Calzada Benza, 1993).

2.2.3.3 POLLINATION

Pollination is one of the main limiting factors in cherimoya production. The cherimoya flower is hermaphroditic and exhibits protogynous dichogamy, female parts being receptive before pollen is shed (Schroeder, 1943; Thakur & Singh, 1965). Dichogamy appears to be the main factor limiting self-pollination (George *et al.*, 1992). Fruit set is also adversely affected by a high degree of pollen sterility, the negative influence of environmental conditions, especially climate, and by the absence of effective pollinators (Jordán & Botti, 1992).

The flowering cycle, which depends mainly on climate, starts with the opening of the flowers, usually between late afternoon and noon of the following day (Rosell & Galan, 1995), and the stigmas being receptive for pollen. This female phase takes about 25 - 35 hours (Soria *et al.*, 1991), depending on environmental conditions. Only in the evening of the following day do anthers release pollen and is the flower in its male phase (Figure 1.13). Some authors state that the rhythm of change from female flower to male stage occurs synchronously and simultaneously at tree level and even at plot level (Rosell & Galan, 1995), preventing simultaneous presence of female and male flowers and impeding insect pollination, while other authors describe the presence of an overlap of the two flowering stages (Garcia del Corral, 1989).

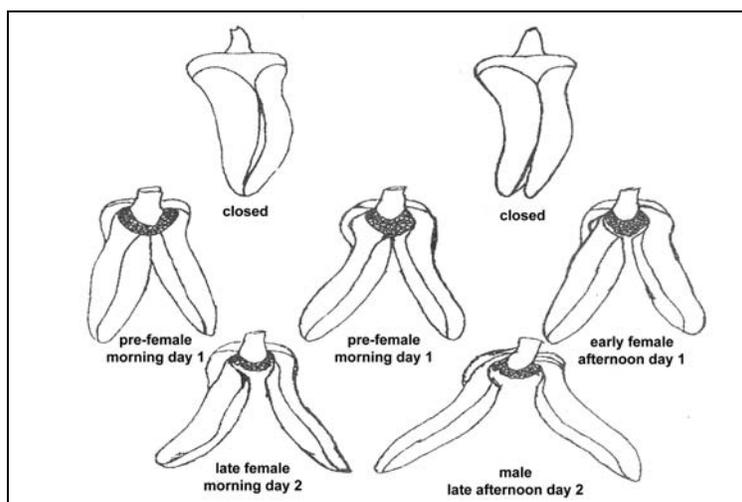


Figure 1.13. Cherimoya flowers at different moments, showing protogynous flowering (Hermoso González *et al.*, 1997)

Outside the centre of origin, natural pollination is low, varying between 0.5 and 5 % of fruit set, with hand pollination being used to increase fruit set (Richardson & Anderson, 1990). There is a lot of uncertainty about natural pollination mechanisms, demonstrated by the heavy fruit set in wild cherimoya stands in the centre of origin. Most authors consider the presence of natural pollinators as the reason for natural fruit set whereas other authors (Gazit *et al.*, 1982; Ibar Albiñana, 1986; George *et al.*, 1989) state that exact environmental conditions of cool temperature and high humidity (> 70 % RH) can prevent the loss of stigma receptivity before anther

dehiscence thus allowing natural self-pollination. Saavedra (1977) showed an increase in fruit set by spraying open flowers with water and Hofmann & Hofmann (1987) observed that in its native home of Ecuador and Peru, cherimoya sets heavily during cool misty weather. Observations on fruit set patterns in Australia have indicated considerable seasonal variation between and within orchards in the same region suggesting possible environmental causes for poor fruit set (George *et al.*, 1989).

Studies in Israel (Gazit *et al.*, 1982), Australia (George *et al.*, 1989) and Florida (Nadel & Peña, 1994) have shown that beetles (Coleoptera) of the family Nitidulidae (genera *Carpophilus* and *Uroporus*) are important pollinators of *Annona* flowers, while in Spain (Hermoso González *et al.*, 1997; Guirardo Sánchez *et al.*, 2001) and Italy (Continella *et al.*, 1996) *Orius* species (Hemiptera) seem to play a role in natural pollination. These insects are capable of transferring pollen from the anthers to the stigmas within the same flower or to the stigmas of other nearby flowers (George *et al.*, 1989).

Hand pollination can increase fruit set considerably (Schroeder, 1941; George *et al.*, 1992), especially when flowers are pollinated with pollen from the same cultivar. This indicates a degree of cross-incompatibility between cultivars (Richardson & Anderson, 1996). Pollen should be collected from male stage flowers, dried and conserved with a maximum of 3 days (du Preez, 1996a), and applied on female stage flowers, using a brush or a puffer pollinator gun (López-Cózar Martínez, 1987). Hand pollination is a common agricultural practice in California (Grossberger, 1999), Spain (Soria *et al.*, 1991; Guirardo Sánchez *et al.*, 2001), Chile (Gardiazabal & Rosenberg, 1993), Australia (George *et al.*, 1987) and New Zealand (Richardson & Anderson, 1990). Advantages include a higher yield of well-formed, easily reachable fruits and possibilities to obtain an early, well-priced harvest. Disadvantages are intensive labour, although some is recuperated by an easier harvest, and the slightly higher seed content due to optimal development of all ovaries (Guirardo Sánchez *et al.*, 2001). Trials with gibberellin treatments (1,000 ppm) on atemoya flowers realised in Florida (Campbell, 1979) showed the possibility to obtain, often seedless, fruit set, but fruit quality and size was inadequate compared to natural and artificial pollination.

2.2.3.4 PESTS AND DISEASES

Cherimoya is susceptible to different pests and diseases, which vary according to country of cultivation and probably to cultivar, but is probably resistant to nematodes (Morton, 1987).

Major plant diseases are bacterial wilt (*Pseudomonas solanacearum*), producing collar rot, tree decline and eventual tree death, Oidium (*Oidium* sp.) and *Botrytis cinerea*. Important fruit diseases are black cancer (*Phomopsis annonacearum*), diploidia rot (*Botryodiplodia* spp.), purple blotch (*Phytophthora* sp.), *Cylindrocladium* spot (*Cylindrocladium* sp.) and *Botrytis cinerea*, all increasing under moist or wet

conditions (Sanewski, 1991; Franciosi Tijero, 1992; PROSEA, 1992). Antracnose (*Colletotrichum gloeosporoides*) seems to be a problem in warmer cherimoya producing zones such as Florida (Cockshutt, 1990) and Australia (Hutton & Sanewski, 1992).

The most important pest is the fruit fly, belonging to the genus *Anastrepha* in Southern America with exception of Chile (Franciosi Tijero, 1992), to the genus *Ceratitis* especially in Europe (Hermoso González *et al.*, 1999), and to the genus *Bactrocera* in Australia (Sanewski, 1991).

Common pests besides fruit fly are the annona seedborer (*Bephratelloides* sp.), which damages fruits, and leaf miner (*Phyllocnistis* sp.) that can destroy considerable amounts of leaves, thus reducing photosynthesis (Farré Massip & Hermoso González, 1987). Minor pests are mealy bugs (*Planococcus* spp. and *Pseudococcus* spp.), spotting bugs (*Amblypelta* spp.) and scales (*Parasaissetia* spp.) (PROSEA, 1992).

2.2.3.5 CULTIVARS

Right from the beginning, farmers and later researchers started a steady process of selecting the best fruits. The combination of this selection and the fact that most commercial cherimoya plantations are based on grafted trees resulted in the establishment of cultivars with more or less fixed properties. A lot of cultivars have been described since the beginning of this century. As the selection of best cultivars, based on fruit quality, yield, pest resistance, harvest time and rusticity, is still going on, some early-developed varieties are not cultivated anymore whereas others gain importance. Some of the most cultivated cultivars are '*Fino de Jete*', which occupies 95 % of the Spanish cherimoya area (Farré Massip & Hermoso González, 1997), '*Bays*', '*Booth*', '*White*' and '*Pierce*' in the United States and Australia (Grossberger, 1999), '*Rereta*' and '*Bronceada*' in New Zealand (Richardson & Anderson, 1993a), '*Bronceada*' and '*Concha Lisa*' in Chile (Gardiazabal & Rosenberg, 1993) and '*Cumbe*' in Peru (Franciosi Tijero, 1992).

Fruit characteristics can vary within a cultivar. Reasons can be wrong identification of cultivar, but are mostly due to genotype-by-environment interactions (Ellstrand, 1997). The influence from the environment can be due to differences in soil properties, climate, exposure, pathogens, time of harvesting, tree training, tree age, pollination and rootstock (George *et al.*, 1987; Richardson & Anderson, 1993a; Ellstrand, 1997; Hermoso González *et al.*, 1997). Recently, isoenzym, RAPD and AFLP analysis proved to be useful tools in correct identification and reclassification of cherimoya cultivars (Ellstrand & Lee, 1987; Pascual *et al.*, 1993; Ronning *et al.*, 1995; Perfectii & Pascual, 1998; Rahman *et al.*, 1998).

Safford (Popenoe, 1974) defined 5 typical botanical forms based on areoles on fruit skin in order to group cultivars and to describe unknown cherimoya cultivars (Figure 1.14):

- forma '*laevis*' (lisa, smooth) : smooth fruit skin, difficult to discern areoles
- forma '*impressa*' (fingerprint) : areoles are U-shaped depressions
- forma '*umbonata*' : small points at the apex of each areole
- forma '*tuberculata*' : large sharp points at the apex of each areole
- forma '*mamillata*' : areoles form large blunt protuberances

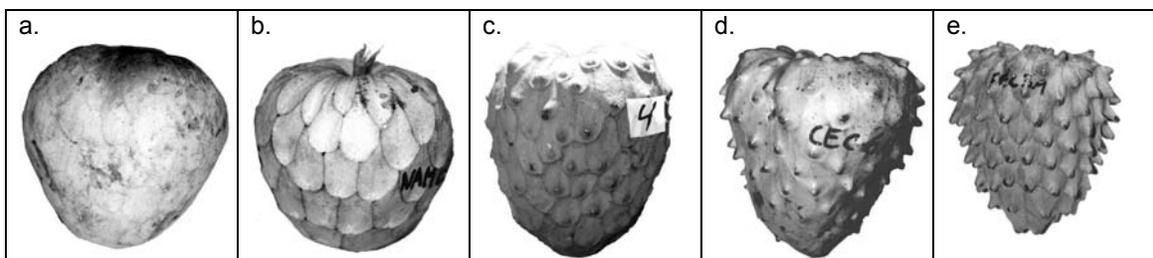


Figure 1.14. Different botanical forms of cherimoya fruits: a: forma '*laevis*'; b: forma '*impressa*'; c: forma '*umbonata*'; d: forma '*tuberculata*'; e: forma '*mamillata*'

2.2.3.6 BREEDING

Genetic improvement of cherimoya is made difficult by its long generation intervals, its highly heterozygous nature and its self-incompatibility (Rasai *et al.*, 1995). Breeding programmes aim at fruits that show good shape, smooth skin, attractive skin colour, low seed content, good flavour and extended post-harvest life and at trees with a healthy growth and ideal branching pattern (Mahdeem, 1990; George *et al.*, 1992).

Australian cherimoya breeding programmes consist, besides evaluation of seedling progeny of cultivars and polyline crosses, generally of interspecific crosses with *A. diversifolia*, *A. reticulata* and *A. squamosa*, with progeny selection and backcrossing. An alternative approach is currently undertaken using mutation breeding, applying γ -radiation or colchicine, to obtain tetraploids, leading to larger fruits, and triploids, resulting in seedless fruits (George *et al.*, 1999). Recently developed *in vitro* techniques could accelerate breeding programmes. These techniques include endosperm culture to obtain triploid plantlets and anther culture combined with chromosome doubling to obtain homozygous diploids that could express recessive genes (Rasai *et al.*, 1995).

Spain is mostly concentrating on germplasm collection and *ex situ* evaluation of numerous accessions (Farré Massip & Hermoso González, 1987). The 'Estación Experimental la Mayora' (Malaga, Spain) actually holds the most inclusive worldwide *ex situ* cherimoya field collection, with about 280 accessions from diverse origin with a particular interest in accessions from Peru and Ecuador (Hermoso González *et al.*, 1999). Other Spanish research aims at marker-assisted selection of accessions and

crosses to allow earlier selection and reduction of population size (Perfectti & Pascual, 1998) and ploidy manipulation to obtain haploid, triploid and tetraploid plants (López Encina *et al.*, 1999). Spain is also conducting preliminary research on genetic transformation, via *Agrobacterium*, to change postharvest characteristics and provide resistance to pests and diseases (López Encina *et al.*, 1999).

In the United States small breeding programmes are undertaken by interbreeding different cultivars by manual cross-pollination and progeny evaluation (Mahdeem, 1990). In Mexico, where cherimoya can be found semi-cultivated, a situation comparable to the Ecuadorian situation, germplasm collection, characterisation and subsequent selection led to the establishment of a commercial plantation with a superior accession, Cortes II-31, showing promising results (Agustín, 1999).

2.2.3.7 POST-HARVEST

Cherimoya, like avocado (*Persea americana*), is a climacteric fruit, characterised by a rapid increase in respiration and ethylene biosynthesis rate (Figure 1.15). It ripens very quickly after harvesting and is, as many subtropical fruits, highly perishable. This feature considerably limits commercial trading of cherimoyas, due to difficulties in long-term transport and storage problems (Sanewski, 1991; Palma *et al.*, 1993).

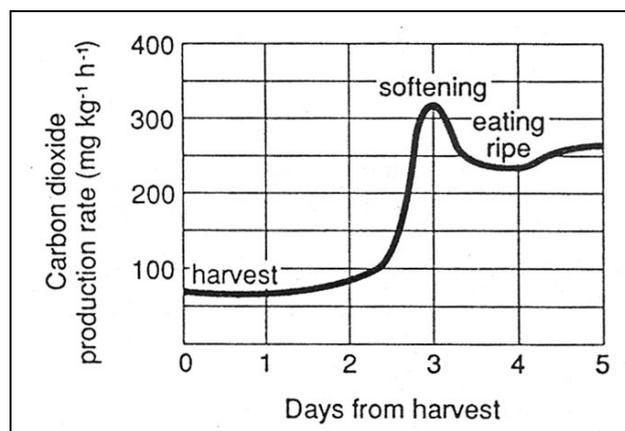


Figure 1.15. Typical respiration curve of *Annona* fruits (Sanewski, 1991)

When fully mature or 'tree-ripe', visible by a yellowish tinge, fruits are picked. They soften, at 20 °C, in 5 to 8 days (Batten, 1990). If harvested too green, fruits will not ripen. Due to its soft skin, cherimoya is highly susceptible to mechanical injury, skin and pulp blemish (George *et al.*, 1987). To overcome these problems, fruits are dipped in fungicide (benomyl) and waxed, and appropriate packing is used.

Cherimoya has a short postharvest life, and can be preserved, depending on the cultivar, less than a week at room temperature (Gutiérrez *et al.*, 1994) and a maximum of 15 – 20 days at cool temperatures (Farré Massip & Hermoso González, 1997). Experiments using controlled atmosphere, with removal of oxygen and

ethylene contents and addition of carbon dioxide (Broughton & Guat, 1979), extend conservation time, but exposure to normal conditions result in a very rapid ripening (Palma *et al.*, 1993). Cold storage at 4 – 5 °C, although used for most fruits, is not suitable for cherimoya, due to chilling injury (Palma *et al.*, 1993; Alique *et al.*, 1994). Storage of mature cherimoya fruits should be at a temperature of 10 °C to avoid blackening (Campbell, 1992).

2.2.4 CROP ECOLOGY

During his search for the origin of cherimoya in northern Peru and southern Ecuador, Guzman (1951) found cherimoya trees at an altitude of 2,200 masl on shallow and stony soils. As altitude got lower, plant number increased to disappear again at altitudes below 1,500 masl. He concludes that altitude is not the limiting factor for cherimoya occurrence, but rather altitude related temperature and water availability. Later studies confirmed that relatively dry and cool areas are optimal for cherimoya. Farré Massip & Hermoso González (1987) state that an annual precipitation of more than 600 mm, and preferably over 1,000 mm, is necessary for wild cherimoya, i.e. without irrigation. Annual precipitation should not exceed 1,700 mm due to phytopathological problems that may arise at high air humidity. Rainfall is essential during flowering and early fruit set but water logging results in flower and fruit drop (George *et al.*, 1987). Temperature seems to be a determining factor. Wild cherimoyas show very small temperature range combined with little seasonal temperature fluctuations (Hermoso González *et al.*, 1999). Mean optimal annual temperature should vary between 17 - 22 °C (Franciosi Tijero, 1992, Hermoso González *et al.*, 1999). Nevertheless, in Spain, an important cherimoya cultivating country, mean monthly temperatures oscillate between 25 °C in summer and 13 °C in winter (Farré Massip & Hermoso González, 1987), proving that especially during flowering temperature is important and should be between 16 and 20 °C (Ibar Albiñana, 1986). Minimum temperature limitations are 13 °C for optimal fruit quality and 1 °C for tree development, whereas maximum temperature is limited to 30 °C because of pollination problems at higher temperatures (Morales, 1993) and limited photosynthetic ability (Higuchi & Utsunomiya, 1999). Cherimoya is frost-sensitive and temperatures lower than -2 °C do seriously damage the tree (Farré Massip & Hermoso González, 1997). Pittman (1956) states that temperature limitations can vary depending on the cultivar. George *et al.* (1987) emphasise the importance of relative humidity during flowering with 70 % being the lower boundary to prevent flower abscission and stigma desiccation and 95 % being the upper boundary where stigma sugar secretion gets too much diluted. Generally, cherimoya prefers cool and relatively dry climates without big temperature variations (Franciosi Tijero, 1992).

Cherimoya is generally shallow-rooted, 98 % of roots situated in the upper 40 cm, with considerable lateral root development (Gardiazabal & Rosenberg, 1993). It grows well in shallow and poor classes of soils. Soil texture preferences are variable but well-drained sandy to sandy loamy soils are preferred (George *et al.*, 1987;

Sanewski, 1991; Morales, 1993) with a pH between 6.5 and 7.6 and organic material content between 1.7 and 2.7 % (Martínez Quésada, 1987).

2.2.5 COMMERCIALISATION

Since indigenous people spread cherimoya seeds over most of Latin America and Spanish explorers took cherimoya seeds to Spain in 1757 (Morton, 1987), cherimoya can be found in most subtropical areas worldwide. In the New World, the biggest concentrations can be found in Peru, with 1,600 ha (Franciosi Tijero, 1992); Ecuador, 1,440 ha (Otero Canelos, 1991); Chile, 1,200 ha (Gardiazabal & Cano, 1999); Bolivia, 1,000 ha (Gardiazabal & Rosenberg, 1993); Mexico, 500 ha (Agustín, 1999); United States, 110 ha (Grossberger, 1999); and Brazil, 100 ha (Bonaventure, 1999). Worldwide, Spain is currently the most important cherimoya grower with 3,266 ha in 1999 (Guirardo Sánchez *et al.*, 2001), up from 106 ha in 1956 (Hermoso González *et al.*, 1999), although only 12 % of the production is exported (Calatrava, 1998). Smaller plantations can be found in other Mediterranean countries such as Portugal (Madeira) (de Freitas Nunes, 1997), Italy (Continella *et al.*, 1996) and Israel (Gazit *et al.*, 1982). Other important cherimoya growing countries are Australia (George *et al.*, 1987), New Zealand (Anderson & Richardson, 1990) and South Africa (du Preez, 1996b). Although the fruit is strongly appreciated in Japan, trials to cultivate cherimoya, in open air and in greenhouses, have not been successful due to high temperature related flowering problems (Higuchi & Utsunomiya, 1999).

Main importers are Europe (Italy, France, Belgium, Netherlands, United Kingdom, Germany), North America (U.S.A., Canada) and Asia (Japan, Hong Kong, Singapore, Saudi Arabia, Kuwait, United Arab Emirates) (Undurraga, 1989; Gardiazabal & Rosenberg, 1993).

An important factor in worldwide commercialisation is the time of harvest and availability. Chile, which is strongly influenced by the southern hemisphere seasonality, is the typical cherimoya exporter when the northern hemisphere, *in casu* Spain and U.S.A., do not produce (Figure 1.16).

Quality norms slightly differ between countries, especially in sizes, but generally healthy fruits with uniform sizes (300 - 600 g), '*laevis*' or '*impressa*' skin types and low seed content are preferred (Franciosi Tijero, 1992). Prices of high quality cherimoya fruits vary between 1 and 2 US\$/kg locally (Franciosi Tijero, 1992) and 3 and 5 US\$/kg FOB (INIA, 1997), depending on the season, for export.

Although cherimoya is considered as a fruit with great appeal, its cultivation has not developed yet into a large industry. Most commonly encountered problems with cherimoya in wholesale and retail markets are: excessive blemish, variable quality, incorrect storage and lack of promotion (Sanewski, 1991). Van Damme & Scheldeman (1999a) state numerous factors which impede commercial development of cherimoya: unfamiliarity by the general public; lack of funding for crop

development , marketing and promotion; limited production volume and seasonability of harvest; financial risk at production and retail level; limited number of cultivars, due to a lack of research; lack of enabling socio-economic environment, lack of registered pesticides for a so-called minor crop and reluctance of policy makers to grant tax exemptions for producers willing to engage in new crop development.

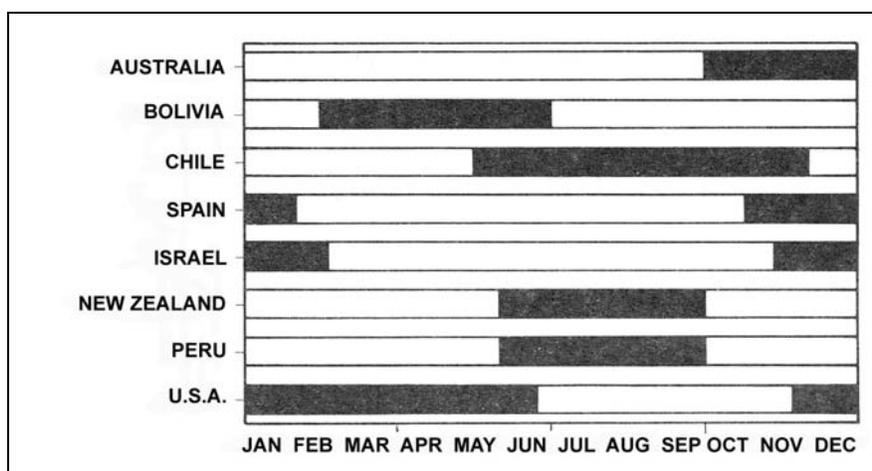


Figure 1.16. Cherimoya harvest periods in different cherimoya producing countries (Gardiazabal & Rosenberg, 1993)

2.2.6 NUTRITIONAL COMPOSITION

The nutritional composition (Table 1.7) of cherimoya fruits is that of a typical sweet fruit but with a high content of carbohydrates and low content of acids. Its vitamin A content is modest, but it is a good source of vitamins thiamine, riboflavin and niacin (National Research Council, 1989) and iron, calcium and phosphorous (Allen, 1967).

Table 1.7. Nutritional composition of 100 g of cherimoya pulp according to different authors

| | Franciosi Tijero, 1992 | De la Rocha, 1965 | Morton, 1989 | Wu Lung, 1970 | Zurita & Lopez, 1980 |
|-----------------------------|---------------------------|----------------------|-----------------|------------------|-------------------------|
| Water (g) | 77.1 | 75.7 | 74.6 | 76.6 | 73.2 |
| Protein (g) | 1.9 | 1.0 | No data | 1.1 | 1.4 |
| Fat (g) | 0.1 | 0.1 | No data | 0.2 | 0.2 |
| Carbohydrates (g) | 18.2 | 22.0 | No data | 21.3 | 24.5 |
| Fibers (g) | 2.0 | 1.8 | 1.5 | 1.9 | 1.3 |
| Ash (g) | 0.7 | 1.0 | 0.61 | 0.8 | 0.7 |
| Calcium (mg) | 32.0 | 24 | 21.7 | 34.0 | 25.0 |
| Phosphorous (mg) | 37.0 | 47 | 30.2 | 35.0 | 31.0 |
| Iron (mg) | 0.5 | 0.4 | 0.8 | 0.6 | 0.8 |
| Vitamine A (IU) | 0.0 | 0.01 | No data | No data | No data |
| Tiamine (vit. B1) (mg) | 0.1 | 0.06 | 0.117 | 0.09 | 0.10 |
| Riboflavine (vit. B2) (mg) | 0.14 | 0.14 | 0.112 | 0.13 | 0.14 |
| Niacine (mg) | 0.9 | 0.75 | 1.02 | 0.90 | 0.94 |
| Ascorbic Acid (vit. C) (mg) | 5 | 4.3 | 16.8 | 17.00 | 29.0 |
| Calories (kcal) | 73 | 81 | No data | 82 | 95 |

2.2.7 USES

Cherimoya is essentially a dessert fruit that is eaten fresh. It can also be used for making ice cream, milk shakes or sorbets and is processed into yoghurt, flan fruit juice and wine (National Research Council, 1989; Gardiazabal & Rosenberg, 1993).

Due to its enzymatic characteristics cherimoya fruits cannot be submitted to thermal processes and its processing should utilise refrigerating or freezing, with addition of antioxidants to avoid enzymatic oxidation and subsequent colouring (Gardiazabal & Rosenberg, 1993; Oleata & Undurraga, 1996). In Chile, freezing of cherimoya is common, especially unsaleable low quality fruit. Frozen cherimoya pulp can be conserved for 120 days at -18°C (Oleata & Undurraga, 1996), can easily be shipped and is used to prepare juices, ice creams, yoghurt, etc. (Franciosi Tijero, 1992). Other processing possibilities include preserving at high sugar concentrations and bottling in modified atmosphere (Gardiazabal & Rosenberg, 1993).

Traditionally, cherimoya seeds are crushed and used as insecticide, mostly to kill lice and cure parasitic skin disorders (Varea, 1922). Human ingestion of resin isolated from the seeds produces symptoms resembling the effects of atropine, although small doses are used as a potent emetic and cathartic (Morton, 1987). Leaves and stem barks of *Annona senegalensis* are used as antidiarrheal drugs, whereas roots and leaves are used in respiratory complaints (Sahpaz *et al.*, 1994).

Biochemically, cherimoya seeds are an important source for acetogenins (Rupprecht *et al.*, 1990), a type of alkaloid, all of which show antiparasitic and cytotoxic activities that are used in pharmaceutical sciences (Sahpaz *et al.*, 1996). The annonaceous acetogenins are a new group of powerful bioactive agents and more than 300 of these compounds have been found to date (Cortès, 1999). Properties attributed have been antimicrobial, antitumor, cardiotoxic and insecticidal (Sanewski, 1991). Some important acetogenins reported in methanolic extracts of cherimoya seeds are annonacin, cherimolin, almenequin, squamocin (Bories *et al.*, 1991; Cortès *et al.*, 1993). The studies of these acetogenins form a promising tool in the development of a future generation of antitumor medicines (Cortès, 1999).

2.2.8 SITUATION OF CHERIMOYA IN ECUADOR

Although situated in the crops' centre of diversity, little detailed information exists on cherimoya cultivation in Ecuador. Otero Canelos (1991), using Ministry of Agriculture data, reports that Ecuador possesses 1,440 ha of cherimoya cultivation, mostly in Pichincha Province. He adds that there is difficulty in estimating the real area covered by cherimoya, due to the numerous wild cherimoya stands and the number of trees in backyard gardens. This fact is confirmed by Farré Massip & Hermoso González (1987). They report a scarcity of fruits on local markets and relate this to the low fruit quality as fruits are heavily infested by pests resulting in low prices and

discouraging local farmers from investing in the fruit crop. León Fuentes (1999) states that in Ecuador, there are no orchards of one single variety and that most orchards consist of plants propagated by seed. The most important phytopathological problem is the fruit fly (*Anastrepha* spp.).

Three national germplasm collections have been reported, one realised in Pichincha Province by Otero Canelos (1991), one conducted in Pichincha and Azuay Provinces by León Fuentes (1999) and one carried out in Loja Province by González Lituma & González Lituma (1980). Two international germplasm collections have been conducted by the Spanish 'Estación Experimental la Mayora' (Farré Massip & Hermoso González, 1987; Farré Massip, 1999). Otero Canelos (1991) found all 31 characterised accessions to be infested with *Anastrepha* spp. Average infestation degree was 25.3 %, indicating that on the 92 fruits characterised one in four was infested with fruit fly. González Lituma & González Lituma (1980) characterised 100 accessions and a total of 22 accessions where infested with *Anastrepha* fruit fly. Cherimoya accessions were collected between 1,520 and 2,370 masl. Tree age varied from 6 to 150 years whereas individual fruit weight varied between 115 and 798 g. Farré Massip & Hermoso González (1987) observed during their collection trips in Loja Province the lack of cultivation practices and considered all trees as strictly wild. They were impressed by the wild cherimoya stands and the age of some of the trees, but warned of a loss of genetic diversity due to land pressure for other agricultural activities.

In 1921, Wilson Popenoe found in the mountains of Loja Province wild thickets of cherimoya and gathered fruits, equal in delicacy and size to the best of the cultivated forms (Fairchild, 1990). Hofmann & Hofmann (1987) state that Loja Province offers a rich potential gene pool to find special qualities for pulp texture, skin thickness, colour, tree shape, cold or drought hardiness, disease resistance, etc., but warn that due to fruit fly problems this valuable resource of genetic material may be lost for the future. Grossberger (1999) also emphasises the importance of cherimoya selection in Loja Province.

Although cherimoyas are found in markets throughout the Andean region, there has been little organised evaluation of the different types, the horticultural or cropping methods used, or the problems growers encounter. Given such attention, as well as improved quality control, the cherimoya could become a more important cash crop for rural villages. With suitable packaging available, a large and lucrative trade with distant cities seems likely. Moreover, increased production will allow processed products, such as cherimoya concentrate for flavouring ice cream, to be produced both for local consumption and for export (National Research Council, 1989).

The National Research Council (1989) highlights as an important area for cherimoya research and development the realisation of a germplasm inventory and collection from natural populations as well as from gardens and orchards, especially throughout the Andes.

2.3 HIGHLAND PAPAYAS (*VASCONCELLEA* SPP.)

2.3.1 BOTANY

2.3.1.1 FAMILY CARICACEAE

Caricaceae is a small family whose species are, with the notable exception of two West African ones, entirely Neotropical. Its largest distribution is recorded in South America where species are abundant (Figure 1.17). Traditionally, the position of Caricaceae within the dicotyledons has been in the order of Violales (Cronquist, 1981). Recently however, use of molecular techniques (APG, 1998) and the presence of mustard-oil glucosides (Rodman *et al.*, 1998) suggest Caricaceae should be placed within the order Brassicales.

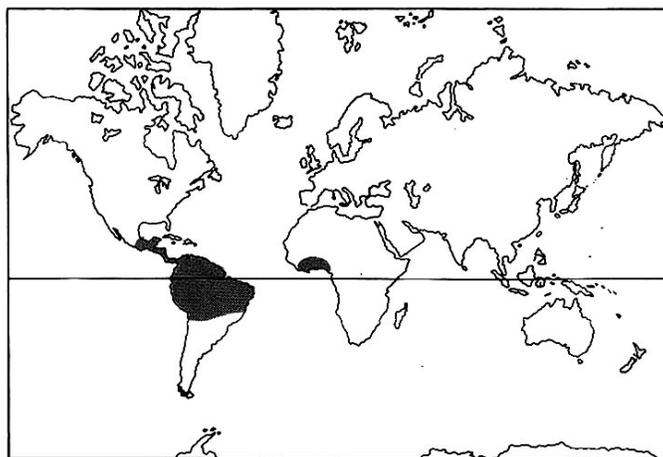


Figure 1.17. Worldwide distribution of Caricaceae (Heywood, 1985)

Caricaceae contains sparsely branched and pachycaul trees, rarely herbs, with soft wood and a well-developed system of articulated laticifers. Leaves are alternate, often spirally arranged at branch tips. They are large, palmately veined and lobed to palmate in shape. Stipules are absent or spine-like. Flowers can be found solitary or in cymes and are rarely bisexual. Plants are mostly dioecious. The regular flower consists of five sepals and five petals with 10 anthers in the smaller male flower and a superior ovary in the bigger female flower. The ovary consists of five fused carpels with many anatropous ovules on parietal placentas. The style is short and crowned by five stigmas. Seeds show a gelatinous coat, the sarcotesta, a straight embryo and oily, proteinaceous endosperm. The fruit is a berry. The stem of *Vasconcellea* and *Carica* species is very unusual in that there is little development of secondary xylem. The wood is formed largely from the phloem, which gives the trunk its rigidity (Heywood, 1985; Mabberley, 1990).

Caricaceae is divided in six genera. The genus *Cylicomorpha* consists of two West African tree species. *Horovitzia* is a monotypic genus of hairy herbaceous plants that can only be found around Oaxaca, Mexico. Genus *Jarilla* consists of three

herbaceous species all endemic to southern Mexico and Guatemala. The genus *Jacaratia* consists of seven widespread tree species, which possess composed leaves. Formerly a genus, later only a section within the genus *Carica* and than again rehabilitated as a genus by Badillo in 2000, the genus *Vasconcellea* now is the most important genus with 21 species. Genus *Carica* is now a monotypic genus but includes the most important Caricaceae, i.e. the papaya (*Carica papaya* L.) (Badillo, 1971; Badillo, 1983; Badillo, 1993; Badillo, 2000).

The family's economic importance resides largely in *Carica papaya*, which has a large, bland juicy fruit and is extensively cultivated throughout the tropics (Heywood, 1985), with Brazil being the world's major producer (FAO, 2002). Its fruit is eaten fresh or may be tinned, crystallised or made into jam, ice cream, jellies, pies or pickles. Green fruits are consumed in South-East Asia but are also used to extract the latex, which contains a complex of proteolytic enzymes. Other Caricaceae are mainly locally consumed, as fresh or processed fruits.

2.3.1.2 GENUS *VASCONCELLEA*

After the rehabilitation of section *Vasconcellea* as a genus, *Vasconcellea* is, with 21 of the 35 Caricaceae species, by far the biggest genus of the family. The rehabilitation, which separated the *Carica* section, containing only *Carica papaya* L., from the *Vasconcella* section, was realised based on morphological characteristics (Badillo, 2000) as well as genetic characteristics (Aradhya *et al.*, 1999). In 2001, the genus name *Vasconcella* was corrected to *Vasconcellea* (Badillo, 2001). Papaya, unlike the *Vasconcellea* species is characterised by a unilocular ovary, a hollow stem, leaves with more than seven veins and lamelliform protuberances on the seed sclerotesta. Besides morphological characterisation and results of molecular marker techniques, its incompatibility to hybridise with *Vasconcellea* species also supports the new classification. *Vasconcellea* and *Carica* species both show a chromosome number of $2n = 18$ (De Zerpa, 1980).

Vasconcellea species are often grouped as 'highland papayas' or 'mountain papayas' (National Research Council, 1989) because of their resemblance with papaya and for their typical ecological preferences for higher altitudes.

Badillo (1993) presented worldwide a total of 20 species within the *Vasconcella* section of genus *Carica*. Recently (Badillo *et al.*, 2000), a new species *Vasconcellea palandensis* (V.M. Badillo *et al.*) V.M. Badillo, found in southern Ecuador was added. This results in a total of 21 described species at this moment. Ecuador holds 15 of these 21 described species (Badillo, 1983; Badillo, 1997; Badillo, 1999) and must unquestionable be considered as one of the 'hot spots' for *Vasconcellea* species. Loja Province alone, covering only 10,790 km², contains at least seven *Vasconcellea* species (Cueva, 1999; Van den Eynden *et al.*, 1999) and is generally acknowledged to be an important centre for *Vasconcellea* research (Soria, 1991). The native *Vasconcellea* species found in Loja Province generally occur over 1,000 masl. They

are *Vasconcellea candicans* (A. Gray) A. DC., *V. cundinamarcensis* V.M. Badillo, *V. monoica* (Desf.) A. DC., *V. parviflora* A. DC., *V. stipulata* (V.M. Badillo) V.M. Badillo, *V. weberbaueri* (Harms) V.M. Badillo and *V. × heilbornii* (V.M. Badillo) V.M. Badillo, a natural hybrid.

V. cundinamarcensis, *V. stipulata* and the natural hybrid between both species *V. × heilbornii* are the most abundant highland papayas and consequently the most used ones in Loja Province (Jiménez *et al.*, 1998). They will form the main focus of this study on highland papayas. Taking into account the economic importance of one cultivar of *V. × heilbornii*, namely *V. × heilbornii* 'Babacó', this cultivar is in some analysis considered separately. Where no specification of cultivar or variety is mentioned, results are applicable for the species *V. × heilbornii*.

2.3.1.3 VASCONCELLEA CUNDINAMARCENSIS V.M. BADILLO (Badillo, 1983; Badillo, 1993; Badillo, 2000)

Synonyms: *Carica candamarcensis* Hook. f., *Carica cestriflora* (A. DC.) Solms, *Carica chiriquensis* Woodson, *Carica pubescens* (A. DC.) Solms, *Carica pubescens* Lenné & C. Koch, *Carica cundinamarcensis* Linden, *Papaya cundinamarcensis* (Linden) Kuntze, *Papaya pubescens* (A. DC.) Kuntze, *Vasconcellea cestriflora* A. DC.

Distribution: Abundant in temperate zones of the Andes (1,500 – 3,000 masl) ranging from Colombia and Venezuela to Bolivia. Cultivated in Chile and introduced in similar climates where it sometimes naturalises.

Vernacular Names (Badillo, 1971; Fouqué, 1973): Eng: mountain pawpaw; Fre: papayer de la montagne; Spa: chamburo, papaya de tierra fría, chihualcán, siglalón, chichuacacón, titi-ish, bonete, papaya de altura, papayuela, tapaculo.

Description (Figure 1.18): Polygamous, monoecious or dioecious plants 3 – 10 m tall with stout succulent, medullose and few-branched stems. Essentially pubescent in all parts, the leaves clustered in a dense terminal crown. Leaf blades palmately divided into 5 or 7 lobes. Male inflorescence is 1 - 15 cm long, many-flowered, with axis and branches pubescent. Female inflorescence is pubescent, short and few-flowered. Bisexual inflorescences similar to the male ones, sometimes only with male flowers in certain seasons. Flowers greenish: calyx lobes somewhat spreading, glabrous or pubescent; corolla pubescent or subglabrous outside, glabrous or pilose within. Fruits obovoid, slightly apiculate, yellow or orange, acid, fragrant, obtusely pentagonous or 5-sulcate, 6 – 15 cm long and 3 – 8 cm wide, sometimes bigger. Seeds numerous, sarcotesta smooth, sclerotesta reddish-brown with wide low interrupted ribs. In some cases, the fruits derived from monoecious plants are rather oblique.

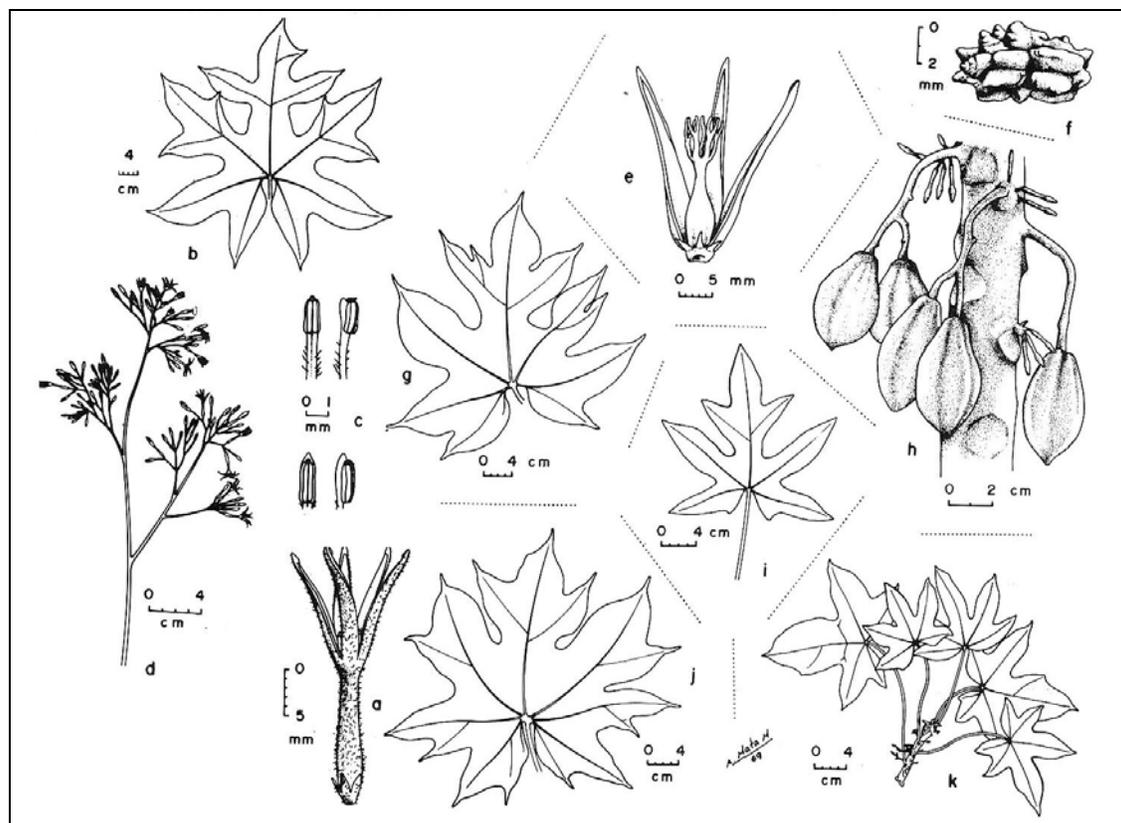


Figure 1.18. *Vasconcellea cundinamarcensis* V.M. Badillo. a) male flower; b,g,i-k) leaf forms; c) upper and lower stamens; d) male or predominantly male inflorescence; e) female flower, two petals removed; f) seed; h) fruits from bisexual inflorescence (Badillo, 1993)

2.3.1.4 *VASCONCELLEA STIPULATA* (V.M. BADILLO) V.M. BADILLO (Badillo, 1983; Badillo, 1993; Badillo, 2000)

Synonyms: *Carica stipulata* V.M. Badillo

Distribution: Only known from the central-southern part of Ecuador (Loja and Azuay Provinces) and the northern part of Peru between 1,600 and 2,450 masl.

Vernacular Names (Badillo, 1971): Spa.: toronche, siglalón silvestre.

Description (Figure 1.19): Dioecious, tall shrubs or arborescent plants, up to 10 m high, sometimes branched, often without leaves when flowering. Leaf scars on both sides with a conic, hard and pointed stipular spine 4 - 7 mm long. Leaf lamina glabrous, 5-nerved and 5-lobate with a stipular spine 1 - 2 mm long on each side of the petiole. Male inflorescences solitary or several from each trunk, peduncle 1.5 – 15 cm long. Female inflorescence short and few-flowered. Flowers orange-red or orange, greenish. Calyx opposed, rarely subopposite or alternate, to the corolla lobes. Corolla glabrous outside, inside pilose. Ovary 5-celled, fusiform. Fruit yellow, fragrant, 5-locular, ellipsoid, 8 – 9.5 cm long and 4 – 4.5 cm wide, truncate at base,

narrowed in upper half with 10 longitudinal furrows, 5 of them deeper. Seeds numerous with 6 - 7 inconspicuous, longitudinal interrupted crests.

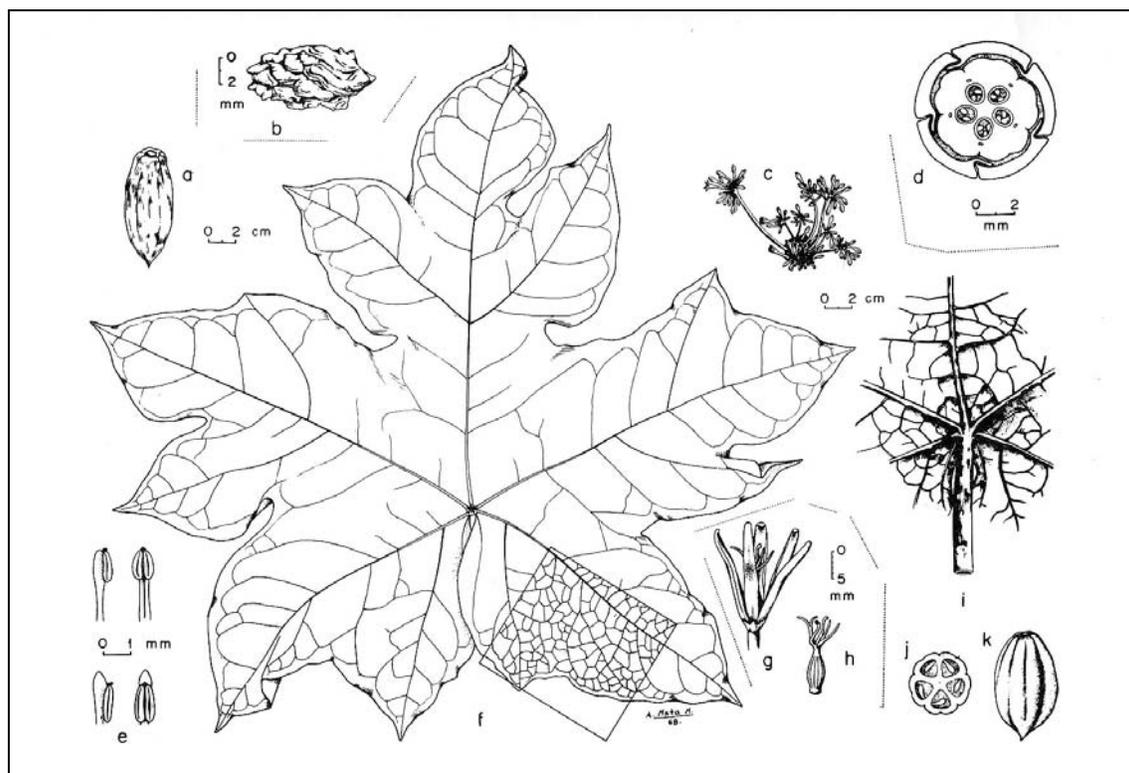


Figure 1.19. *Vasconcellea stipulata* (V.M. Badillo) V.M. Badillo. a) fruit; b) seed; c) male inflorescence; d) cross section of a female flower in flower bud showing corolla and ovary; e) upper and lower stamens; f) leaf; g) female flower; h) gynoecium of female flower; i) detail of abaxial leaf-petiole connection; j) cross section of fruit; k) lateral view of fruit (Badillo, 1993)

2.3.1.5 *VASCONCELLEA* × *HEILBORNII* (V.M. BADILLO) V.M. BADILLO (Badillo, 1971; Badillo, 1983; Badillo, 1993; Badillo, 2000)

Synonyms: *Carica* × *heilbornii* V.M. Badillo

Distribution: *V. × heilbornii* originated in the southern and central part of Ecuador, where the parent species grow at altitudes between 1,600 and 2,800 masl.

Description: Hybrids derived from crosses between *V. cundinamarcensis* and *V. stipulata* generally showing some degree of parthenocarpy (up to complete absence of seeds). Dioecious, monoecious or polygamous plants, somewhat pubescent to completely glabrous, stipular spines present or, sometimes, absent. Flowers orange, green or greenish-yellow, calyx lobes alternating with corolla lobes. As the plants are reproduced by clonal means the male flowers are in some cases unknown.

Different varieties (previously nothomorphs) of *V. × heilbornii* have been described, differing in stipules, female flowers and inflorescences. Actually two varieties and one

cultivar are withheld in the latest Caricaceae revision. Nevertheless, Badillo (1993) states that several other undescribed varieties can be found in Ecuador.

- *Vasconcellea* × *heilbornii* 'Babacó' (V.M. Badillo) V.M. Badillo

Synonyms: *Carica* × *heilbornii* nm. *pentagona* (Heilborn) V.M. Badillo; *Carica pentagona* Heilborn

Vernacular names (Badillo, 1971; Fouqué, 1973): Spa: babaco.

Characteristics: Plant 1(-2 m) high, rarely ramified, stipular spines absent, solitary short-peduncled female flowers green or greenish-yellow inside, prismatic parthenocarpic fruits 1-locular up to 50 cm long, with no or few seeds.

- *Vasconcellea* × *heilbornii* var. *chrysopetala* (Heilborn) V.M. Badillo

Synonyms: *Carica* × *heilbornii* nm. *chrysopetala* (Heilborn) V.M. Badillo; *Carica chrysopetala* Heilborn

Vernacular names (Badillo, 1971; Fouqué, 1973): Spa: chamburo, higacho, toronchi.

Characteristics: Treelike plants up to 7 m, stipular spines present but small, solitary flowers yellow-orange especially the upper part, the rest green, fruits ovoid, 5-locular, 11 - 20 cm long and 6 – 8 cm wide with obtuse apex, few to very few seeds, but seldom absent.

- *Vasconcellea* × *heilbornii* var. *fructifragrans* (García-Barr & Hern. Cam.) V.M. Badillo

Synonyms: *Carica* × *heilbornii* nm. *fructifragrans* (García-Barr. & Hern. Cam.) V.M. Badillo; *Carica fructifragrans* García-Barr. & Hern. Cam.

Vernacular names (Badillo, 1971): Spa: chamburo, chihualcán.

Characteristics: Very similar to *Vasconcellea* × *heilbornii* var. *chrysopetala* but showing some notable differences, like well-developed stipular spines, greenish-yellow flowers, subpentagonous almost cylindrical fruits of 12 - 14 cm long, sometimes longer.

2.3.1.6 INTERSPECIFIC HYBRIDISATION

Vasconcellea species tend to hybridise, as clearly indicated by the different undescribed natural *Vasconcellea* × *heilbornii* varieties (Badillo, 1971). These *V.* × *heilbornii* varieties, some of which were described as species previous to their taxonomical revision, all show a degree of parthenocarpy, resulting in seedless (babaco) fruits or fruits with very low seed content and are therefore generally propagated by cuttings. Numerous locally cultivated forms exist, especially in southern Ecuador, which have not been described yet (Horovitz & Jiménez, 1967; De Zepa, 1980; Badillo, 1993). Soria & Soria (1992) experimented with crosses between babaco (*V.* × *heilbornii* 'Babacó') and *V. cundinamarcensis* and obtained variable backcrosses with intermediate characteristics. Horovitz & Jiménez (1967) describe the characteristics of progeny of *V.* × *heilbornii* var. *chrysopetala* seeds to be very variable and attribute these differences to the pollen source. This shows the

possibility of obtaining new 'varieties' which, after a careful selection, could be used in commercial cultivation. Genetic analysis of *V. × heilbornii* and its supposed progenitors, *V. cundinamarcensis* and *V. stipulata*, using AFLP analysis (Van Droogenbroeck *et al.*, 2002), did show a wide variability in *V. × heilbornii* and *V. stipulata* accessions, indicating a continuous natural process of hybridisation and backcrossing. With the exception of the hybrid between *V. cundinamarcensis* and *V. stipulata*, no other natural hybrids have been described, however.

Within genus *Vasconcellea*, numerous artificial hybrids are described, illustrating the ease of hybridisation. Different interspecific hybrids have been reported (Horovitz & Jiménez, 1967; Badillo, 1971; Mekako & Nakasone, 1975) with different and often contradictory success rates. This indicates the importance of environmental factors in relation to hybridisation. Seed viability or seedlessness of fruits obtained by crossings, is the main limiting factor in obtaining artificial hybrids (Mekako & Nakasone, 1975).

Intergeneric artificial hybrids with *Carica papaya*, have been generated to obtain introgression of desired traits like disease resistance or cold tolerance. This hybridisation with *C. papaya*, however, is prevented due to genome incompatibility barriers, which have lead to abortion of hybrid seeds soon after their formation (Magdalita *et al.*, 1997). This was one of the arguments in the rehabilitation of the genus *Vasconcellea*. Embryo rescue techniques are often used to overcome these incompatibility barriers (Drew *et al.*, 1998).

Natural or artificial interspecific hybridisation could lead to the introduction of specific traits such as the pleasant fragrance of *V. stipulata* or babaco, the monoecious habit of *V. monoica*, the cold-tolerance of *V. cundinamarcensis* and *V. stipulata*, and the ornamental qualities of pink-flowered *V. parviflora* (Manshardt & Wenslaff, 1989b). All species mentioned are present in southern Ecuador.

2.3.2 ORIGIN

Vasconcellea species, like most of the family Caricaceae members, originate in South and Central America (Badillo, 1993). *V. cundinamarcensis* is common in the Andes, at higher elevations between 1,500 and 3,000 masl, from Colombia and Venezuela up to Bolivia. *V. stipulata* is more rare and is only present in central and southern Ecuador and northern Peru between 1,600 and 2,450 masl. The hybrid between these species, *V. × heilbornii*, can only be found in the same restricted area where *V. stipulata* is found.

V. × heilbornii 'Babacó' is currently the most important *Vasconcellea* species. It is cultivated in some subtropical countries and is considered as native from the valleys of Loja Province (Cossio, 1988; National Research Council, 1989; Merino Merino, 1989).

2.3.3 CULTIVATION

2.3.3.1 PROPAGATION

Propagation of *Vasconcellea* species can be realised in different ways. Generative propagation needs viable seeds which is often a problem in *V. × heilbornii* varieties. The hybrid character of *V. × heilbornii* implies vegetative propagation, giving rise to typical phytopathological problems. Solution of a number of propagation problems lies in the use of *in vitro* propagation (Litz & Conover, 1980; Vega de Rojas & Kitto, 1991; Jordán & Velozo, 1996). Jiménez *et al.* (1998) report a germination rate of 5 % for a seed mixture of different *V. × heilbornii* varieties, indicating the rare presence of some viable seeds in some varieties.

Seed propagation is recommended for *V. cundinamarcensis*, avoiding the typical problems of availability of plant material, which occurs in a number of *V. × heilbornii* varieties (CAF, 1992). Seeds are extracted from mature, selected fruits. To remove the sarcotesta, a gelatinous aril covering the seeds, seeds are soaked in water for 48 hours, which facilitates removal of the sarcotesta that ferments during this treatment. The clean seeds are dried and are then ready for sowing or conservation (CAF, 1992). Only a few studies on germination of *Vasconcellea* species exist. Alarcón *et al.* (1997) studied germination of *V. cundinamarcensis* at different temperatures and different storage periods. No influence on germination was noticed at temperatures between 15 – 30 ° C. Germination increased considerably, up to a maximum of 87 %, by varying storage times up to 12 months. Recently extracted seeds showed low germination, while a storage time of four months considerably improved germination. After twelve months of conservation however, vigour dropped considerably. FAO (1992) reports a germination rate of 60 % in *V. cundinamarcensis* whereas Jiménez *et al.* (1998) report germination rates of 68 % for female *V. cundinamarcensis* plants. Due to the limited range and lack of real cultivation, little information is available on generative propagation of *V. stipulata*. Jiménez *et al.* (1998) found a germination of only 3 % in a mixture of *V. stipulata* accessions.

An important constraint in generative propagation of *Vasconcellea* species is the presence of dioecism in *V. stipulata* and a complicated sex-determining genetic system in *V. cundinamarcensis* (Horovitz & Jiménez, 1972), both resulting in the presence of numerous sterile male or monoecious plants. In papaya, this problem can be resolved using microsatellite analysis of seedlings (Parasnis *et al.*, 2000).

Germination of papaya has been well-investigated. Germination percentages are very variable and range from 15 % (Bertocci *et al.*, 1997) to 92 % (Andreoli & Khan, 1993). Ellis *et al.* (1985b) mention severe dormancy problems and report slow, erratic and incomplete germination. Some partly successful dormancy-breaking treatments include pre-chilling, removal of sarcotesta, pre-soaking in water, pre-application of gibberellic acid (10 – 10,000 ppm) and pre-application of potassium nitrate. Differences in domestication level were described by Paz & Vazquez Yanes (1998),

who found higher germination (55 %) with cultivated plants than with wild papaya plants (24 %).

Vegetative propagation has only been described for babaco. Commercially, cuttings are the most important way of propagating babaco plants. They are obtained when the plant has finished producing (Soria & Viteri, 1999). Cuttings are 15 – 20 cm (Soria & Viteri, 1999) up to 25 - 30 cm long (Merino Merino, 1989). Rooting is best realised in a substrate of sand pumice and humus or some other aerated substrates as sawdust or *Sphagnum* moss (Guerrero & Castro, 2000). Merino Merino (1989) mentions rooting percentages of up to 95 %. Application of plant hormones has not led to significant improvement (Guerrero & Castro, 2000). It is recommended to dry the cuttings for four days to eliminate latex and let the wounds dry. Disinfection with fungicides may prevent development of fungi (INIAP, 1992). The major problem in babaco propagation is to obtain healthy material. Due to the small, often unbranched habit of babaco plants, the number of cuttings that can be produced from any plant is limited (Endt, 1981). Thus distribution of babaco from its centre of origin has been slow (Kempfer & Kabaluk, 1996). A babaco plant of 2 m can only give rise to an average of 10 cuttings. Green cuttings or young lateral shoots, obtained by eliminating apical dominance (Merino Merino, 1989), are an easy alternative for vegetative propagation although this method interferes with fruit production. Jiménez *et al.* (1998) report for rooting of stem cuttings success rates of approximately 50 % for babaco and other *V. × heilbornii* varieties, whereas *V. cundinamarcensis* and *V. stipulata* showed difficulties in rooting with rates of 11 and 4 % respectively.

Grafting of *Vasconcellea* species is also possible but more labour intensive. The method consists of a simple cleft graft and success rates are very high (Jiménez, 1959). Despite the higher cost of grafted plants, interest is rising, as other *Vasconcellea* species, less susceptible to root diseases than babaco (Ochoa *et al.*, 2000), can be used as rootstock and thus lead to resistant babaco plants (Fabara *et al.*, 1985). As with cuttings, obtaining adequate grafts, preferably tips of 1 – 1.5 cm diameter (Soria & Viteri, 1999), can be a problem due to the unbranched habit of babaco. Soria (1983) reports a 90 % take of babaco on *V. cundinamarcensis* grafts.

In vitro propagation techniques gain more importance although plant prices are significantly higher than for traditionally vegetatively propagated plants. Disadvantages of traditional vegetative propagation, as lack of large numbers of healthy cuttings, can easily be overcome by *in vitro* propagation. In 1987 some 400,000 plants have been propagated through *in vitro* in Italy alone (Cossio, 1988), a number impossible to reach through propagation by cuttings. Micropropagation of babaco consists of cultivating meristematic axillary buds in a suitable medium (Cossio, 1988). Jordán & Velozo (1997) report difficulty with *in vitro* regeneration of babaco due to low morphogenic responses. Somatic embryogenesis of babaco and *V. cundinamarcensis* from morphogenic callus and callus-derived cell suspensions allowed initiation of a large number of somatic embryos (Jordán & Velozo, 1996; Jordán & Piwanski, 1999). Vega de Rojas & Kitto (1991) obtained somatic embryogenesis from babaco ovules. Cell suspension cultures of callus derived from

peduncle explants led to somatic embryogenesis in *V. stipulata* (Litz & Conover, 1980).

2.3.3.2 CULTIVATION PRACTICES AND CROPPING

As babaco (*V. × heilbornii* 'Babacó') is the only highland papaya that is cultivated, even though on a small scale, at commercial level worldwide, most information on cultivation practices, in greenhouse or open air, are focused on this species. Limited information is available for *V. cundinamarcensis* and is only based on info from Ecuador. No data on cultivation of *V. stipulata* have been published, due to the lack of research and cultivation of this species.

Babaco plants, propagated by cuttings, grafting or tissue culture, are planted in well-drained soils at 1.5 m x 1.5 m (4,444 plants/ha) or 1.2 m x 1.5 m (5,500 plants/ha) (Fabara *et al.*, 1985; Soria & Viteri, 1999). Merino Merino (1989) mentions planting densities of 3,400 to 6,600 plants/ha in greenhouses in Spain. Plant distances for *V. cundinamarcensis* are 1.5 m between plants and 2 m between rows, resulting in 3,000 – 3,500 plants/ha (CAF, 1992) although FAO (1992) reports a higher plant distance of 3 m x 3 m, reducing plant density to 1,111 plants/ha. In the dioecious species *V. cundinamarcensis* and *V. stipulata* it is recommended to eliminate male plants, leaving only one male for ten female plants to ensure pollination (CTIFL, 1992).

Babaco plants are pruned, leaving one, two or three branches depending on planting distances, nutrition, type of plantation (greenhouse vs. open air) and desired fruit size. Single-branched plants yield larger, heavier fruits, often unsuitable for commercialisation, than multi-branched trees (Soria & Viteri, 1999). Cossio (1988) confirms the preference of V-shaped plants to obtain suitable fruit sizes for European markets. *V. cundinamarcensis*, generally showing higher and multi-branched trees, needs more severe pruning in order to ease harvest activities and plant management and reduce phytosanitary problems, while heavy fruiting sometimes needs fruits to be eliminated, leaving two fruits per peduncle (CAF, 1992).

Fertilisation depends on the nutritional status of the soil. Recommended average fertilisation applications for babaco are 100 - 250 kg/ha N, 100 - 200 kg/ha P₂O₅, 200 - 400 kg/ha K₂O, 150 - 200 kg/ha Ca, 100 - 150 kg/ha Mg and 30 - 50 kg/ha S. This must be higher in greenhouse conditions due to a faster development and more intense production (Soria & Viteri, 1999). Organic fertilisation is recommended every six months (CTIFL, 1992).

A good irrigation management is essential for successful babaco cultivation. Babaco plants are demanding in water but excess soil water can be very harmful (Merino Merino, 1989). Averagely, dependent on soil and climate conditions, babaco needs between 600 and 1,000 mm water per year (Fabara *et al.*, 1985). Irrigation is best realised by drip-irrigation, in order to obtain optimal water use and to avoid spreading

of root fungi. *V. cundinamarcensis* requires 800 – 1,500 mm water per year (CAF, 1992).

Few fruit species are capable of producing in such a short time such large quantities of fruit as babaco (Merino Merino, 1989). When babaco plants have passed their juvenile period, two to three months after transplanting, flowers appear and the first harvest starts nine to ten months after transplanting (Soria & Viteri, 1999) (Figure 1.20). Generally, fruits need eight to ten months from flower to mature fruit (Cossio, 1988).

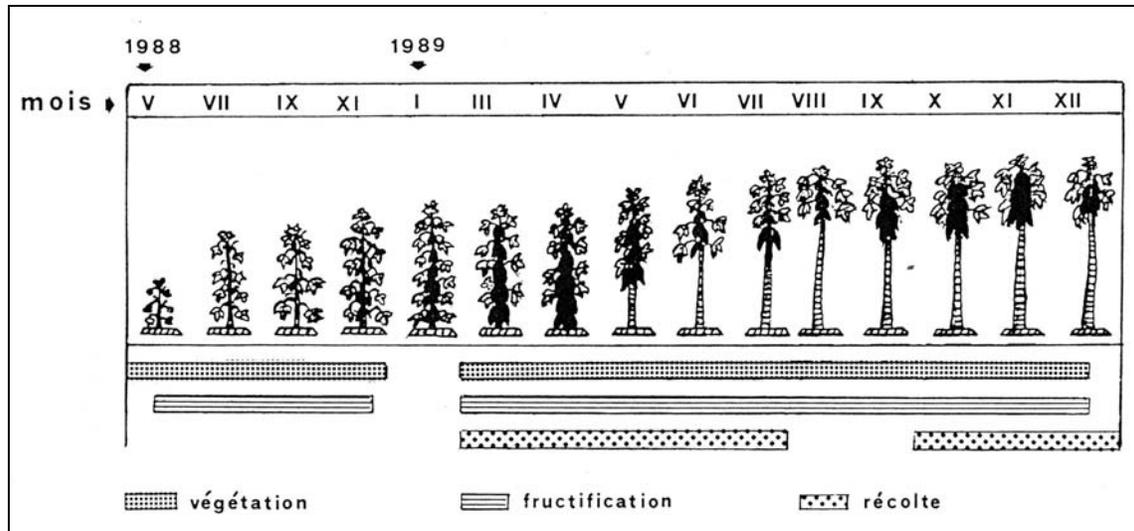


Figure 1.20. Plant evolution of babaco plants planted on 27th of April 1988, grown under greenhouse conditions in Switzerland (Evéquo, 1990)

Average fruit weight is 1 kg (Soria & Viteri, 1999) but varies between 300 g and 1,200 g (Merino Merino, 1989). Data on yields are very variable and largely depend on plant density, irrigation, type of plantation, etc. Merino Merino (1989) mentions Spanish yields of 15 – 20 kg/plant.year resulting in yields between 40 and 120 ton/ha.year whereas Cossio (1988) reports average yields of 45 to 70 ton/ha.year in Italy. In New Zealand, the first country to have introduced babaco cultivation, yields are projected between 40 to 45 ton/ha (Endt, 1981). These yields are comparable to, and slightly higher than papaya yields which average 40 ton/ha.year (Morton, 1987). Kempler & Kabaluk (1996) report babaco yields of 250 ton/ha.year in highly technified greenhouses whereas Van Oosten (1986) describes yields of 320 ton/ha. Wiid (1994) mentions average yields of 35 ton/ha.year in South Africa but reports a drop to 17 ton/ha in the third year. In Ecuador, INIAP (1992) estimates a productivity of 100 ton/ha.year during a lifetime of two years. Merino Merino (1989) agrees with the production time of two to three years. At this time, plants reach a height of 2.5 to 3 m, at which production decreases and plant management becomes difficult. Depending on the state of the rooting system, a second production cycle can be started by cutting the plant up to 30 cm above ground level, or else new plants can be planted (Merino Merino, 1989). *V. cundinamarcensis* plants start producing 10 –

15 months after planting and show a continuous production throughout the year (CAF, 1992). Little to no exact yield data are available for *V. cundinamarcensis* and *V. stipulata*, but FAO (1992) reports *V. cundinamarcensis* fruit production of up to 200 fruits per plant, which would lead to approximately 60 ton/ha.year. Estimated biological life cycle is 5 years (FAO, 1992). CAF (1992) to the contrary, reports yields which are considerably lower, i.e. between 3 and 10 tons/ha.year with a lifecycle of 20 – 25 years. These contradictions illustrate the lack of exact knowledge on highland papayas.

2.3.3.3 POLLINATION

Unlike papaya, where different floral types, ranging from strictly pistillate to strictly staminate flowers with different intermediate forms (Lassoudière, 1969a), exist, *Vasconcellea* species possess only female or male flowers and do not have bisexual flowers (Horovitz, 1954). Horovitz (1954) states that dioecism is the oldest condition in Caricaceae, confirmed by dioecism in wild papaya plants (Badillo, 1971), but that due to domestication different flower types developed. *V. stipulata* is a strictly dioecious species whereas in *V. cundinamarcensis* both dioecious and monoecious plants, showing both pistillate and staminate flowers, occur (Badillo, 1993).

Papaya pollination remains confusing. Most authors (Ronse Decraene & Smets, 1999; Badillo, 1971) report insect pollination although wind pollination (PROSEA, 1992) and self-pollination, in case of bisexual flowers (Morton, 1987), have also been described. Arguments in favour of insect pollination are the potential rewards that are present in, especially male, flowers. These potential rewards, described for *Carica papaya*, consist of pollen, calcium oxalate crystals and nectar (Ronse Decraene & Smets, 1999). In the case of the hybrid *Vasconcellea* × *heilbornii* which is often seedless, fruit formation shows a high degree of parthenocarpy (Badillo, 1993).

2.3.3.4 PESTS AND DISEASES

Most pests and diseases described for *Vasconcellea* species have been documented for *Vasconcellea* × *heilbornii* ‘Babacó’, the most commercial species, whereas it has been added that wild *Vasconcellea* species are probably less susceptible to phytopathological problems (Soria & Viteri, 1999). According to CTIFL (1992) no important phytopathological problems occur in *V. cundinamarcensis*.

The most important fungal diseases for babaco are *Phytophthora* spp., occurring mainly during multiplication by cuttings and causing losses up to 100 %, and the soil-borne *Fusarium oxysporum* (Ochoa et al., 2000), the most important disease in babaco cultivation. *Fusarium* causes high losses during production and is especially dispersed through gravitational irrigation. Less important fungal diseases are *Oidium* spp., *Alternaria* spp. and *Mycosphaerella* spp. Babaco is also susceptible to a mosaic virus called ‘babaco mosaic virus’ (Cossio, 1988; Merino Merino, 1989; Soria & Viteri,

1999). Obviously, propagation by cuttings easily spreads these diseases and healthy material must be taken. *V. cundinamarcensis*, mostly propagated by seeds, is susceptible to damping-off especially with excess humidity (CAF, 1992).

Besides root fungi, phytoparasitary nematodes (*Meloidogyne incognita*) are another phytopathological problem in babaco. They may reside for years in the soil without being noticed or only slightly delaying plant development. Severe infestation leads to leaf and fruit fall, but may also induce subsequent fungi attacks. Anticipation of nematode development is the best way to avoid these problems. Preventive measures may consist of crop rotation, soil sterilisation, use of trap plants (*Tagetes* spp.), use of natural fungal or bacterial enemies, or use of resistant rootstocks (*V. monoica* or *V. weberbaueri*) (Cossio, 1988; Soria & Viteri, 1999).

Pests include acari (*Tetranychus urticae*), especially in Spain (Merino Merino, 1988), and aphids (*Aphis* spp.).

2.3.3.5 CULTIVARS

There are different views on the presence in Ecuador of babaco cultivars. Ecuadorian authors mention that due to vegetative propagation methods only one variety exists. They do however point out different ecotypes that vary especially in fruit characteristics, but these are not enough differentiated to be given another taxonomical status (Soria & Viteri, 1999). Differences may only be the result of environmental conditions (Fabara *et al.*, 1985). In Spain different clones have been introduced, which differ mainly in sugar content, of which '*Dulce Babaco Hortex*' is the most important (Merino Merino, 1989). In New Zealand, a clone called '*Connops Sweet*' with interesting characteristics is cultivated (Cossio, 1985). Molecular techniques should be applied to try to discern genotypical variability and to determine the existence of cultivars. The National Research Council (1989) reports a variety of *V. × heilbornii* var. *chrysopetala* called '*Lemon Crème*' cultivated in New Zealand.

No commercial cultivations of *V. cundinamarcensis* and *V. stipulata* exist, and no cultivars have been described or developed. CAF (1992) confirms the absence of cultivars for *V. cundinamarcensis*.

2.3.3.6 BREEDING

The absence of real cultivars for *Vasconcellea* species indicates the lack of breeding. Breeding efforts using *Vasconcellea* species are mainly aimed at papaya breeding. Desired traits to be incorporated into papaya genotypes include mainly resistance genes (Manshardt & Wenslaff, 1989a; Magdalita *et al.*, 1997; Drew *et al.*, 1998) but cold tolerance and organoleptic characteristics (Manshardt & Wenslaff, 1989b) are also targeted. Interspecific gene flow between *Carica papaya* and *Vasconcellea*

species shows considerable postzygotic barriers making the use of embryo rescue indispensable (Mekako & Nakasone, 1975; Drew *et al.*, 1998).

In practice, breeding within genus *Vasconcellea* is mainly experimental breeding to obtain new hybrids, aimed at obtaining smaller and seedless fruits (Soria & Soria, 1992) but no reports of real targeted breeding programmes have been reported. The wide variability, especially present in Ecuador, and the ease of hybridisation within the genus leave a lot of underused potential (Cossio, 1988). According to Kempler & Kabaluk (1996) babaco breeding is possible through somatic embryogenesis or organogenesis, or by trying to find natural mutations. The National Research Council (1989) states that 'the great adaptability and high yield of these 'new' papayas – given genetic selection and improvement – should be a bonanza'.

2.3.3.7 POST-HARVEST

A first babaco harvest is obtained 10 – 12 months after transplanting. Normally, colour change, from green to golden yellow, is used as an indicator of ripeness. Fruits reach physiological maturity when skin colour starts presenting yellow patches. At the same time soluble solids content increases slightly from 5 to 8 °Brix (Harman, 1983). Fruits are harvested by cutting the peduncle (Izurieta, 1989). As fruits are very sensitive to the touch, handling has to be realised with care to avoid bruising. Optimal ripeness for harvest is when the fruit develops 5 % of yellow colour on the skin (Izurieta, 1989), although Merino Merino (1989) reports harvest at 10 – 40 % colouring. As babaco is a climacteric fruit, similar to other members of the Caricaceae such as papaya, ripening continues after harvesting (Harman, 1983). Harvesting of *V. cundinamarcensis*, 10 – 15 months after transplanting, also starts when yellow colouring is noticed on the fruits.

Ripening of babaco occurs in different phases: a first phase starts when yellow skin colouring reaches 15 % of total surface area. In a second phase, respiration and CO₂ production rise considerably to start the development of the typical organoleptic fruit characteristics at 30 – 40 % of skin colouring. Finally, full organoleptic characteristics are reached at 80 % skin colouring. To consume or process the fruits, it is advised that skin colouring should have reached 50 % (Izurieta, 1989).

Packing is realised in wooden boxes containing 12 – 15 fruits (Izurieta, 1989). In Ecuador fruits are classified in three classes: big (more than 1,000 g), intermediate (700 – 1,000 g) and small (300 – 700 g) (Izurieta, 1989). The smaller *V. cundinamarcensis* fruits are packed in wooden or cardboard boxes, wrapped in silk paper. Classification is also realised according to weight: special (between 125 – 150 g), fancy (100 – 125 g) and extra (60 – 100 g), with more fruit deficiencies allowed as class decreases (CAF, 1992).

Babaco, and most of the other *Vasconcellea* species, are very suitable for processing. Typical processed products include preserves, frozen products, juices

and dehydrated products (Izurieta, 1989; National Research Council, 1989). Use in sorbets and jams has also been reported (CTIFL, 1992). Canned preserves of *V. cundinamarcensis* are marketed in Chile while *V. stipulata* are said to be the best highland papaya for jams and sauces (National Research Council, 1989).

Another attractive feature of babaco fruits is their excellent keeping quality (Endt, 1981). Ripe fruits can be conserved for approximately three weeks at room temperature (CTIFL, 1992) or up to five weeks when harvested at 10 % colouring (Merino Merino, 1989). Harman (1983) experimented with different conservation times and temperatures and concluded that fruits are chill-sensitive and can not be conserved below 3 °C, whereas at 6 °C babaco had a storage life of 5 weeks. In all cases, fruit colouring gives a good indication of the ripening stage.

2.3.4 CROP ECOLOGY

Growth condition specifications for *Vasconcellea* species mainly focus on babaco. As little studies have been realised on *V. cundinamarcensis* and *V. stipulata* crop ecology of these species is not well described.

Climatic conditions for babaco production are mainly based on conditions occurring in its centre of origin, *in casu* southern Ecuador. There it can be found at altitudes between 800 and 2,600 masl (Soria & Viteri, 1999) although Cossio (1988) reports altitudes between 1,500 and 3,000 masl. According to Merino Merino (1989) mean temperature at these sites oscillates between 15 and 20 °C with average minimum and maximum temperatures at respectively 12 and 24 °C. Babaco is susceptible to low temperatures and temperatures below 1 – 2 °C can already cause plant damage. Merino Merino (1989) advises temperatures above 8 °C and below 30 °C. Ideal temperature in Ecuador ranges 13 – 22 °C (Cossio, 1988). Kempler & Kabaluk (1996) report greenhouse minimums of 10 °C at night and 12 °C during the day, rising to a minimum of 18 °C for fruit to ripen quickly and uniformly. This is contradicted by Soria & Viteri (1999) who define optimal greenhouse temperatures between 20 – 25 °C. *V. cundinamarcensis*, generally found at slightly higher elevations, up to 3,000 masl (National Research Council, 1989), is reported to support minimum temperatures down to –3 °C (CTIFL, 1992) and prefers a mean temperature between 14 – 18 °C (CAF, 1992).

In Ecuador, optimal annual precipitation for babaco is ranging 600 – 1,000 mm (Cossio, 1988) although Soria & Viteri (1999) report annual precipitations of 400 – 1,000 mm in the interandean valleys and 1,200 – 1,500 mm at the foothills, illustrating the adaptability of babaco. When this precipitation is evenly distributed there is no need for additional irrigation. At lower precipitation, cultivation needs irrigation (Merino Merino, 1989). Greenhouse cultivation is also possible at sites with higher precipitation (Soria & Viteri, 1999). According to CTIFL (1992), *V. cundinamarcensis* is more drought resistant than babaco and wild populations are found at annual precipitation levels between 800 and 1,500 mm (CAF, 1992).

Illumination, typical for tropical highlands, also seems to be an important factor for ideal crop development. Well-illuminated plants produce a higher fruit number (Cossio, 1988). Ideal relative humidity is between 70 - 80 %, especially during the first months of cultivation. This relative high humidity is necessary to overcome the large transpiration losses due to big leaf surfaces and limited root development. This poor root development is also responsible for the susceptibility to wind (Merino Merino, 1989).

Babaco adapts to the majority of soils if and when they are well drained (Soria & Viteri, 1999). Optimal soil texture is loam to sandy loam. Sandy soils give good plant development but humidity is more difficult to control and presence of nematodes is more likely. Clay soils, in contrast, give rise to poorly developed plants with fewer fruits (Merino Merino, 1989). Babaco plants develop in variable soil acidity with pH values between 5.8 and 8.2, but a neutral pH between 6.5 and 7.2 is optimal (Cossio, 1988). Plants develop well in soils with organic matter contents between 3 and 5 % (Soria & Viteri, 1999). Edaphic conditions for *V. cundinamarcensis* are similar to those of babaco, although an organic content above 5 % is preferred (CAF, 1992).

2.3.5 COMMERCIALISATION

Only babaco, *V. × heilbornii* 'Babacó', is commercially developed worldwide. It was introduced as a crop in New Zealand in 1973 (Endt, 1981; Harman, 1983) from where it spread during the eighties to Australia (Cossio, 1988), Italy (Cossio & Bassi, 1987; Ferrara *et al.*, 1993), Spain (Merino Merino, 1989), France (CTIFL, 1992), South Africa (Wiid, 1994) and even Switzerland (Evéquo, 1990; Evéquo, 1994), Canada (Kempner & Kabaluk, 1996) and Holland (Heij, 1989) where greenhouse trials have been initiated.

In New Zealand, first fruits were initially sold at 2 euro/fruit although projected acceptable prices are 0.5 – 1.0 euro/fruit (Endt, 1981). In Spain, fruits were sold in 1988 at 1.5 euro/kg (Merino Merino, 1989). In Italy, initial prices fluctuated around 5 euro/kg (Cossio, 1988) but dropped to 0.5 euro/kg in 1987 (Ferrara *et al.*, 1993) to stabilise, aimed at specialised channels, again between 1.5 and 2.5 euro/kg (Cossio, 1988). Ecuadorian fruits are sold in Europe at 2 – 3 euro/kg (Soria & Viteri, 1999).

Cossio (1988) mentions quality norms for babaco as developed in New Zealand. Fruits must be clean, regular, free of diseases, harvested at the start of yellow colouring and weight must be less than 700 g.

Most of the introduction trials outside of Ecuador have failed. This was always due to commercialisation problems. In New Zealand, the main constraints were the novelty, the unfamiliarity with fruit utilisation and the lack of promotion. The large fruit size made them too expensive for the consumer wanting to try something new (Hewett, 1993). In Italy, where in 1986 a massive plant sale promotion took place, fruit sale did not reach expected values due to qualitative characteristics (unfamiliar aroma and

taste, elevated acidity and low sugar content), oversized fruits and a lack of promotion in the press and to consumers (Ferrara *et al.*, 1993). Kempler & Kabaluk (1996) report rapid overproduction and deteriorating fruit quality, partly due to open-air cultivation, as the main reason for commercialisation failure in New Zealand and Italy. In Holland, where a greenhouse of 6,000 m² was established, production was too high, resulting in decreasing prices and lack of profit (Stijger, 2001). In Switzerland, production caused no problems but elevated production costs, especially greenhouse heating, made babaco cultivation not profitable when compared to imported fruits, while regular-sized fruits were difficult to obtain (Evéquo, 1994).

In spite of these unsuccessful stories, interest in babaco still holds. As exotic fruits are increasingly popular, with kiwi and mango as well-known success stories, babaco still has the potential to figure as a niche crop. Heij (1989) classifies babaco with crops that are promising for commercial cultivation, while Evéquo (1994) foresees easy commercialisation due to the exotic and decorative aspect of babaco fruits. Most introductions failed due to a high initial production, causing price drops, combined with a lack of familiarity of the consumer with the fruit, aggravated by its large size (Izurieta, 1989). Market development and quality control are the most important prerequisites to turn babaco into an economically rewarding crop (Kempler & Kabaluk, 1996).

Recently, *V. cundinamarcensis*, produced in northern Chile (National Research Council, 1989), has been introduced in the USA as 'ababai' and is expected to gain importance once it catches on (IFC, 2002). In the year 2002, *V. cundinamarcensis*, cultivated in Chile, was included in the offer of the Oxfam Fair Trade shops in Belgium (Oxfam Wereldwinkels, 2002) and sold as mini papaya.

2.3.6 NUTRITIONAL COMPOSITION

Analyses of nutritional composition, mainly of babaco (Table 1.8), show low amounts of sugar, making fruits less suitable for direct consumption. Potassium and vitamin C levels are rather elevated (CTIFL, 1992) while acidity levels are low with pH ranging 4 – 5 (Ferrara *et al.*, 1989). Soluble solids may vary a lot. Ferrara *et al.* (1993) report figures ranging 2.0 - 7.6 °Brix, a variation that cannot be clearly explained, but that is probably due to differences in ripeness stage and cultural practices.

Table 1.8. Nutritional composition of 100 g of babaco according to different authors

| | Cossio, 1988 Babaco, Italy | Cossio, 1988 Babaco, Ecuador | Cossio, 1988 Babaco, New Zealand | CORPEI, 2000 Babaco, Ecuador |
|-----------------------------|-------------------------------|------------------------------------|--|------------------------------------|
| Water (g) | 94.4 – 95.1 | 93.3 | 93 | 95 |
| Protein (g) | 0.74 – 0.95 | 0.90 | 0.90 | 0.7 |
| Fat (g) | 0.1 – 0.2 | 0.1 | 0.2 | 0.1 |
| Carbohydrates (g) | 2.4 | 5.4 | 6 | 6 |
| Fibers (g) | 0.8 – 1.5 | 0.5 | 0.7 | 1.1 |
| Ash (g) | 0.5 – 0.7 | 0.4 | 0.69 | No data |
| Calcium (mg) | 27 - 36 | 11 | 12 | 13 |
| Potassium (mg) | 281-387 | No data | 220 | 165 |
| Phosphorous (mg) | 6.9 | 14 | 17 | 7 |
| Iron (mg) | No data | No data | 0.9 | No data |
| Vitamine A (IU) | No data | No data | 0.09 | 0.09 |
| Tiamine (vit. B1) (mg) | No data | 0.02 | 0.02 | 0.03 |
| Riboflavine (vit. B2) (mg) | No data | 0.06 | 0.03 | 0.02 |
| Niacine (mg) | No data | No data | No data | 0.5 |
| Ascorbic Acid (vit. C) (mg) | No data | 29 | 31 | 28 |
| Calories (kcal) | No data | 23 | 28 | No data |

2.3.7 PAPAIN

All members of family Caricaceae possess laticifers. When a plant is damaged, it releases a latex that works as a defence mechanism to fend off predators (Dussourd & Eisner, 1987). This latex contains diverse proteins such as chitinases (Azarkan *et al.*, 1997) and protease inhibitors. Caricaceae typically store tremendous amounts of cysteine proteinases in their laticifers. These proteinases show the ability to decompose proteins. Different Caricaceae show different compositions of proteinases. Proteinases of papaya (*Carica papaya*) have been studied in detail, as papaya is an important source of proteinases and is cultivated worldwide for this purpose. The most important proteinases of papaya are papain, chymopapain, caricain (formerly known as proteinase Ω) and glycy endopeptidase (also known as papaya proteinase IV) (El Moussaoui *et al.*, 2001). Although it is only one of the proteinases, papain is often used as a general term to describe proteinases extracted from papaya. Papaya latex enzyme research flourished during the last decade due to the application of better, often molecular, techniques that can study in detail the structure and function of each enzyme (El Moussaoui *et al.*, 2001). Papain, chymopapain and caricain are broad-spectrum endopeptidases whereas papaya proteinase IV is a highly specific proteinase for a peptide linkage with glycine (Buttle, 1994).

Studies on proteolytic enzymes in *V. cundinamarzensis* showed the presence of four proteinases, CC-I to CC-IV, with CC-I and CC-III suggested to correspond respectively to papain and chymopapain of *C. papaya* (Walraevens *et al.*, 1993). Pereira *et al.* (2001) suggest the possibility that *V. cundinamarzensis* latex consists of six to seven cysteine enzymes, some of them with a transient existence.

Given the importance of papain extraction from *C. papaya* latex, a lot of information exists on this topic. Although latex is exudated by several parts of the plant including stems, branches and petioles, the amount recoverable from such parts is much less than that obtainable from immature fruits (Foyet, 1972). The productivity and proteolytic activity of the latex is affected by the variety and sex of the plants, fruit maturity, lancing operations, cultural practices and other factors (Becker, 1958). Extraction is realised by making 2 mm deep incisions in the immature fruits (Skelton, 1969). Contradictory indications exist about the best moment of tapping. Different authors (Patron, 1952; Lassoudière, 1969b; Foyet, 1972) report the morning during cloudy weather as the best moment, although Madrigal *et al.* (1980) did find only differences in yield while total activity remained the same, suggesting that in the morning latex only has increased moisture content. The liquid latex is sun-dried, oven-dried at temperatures of 50 - 55 °C for 6 hours (Ortiz *et al.*, 1980) or preferably spray- or freeze-dried (Baines & Brocklehurst, 1979; Baeza *et al.*, 1990). According to Becker (1958) 1 kg fresh latex yields 200 g crude papain, while Krishnamurthy *et al.* (1960) report total solids contents ranging 11.1 to 54.9 %. Liaquat & Mazumdar (1994) report increase in both quantitative and qualitative latex yield by applying ethephon, indole-3-butyric acid (IBA) and gibberellic acid (GA₃). Lassoudière (1969b) reports yields of approximately 100 kg dried latex/ha.year. To preserve its activity, the dried latex must be stored at low temperatures in the dark in airtight containers (Poulter & Caygill, 1985). Activities of dried latex are measured by different methods resulting in comparison problems. Methods are based on hydrolysis of low molecular weight synthetic substrates, on hydrolysis of proteins (casseine) or based on milk-clotting ability (Flynn, 1975).

Crude papain is the commercial name given to the dried latex, which consists of a mixture of several proteases (Madrigal *et al.*, 1980). Purified papain is used in textile industry for reducing shrinkage of certain types of wool, in beer industry for clarifying beer, in tanning for bating hides and in food industry mainly as a meat tenderiser but also for dough conditioning, cheese preparation and protein enrichment of cereals (Becker, 1958; Poulter & Caygill, 1985). Papain has a number of pharmaceutical applications as a digestive aid and for external treatment of skin diseases such as wart removal, scar treatment and skin cleansing (Poulter & Caygill, 1985). Starley *et al.* (1999) report the use of mashed papaya flesh as a cheap remedy to treat burns while more recently, chymopapain, another protease from *C. papaya*, has proved to act therapeutically in patients suffering from spine disc disorders (Jaziri *et al.*, 1994).

After 1945, Sri Lanka became the major supplier of papain to Western markets, until papaya mosaic virus seriously decreased production and East Africa and Zaire became the major suppliers. In 1973, Zaire produced 59 % of the world's crude papain equivalent (Flynn, 1975) and this proportion rose until 1980, when supplies became tight and prices high. Subsequently alternative uses for papain were developed and other suppliers such as India entered the market. In 1985, world market figured values between 200 and 400 tons, including papain of all types and qualities, with the USA, Japan, UK, Belgium and France as the major importers (Poulter & Caygill, 1985).

Little research on commercial potential of latex of *Vasconcellea* species has been realised. FAO (1992) reports the use of latex of *V. cundinamarcensis* as a meat tenderiser, by rubbing it in the meat, while the National Research Council (1989) speculates that *V. stipulata*, and other highland papayas, might be grown as a source of papain. Baeza *et al.* (1990) evaluated in detail the latex of *V. cundinamarcensis* and found that the proteolytic activity of freeze-dried latex was between five- and eight-fold higher than that of *C. papaya* latex. Spray-drying resulted in a 70 % loss of activity while oven-drying retained only 49 % of proteolytic activity. Baeza *et al.* (1990) reported also a water content in fresh latex of 80 % (weight percentage), which is 8 % lower than that of papaya, and demonstrated that stem extracts showed even higher proteolytic activities, offering even more possibilities. Recently studies on babaco (Dhuique Mayer *et al.*, 2001) showed proteolytic activity of the latex equivalent or slightly higher than that of papaya.

2.3.8 USES

The National Research Council (1989) classifies the potential of *Vasconcellea* species at three levels: (1) direct use of the tasty, high quality fruits; (2) use of the genetic variability as 'raw' material for the creation of new *Vasconcellea* fruits; and finally (3) use in breeding programmes for papaya improvement in order to extend cultivation range, by using genetic endowment for cold adaptability, and improve papaya production, by using resistance genes from highland papayas.

Direct use of highland papayas is common in the Andes where fruits, mainly pulp, are eaten fresh, roasted, processed in juices, marmalades, preserves and dairy products or even cooked in sauces, pie fillings and pickles (National Research Council, 1989; CAF, 1992; Van den Eynden *et al.*, 1999). Endt (1981) describes the taste of babaco as a unique blend of strawberry, pineapple and papaya. Kempler & Kabaluk (1996) state that due to low soluble solids content, sugar must be added to processed and even fresh fruits. According to National Research Council (1989) *V. stipulata* fruits are the best highland papayas for jams and sauces.

Little is known about medicinal values of *Vasconcellea* species, although FAO (1992) mentions use of fruit to treat arterial sclerosis and use of latex against skin mycosis and verruca plane. The latex is also reported to be antihelmintic and is used to cure enteritis, diabetes and liver diseases.

The National Research Council (1989) states that the potential of highland papayas is only exploited at smallholder level in Andean countries but that even there, given adequate quality control, it might be possible to develop both fresh-fruit export business and an Andean papain-extraction industry. Even in cooler parts of the developing world, highland papayas could provide a range of future fruits. With their extreme variability, they could become a successful backyard crop from Morocco to Papua New Guinea and develop from local trade over small- or large-scale domestic trade to export.

According to the National Research Council (1989), natural hybridisation of *V. × heilbornii* varieties with *V. stipulata* results in many intermediate forms with different flavours and varying amounts of seeds. This is confirmed by Soria & Soria (1992) who artificially developed some hybrids between *V. × heilbornii* and *V. cundinamarcensis*. Mekako & Nakasone (1975) report variability and even some heterosis effects in other *Vasconcellea* interspecific hybridisation.

Use of highland papaya genes in papaya breeding is especially useful in programmes that focus on the incorporation of resistance against papaya ringspot virus. *V. candicans*, *V. cundinamarcensis*, *V. stipulata* and *V. × heilbornii* are Ecuadorian *Vasconcellea* species that have been reported as being resistant against papaya ringspot virus (Manshardt & Wenslaff, 1989b). In a more recent study however, Manshardt (1992) mentions *V. × heilbornii* 'Babacó' as susceptible to papaya ringspot virus, illustrating the lack of knowledge on *Vasconcellea* species and their properties.

2.3.9 SITUATION OF VASCONCELLEA IN ECUADOR

At this moment, Ecuador is the only country in the world where babaco is cultivated successfully at a commercial level. Due to local climatologic characteristics, babaco produces the whole year round (Cossio, 1988). In Ecuador, it is a very popular ingredient in fruit juices consumed in restaurants and at family level. Babaco cultivation developed probably in southern Ecuador (Fabara *et al.*, 1985) and spread throughout the higher elevations by human distribution of plant material. Babaco was cultivated by the local population before the Spanish conquest (National Research Council, 1989) whereas cultivation at commercial level has been reported for over 80 years around the city of Baños (Tungurahua Province) (Cossio, 1988). Plantations can be found in open-air but recently greenhouses have gained considerably in importance. In 1962, Ecuador possessed 54 ha of babaco, rising to 120 ha in 1982 and 200 ha in 1988 (Cossio, 1988). Soria & Viteri (1999) report a total area of 79 ha with a production of 632 tons in 1996, decreasing mainly due to intensification of greenhouse cultivation and *Fusarium* problems (Ochoa *et al.*, 2000) to 25 ha producing 419 ton in 1997. Main cultivation zones are Tungurahua and Loja Province (CORPEI, 2002), while Pichincha, Azuay and Cotopaxi Provinces hold some high-input greenhouse cultivations (Soria & Viteri, 1999). In 1999, 16,880 US\$ FOB of babaco have been exported mainly to the Netherlands, Colombia, Germany and Switzerland (SICA, 2002). Unfortunately, due to phytosanitary restrictions fresh babaco fruits cannot be exported to the USA (SICA, 2002). Prices in Ambato, an important Ecuadorian centre of babaco cultivation, are around 8 euro/12 fruits (Coppens d'Eeckenbrugge, pers. comm.).

Vasconcellea cundinamarcensis is cultivated throughout the Ecuadorian Andes on a small-scale level and is marketed locally (CAF, 1992), while *V. stipulata* can only be found in southern Ecuador, restricted to sporadic presence in home gardens.

Recently, commercialisation of *V. stipulata* has been attempted by farmers near Ambato, because of its excellent flavour and smaller size (Coppens d'Eeckenbrugge, pers. comm.).

3. PROBLEMS AND OBJECTIVES

3.1 PROBLEM FORMULATION

3.1.1 IMPORTANCE OF PLANT GENETIC RESOURCES WORLDWIDE

Plant genetic resources for food and agriculture provide the biological basis for world food security. They serve as the plant breeder's most important raw material and the farmer's most essential input. Under-conserved and under-utilised plant genetic resources are considered of great importance by the United Nations (FAO, 1996a) and by the leading international research organisations such as the CGIAR (Swaminathan, 1999). Their importance includes different fields: they can play an important role in food security, show a large adaptation to marginal areas, often include multiple uses (including also cultural aspects) and contribute to a global diversity. The conservation and sustainable utilisation of these resources and the fair and equitable sharing, taking into account the value of traditional knowledge, of the benefits has been discussed frequently in recent years by international organisations and has been regulated in Global Agreements as the Convention on Biological Diversity (United Nations Environment Programme, 1992).

Of the estimated 300,000 to 500,000 higher plant species on earth, 30,000 are considered edible, though mankind has utilised only 7,000 of these. Given this enormous repository, it is remarkable that as few as 30 plant species account for about 95 % of the world's calories and proteins uptake. Only three species, maize, rice and wheat, provide more than half of the plant-derived energy intake (FAO, 1996a). Genetic variability of these species is often low due to the extension of modern agricultural systems and the adoption of high-yielding, universal and uniform cultivars (Altieri & Merrick, 1987).

Recently, plant genetic resources have received a growing recognition for their role in food and nutritional security, income generation and environment conservation. Their sustainable conservation and use, linked to access, equitable sharing of benefits and farmer rights, have even become political issues, often causing conflicts between developing and developed countries. Recently, the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2001) ensures that plant genetic resources of economic and/or social interest, particularly for agriculture, will be explored, preserved, evaluated and made available for plant breeding and scientific purposes, thus forming a first step towards a transparent use of plant genetic resources.

At this moment, especially developed countries increase their efforts to safeguard existing diversity through germplasm conservation. This conserved germplasm may provide plant breeders with the genetic resources needed for developing new crops which are more resistant to diseases, insect pests, poor soils and harsh weather, thus enabling farmers to maintain high yields (Plucknett *et al.*, 1983). Germplasm conservation mainly consists of *ex situ* storage of seed and vegetative material and does not provide a panacea for conserving natural sources of crop genetic resources

(Oldfield, 1989). Moreover, about 80 % of the *ex situ* conserved diversity belongs to major crops and their wild relatives (Padulosi *et al.*, 1999). *Ex situ* conservation poses a variety of problems, such as lack of representation in gene banks of the whole range of genetic diversity and genetic changes due to storage conditions and grow-out procedures. *In situ* conservation allows plant species to continue their dynamic evolution, often influenced by human intervention, and adaptation to the environment. This phenomenon is particularly important for crops in areas under traditional agriculture where they often become enriched by gene exchange with wild or weedy relatives (Altieri & Merrick, 1987).

Major crops are known as being of great importance for food security in one or several regions and consist mostly of a limited number of, often staple food, crops cultivated around the world. A species that has not been fully exploited in terms of its potential is by distinction under-utilised. The main reason this potentially useful species rarely attract interest from agricultural research is the financial constraint: funding agencies are not in favour of work on crops of unproven potential and unknown commercial value (Morico *et al.*, 1999). Padulosi *et al.* (1999) mention an increasing interest in neglected and under-utilised crop species, reflecting a growing trend within agriculture to identify and develop new crops for export and domestic markets.

In developing countries production of subtropical and tropical fruit has generally only had a marginal or subsistence role. Only recently, with increasing tourism in tropical countries and commercial exchanges with developed countries, fruit growing has developed from being a traditional management activity to an industrial one. This is the case for some countries of South-east Asia and Central America, which have adequately sustained agricultural research on this topic (Morico *et al.*, 1999). Less than five percent of edible fruits native to tropical areas are cultivated and marketed on a commercial basis (Wijeratnam, 2000). For the many minor tropical fruit and nut species that occur in their centre of diversity, little or nothing is done to collect, preserve and characterise this enormous amount of unique genetic diversity. It is expected that a very diverse genetic variability exists in the wild range of indigenous fruits yet to be discovered, as there are so many seedling trees that still occur in wild or semi-wild conditions. Besides the imperative to conserve as much as possible the indigenous fruit species, their potential could be exploited, namely as new fruit trees, as multi-purpose trees, as rootstocks and as sources of germplasm for improving existing crops (Morico *et al.*, 1999). Under-utilised fruit crops may even contribute on a wider level as they often show multiple uses such as medicinal and ornamental value (Padulosi *et al.* 1999). Wijeratnam (2000) mentions the complex circumstances encompassing tropical fruit production (often accompanied with heavy losses) and marketing (due to inadequate infrastructural facilities). Problems at commercialisation level (failure to market the products in the most optimal way) and at consumer level (ignorance about fruits and their usage) also accounts for difficulties in developing minor fruit crops (Rajat, 2000).

An example of a successfully developed minor fruit crop is kiwi (*Actinidia deliciosa*), originating from China (Hewett, 1993). Its production has increased in the last 20 years from 2,500 ton in 1971, all production proceeding from New Zealand, up to 977,399 ton in 2001, coming mainly from Italy (36 %) and New Zealand (27 %) (FAO, 2002). Striking is the absence of commercial production in its country of origin. Another example is pistachio (*Pistacia vera*) with a production rise in the last 20 years of over 700 %, which can mainly be attributed to Iran, Syria and Turkey, its centre of origin (FAO, 2002). Remarkable is the recent rise of pistachio cultivation in the USA, making the USA one of the biggest pistachio producers. As this production is based on just one variety ('Kerman' originating from Iran) it is an example of the possibilities of minor crops but also includes potential danger of relying on a small genetic base and neglecting the genetic diversity.

3.1.2 IMPORTANCE OF PLANT GENETIC RESOURCES IN THE ANDES

Once the home to some of the world's most advanced cultures, the Andean region is now one of the world's poorest areas because of a decline in farming, high rates of population growth, migration and misuse of natural resources (Izquierdo & Roca, 1998). Andean farmers domesticated numerous species of edible roots and tubers, grains, vegetables and fruit crops, all together more than 70 food crops (National Research Council, 1989). Botanical colonisation sealed off a major centre of crop diversity from the rest of the world. Food plants of Asia, Mexico and especially of Europe became prominent; those of the Andes were largely lost. Potatoes are the only exclusively Andean crop to have gone worldwide. Tomato (*Lycopersicon*) species have their origin in the Andes but were not a food of the Incas and were in fact domesticated elsewhere (National Research Council, 1989).

However, in terms of crop diversity the Andean ecoregion, one of the centres of domestication, according to Vavilov, is very rich and much of this diversity is indigenous and selected by man (Castillo, 1995). Little known outside the Andes, many of the under-exploited Andean crops, as well as the associated indigenous knowledge, are fast disappearing. They may prove to have an important role worldwide as food, as well as for medicines and industrial uses. Some are grown as food in Brazil, and as far as New Zealand and Vietnam, whereas others are found in Europe and the United States as gourmet foods (Izquierdo & Roca, 1998).

Fruits form an important part of these Andean under-utilised crops (Campbell, 1996). Some did already find their way outside of the Andes. This is for example the case for goldenberry (*Physalis peruviana*), tree tomato (*Cyphomandra betacea*) and cherimoya (*Annona cherimola*). Other species like naranjilla (*Solanum quitoense*), pacay (*Inga* spp.) and most highland papayas (*Vasconcellea* spp.) are much appreciated locally but are virtually unknown outside of the Andes (National Research Council, 1989; Izquierdo & Roca, 1998). Paradoxically some of these Andean fruit crops, which are cultivated outside the Andes, e.g. in New Zealand, are

exploited at such a commercial level that they form a source of income that is more important than in the area of origin (Libreros & Lastra, 1999).

3.2 OBJECTIVES

The 'Global plan of Action for the Conservation and Sustainable Utilisation of Plant Genetic Resources for Food and Agriculture' (FAO, 1996b) recommends among other things to focus both on *in situ* conservation and development of plant genetic resources and on promoting development and commercialisation of under-utilised crops and species as key activities in future plant genetic resources activities.

In situ conservation and development through on-farm management and improvement needs multi-disciplinary scientific research consisting of four basic types (FAO, 1996b):

- (i) ethnobotanical and socio-economic research to understand and analyse farmer knowledge, selection/breeding, utilisation and management of plant genetic resources;
- (ii) population and conservation biology to understand the structure and dynamics of genetic diversity in local landraces/farmers' varieties;
- (iii) crop improvement research, including research in mass selection and simple breeding; and
- (iv) research and extension studies on little-known crops, including seed production, marketing and distribution.

Promoting the development and commercialisation of under-utilised crops and species also needs specific research and capacity building (FAO, 1996b):

- (i) under-utilised species with potential for increased sustainable use need to be identified;
- (ii) sustainable management practices must be developed and implemented;
- (iii) post-harvest processing methods should be provided; and
- (iv) marketing methods need to be developed.

In its annual report the International Plant Genetic Resources Institute emphasises the need for a search for alternative profitable fruit crops (IPGRI, 2000). As the global demand for tropical fruits is expected to increase by 40 % by 2005, efforts to develop these fruits are justified. Fruits form an important source of vitamins and micronutrients and can play an important role in income generation. At this moment banana, mango, pineapple, avocado and papaya, account for over 90 % of fruit exports. A starting point in this search is the assessment and conservation of the existing diversity. IPGRI (2000) and Padulosi *et al.* (1999) stress the need to concentrate on under-utilised crops, which are often neglected by researchers and mention the lack of research on genetic diversity and the lack of knowledge on local uses as a major constraint to their development.

Heywood (1999) summarises the trends in agrobiodiversity studies. He highlights the need for inventarisation of plant resources that may be used in agricultural development, the conservation of genetic resources, the need to broaden the genetic base of crops, the need to cultivate a diversity of crops (new and under-utilised crops) and the importance of traditional knowledge.

The National Research Council (1989) states that to promote the 'lost crops of the Incas' very basic research is required:

- (i) germplasm collection, especially in remote areas;
- (ii) germplasm characterisation and selection;
- (iii) analysis of agronomical practices;
- (iv) plant genetics;
- (v) post harvest;
- (vi) nutritional studies; and
- (vii) pest and disease control.

Recently, Padulosi *et al.* (2001) consider greater efforts in gathering information on distribution, use and traditional knowledge on under-utilised crops as very valuable to improve future access to this material by researchers and other users.

The Andes is considered to be an important centre of plant genetic resources. The latter often comprise under-utilised crop species under increasing threat of disappearing. As a consequence, local native fruit species in southern Ecuador were targeted to be studied on a multi-disciplinary level. Two crops, known as under-utilised though showing an important potential were selected from an initial ethnobotanical study realised in southern Ecuador (Van den Eynden *et al.*, 1999). These were on the one hand one fruit species, i.e. cherimoya (*Annona cherimola*) and on the other a complex of highland papayas (*Vasconcellea* species). Both are abundant in Loja Province though endemic only to a small area in southern Ecuador and northern Peru. Hence they are little studied especially in their centre of origin. The selected species are generally acknowledged to show a huge potential but to be in need of further research (National Research Council, 1989; FAO, 1992; Castillo, 1995; Campbell, 1996; Libreros & Lastra, 1999).

The National Research Council (1989) advises a germplasm collection of natural populations of cherimoya and highland papayas as an urgent research need in order to discover or develop better types and in order to avoid the danger of losing unique and potentially valuable material. Nuez *et al.* (1999) mention that in southern Ecuador, less-known cultivated species and wild species related to main crops remain uncollected and have not been studied.

Taking into account the general agreement on the need for research on under-utilised fruit crops, but faced with limitations due to a lack of previous studies and the relatively short period to conduct research on perennial crops, multi-disciplinary studies were planned aimed at obtaining a wide range of preliminary data that should

form a basis for further research and policy planning. Research on the targeted fruit species was concentrated on:

- (i) traditional knowledge and uses (ethnobotanical study), in order to have an idea on the local status and constraints;
- (ii) crop ecology, to establish crop requirements and determine potential cultivation zones;
- (iii) germplasm characterisation and selection, to assess the local variability and demonstrate the existing potential;
- (iv) generative propagation, to overcome germination problems and facilitate future seed conservation and management; and
- (v) alternative uses (papain) of the harvested products, to wide the potential uses of some species.

Due to the basic local research circumstances, typically for remote areas in developing countries, the methodologies used, are mostly simple and sometimes rudimentary and therefore results must be considered as preliminary. However, as the results obtained are expected to raise interest at national and international level and lead to further detailed research as well as conservation this study forms the necessary first step . As a general objective, all results obtained, both through this and future studies, should lead to cultivation of native fruits species and could lead to the improvement of the economic situation of local farmers in the region.

4. MATERIALS AND METHODS

4.1 ETHNOBOTANICAL SURVEY

Etnobotanical surveys may give valuable information on the actual situation of the fruit species and may be used to refine add-value strategies for under-utilised species. This ethnobotanical survey aims at assessing the actual status of the targeted fruit species in Loja Province. The local population was interviewed on vernacular nomenclature, uses and knowledge on the fruits together with agricultural practices applied and existing commercialisation systems.

Cherimoya (Annona cherimola)

A survey on cherimoya was realised between July and August 1999. The survey targeted farmers who possessed cherimoya trees. In the zone where these trees are abundant, a total of 162 persons were surveyed in 13 municipalities belonging to eight counties (Figure 4.1).

The survey in the selected zones consisted of a walk through the village and surroundings, asking farmers if they owned cherimoya trees. In case of a positive answer, the farmer was further questioned using a semi-structured interview (Martin, 1995). After finishing all surveys, data were classified and processed using the statistical software SPSS 9.0.

Of the 162 persons surveyed, the majority was male (62 %) with a higher proportion of older population (Figure 4.2). This is mainly due to the methodology used. Surveying farmers in and around the farms often excludes younger farmers working in far-away fields.

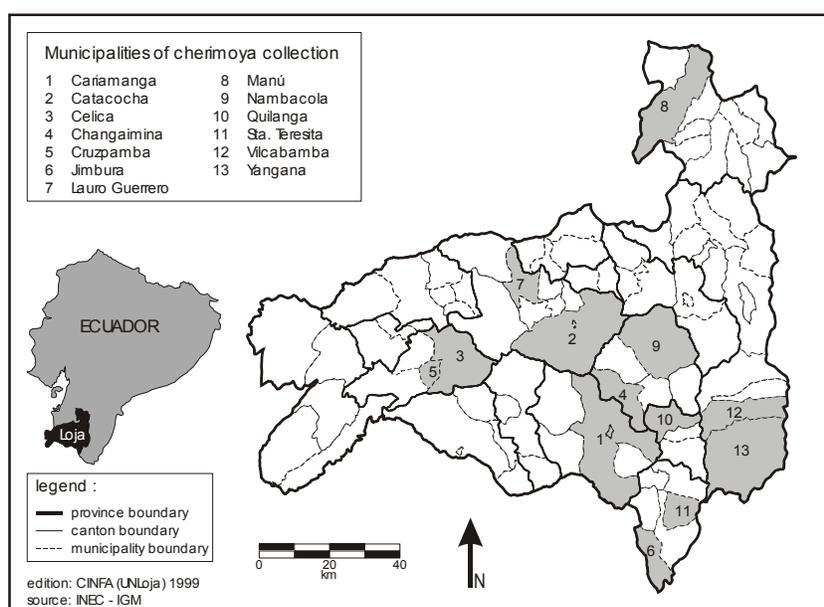


Figure 4.1. Map showing municipalities in Loja Province where the ethnobotanical survey on cherimoya was realised

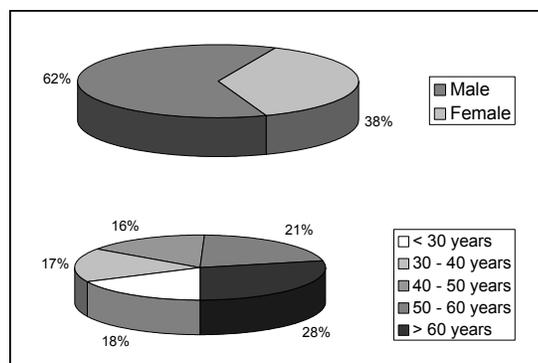


Figure 4.2. Composition of population surveyed (162 farmers) during the ethnobotanical study on cherimoya, according to gender and age

Highland Papayas (*Vasconcellea* spp.)

A survey on highland papayas was realised between August and October 1998. The targeted area included different areas where highland papayas occur abundantly in the wild and/or in (home)gardens, as well as Catamayo county, which is well-known for its commercial activities on agricultural products. A total of 660 persons, belonging to all layers of the population, were surveyed in seven county capitals (Figure 4.3).

The survey was realised at market places during market days. A table with ten *Vasconcellea* samples (*Vasconcellea* × *heilbornii* ‘Babacó’, *V.* × *heilbornii* var. *chrysopetala*, five yet unclassified *V.* × *heilbornii* varieties, two *V. stipulata* accessions and one *V. cundinamarcensis* accession; Figure 4.4) was placed on a clearly visible site in the market place. People were invited to approach the table and were questioned using semi-structured interview (Martin, 1995). After finishing all surveys, data were classified and processed using the statistical software SPSS 9.0.

Of the 660 persons surveyed, the majority was male (63 %) with a higher proportion of younger people (Figure 4.5). The majority of the surveyed people were farmers (20 %), with high participation of housewives, i.e. not active, (16 %), salesmen (17 %) and teachers (12 %). The majority received primary education (43 %) with an important group of people that received secondary education (31 %) and higher education (22 %). Only a minority (4 %) did not receive any education at all.

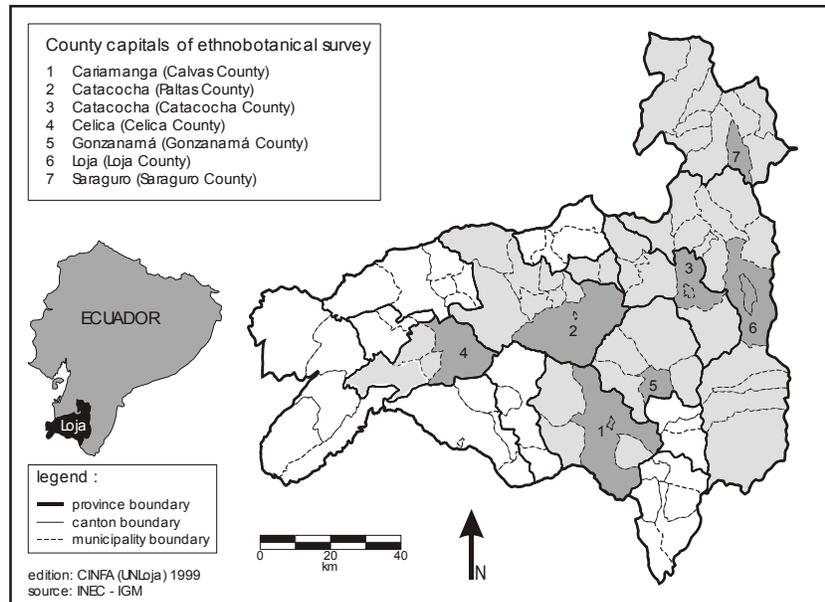


Figure 4.3. Map showing county capitals (dark grey) and corresponding counties (light grey) in Loja Province where the ethnobotanical survey on highland papayas was realised

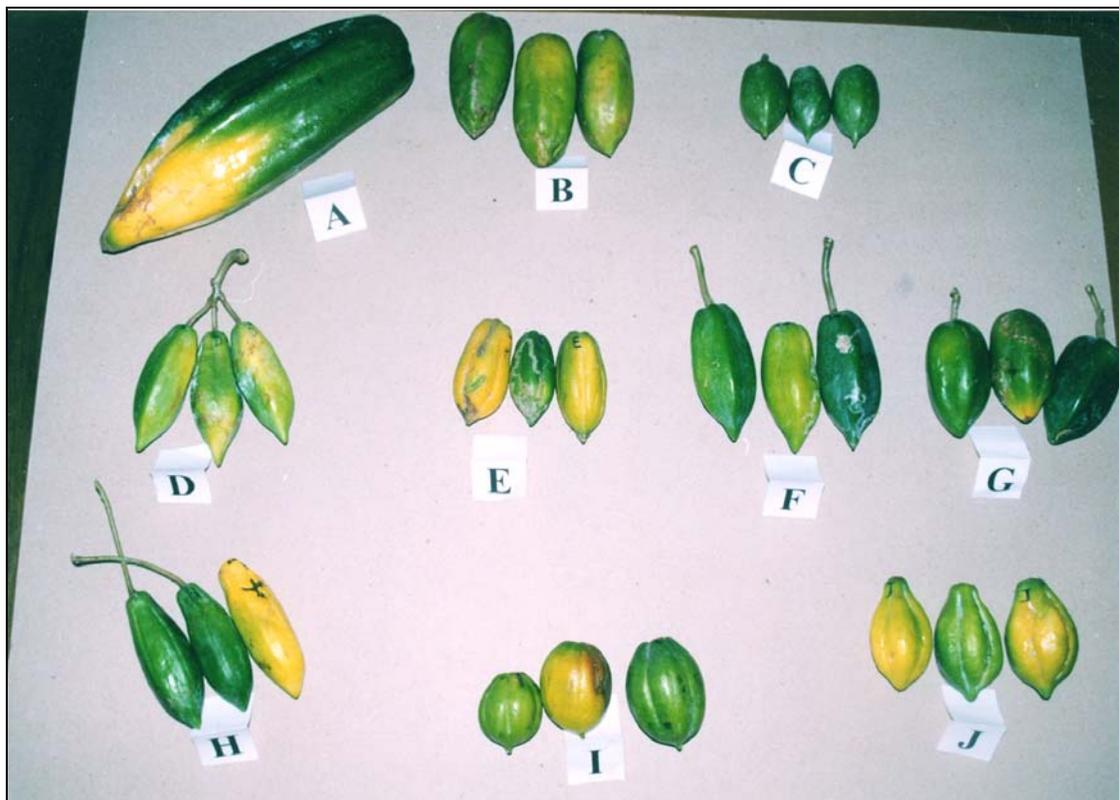


Figure 4.4. *Vasconcellea* samples used for the study on knowledge on highland papayas in Loja Province : A : *Vasconcellea* × *heilbornii* ‘Babacó’; B : *V.* × *heilbornii* var. *chrysopetala*; C – G : unclassified *V.* × *heilbornii* varieties, H – I : *V. stipulata* accessions; J : *V. cundinamarcensis*

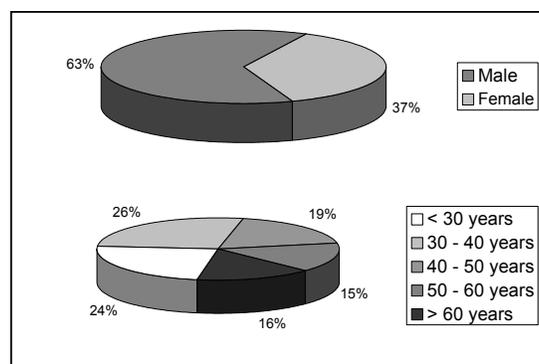


Figure 4.5. Composition of population surveyed (660 persons) during the ethnobotanical study on highland papayas, according to gender and age

4.2 CROP ECOLOGY

A first step in crop domestication is the determination of the optimal edaphoclimatic parameters for the species concerned. Most crop species show specific climate and soil preferences and optimal yield under these specific conditions. Once the edaphoclimatic characteristics are determined, similar conditions elsewhere can be located using a Geographic Information System (GIS). In the case of Loja Province which is the primary target zone for establishing cherimoya and highland papaya cropping, there exists a lot of variability in soil and climate. Therefore, more zones than the surveyed ones may hold optimal growth conditions.

Determination of optimal growth conditions is mostly based on the critical evaluation of yield differences under different edaphoclimatic conditions (Sys *et al.*, 1991). Ecological conditions leading to the highest yields are considered as optimal. As in this study the targeted fruit species are perennial tree crops, establishing yields under different environmental conditions would require a considerable amount of time and space. Therefore the existing methodology was adapted and plant frequencies under different edaphoclimatic conditions were used instead of yields as a criterion to assess the range of edaphoclimatic parameters and their optimums. As the targeted fruit species are mostly found in wild or tolerated in gardens, with their distribution being the result of natural dispersion and natural selection, high plant frequencies at certain edaphic or climatic ranges are considered to be an indication that they occur near or in the optimum conditions.

Different sites with or without presence of the targeted fruit species were selected on the basis of past field visits and surveyed. Each of these sites was geographically located using a GPS (Garmin® GPS45). Altitude was determined using a digital altimeter (Thommen® Altitronic Traveler). Mean annual temperature, annual precipitation and soil formation unit were derived, using the geographical coordinates, from respectively the digitalised (resolution 100 x 100 m) isohyete map, isotherm map and soil map (Organización de los Estados Americanos, 1994) using the prevailing characteristics, as indicated by the map unit. A map unit is defined as a

class used by a thematic map, e.g. map unit '10 – 12 °C'. Soil characteristics were determined using soil sample analysis. Soil sampling was realised using a core sampler. The following soil characteristics were determined at the Soil Laboratory of the 'Universidad Nacional de Loja': texture (method of Boyoucos), organic matter (method of Walkley & Black), pH (H₂O), stoniness, CEC (method of Chapman), available nitrogen (Kjeldahl method), available potassium (flame photometry) and available phosphorous (method of Olsen). Soil parameters were recalculated to an average value for the profile, using weight factors decreasing with depth, as upper layers are considered more important for root development (Sys *et al.*, 1991) (Table 4.1). For each edaphoclimatic characteristic a histogram was calculated, using SPSS 9.0. This histogram shows the range over which data were collected with their accompanying plant frequencies and was also used to determine the optimal range. This optimal range was defined as the range coinciding with 75 % of species occurrence.

Zonal classification, identifying zones with similar edaphoclimatic characteristics, was realised using an adaptation of the simple limitation method described by FAO (Sys *et al.*, 1991). All geographic calculations and classifications were performed using the Geographic Information System (GIS) software package IDRISI 32. For each characteristic (mean annual temperature, annual precipitation and soil formation unit) plant frequency at each map unit was determined and attributed to the respective map unit, using a reclassification of the thematic map. This resulted in a Temperature Frequency Map, a Precipitation Frequency Map and a Soil Formation Frequency Map. A combination of these maps, always keeping the lowest plant frequency as the final plant frequency, results in a Climate Frequency Map (Temperature Frequency Map and Precipitation Frequency Map) and an Overall Frequency Map (Climate Frequency Map and Soil Formation Frequency Map). Final plant frequencies give an indication about the abundance of the targeted species under the local edaphoclimatic conditions. Figure 4.6 gives a short schematic overview of the methodology followed with an example for *V. stipulata*.

Cherimoya (*Annona cherimola*)

Fieldwork for the edaphoclimatic study of cherimoya was realised in the period August - October 1995. Based on a previous cherimoya germplasm collection (González Lituma & González Lituma, 1980) and local knowledge at the 'Universidad Nacional de Loja', 20 sites with presence of wild cherimoya stands, in forests, were selected (Figure 4.7). As in these forests cherimoya plants were abundant, no exact plant number could be obtained and frequencies were determined based on number of sites where *Annona cherimola* was occurring. In case of cherimoya, additionally relative humidity and mean minimum and maximum temperatures were taken into account. Relative humidity data were obtained for a number of climatic stations (period 1971-1986) in Loja Province (Maldonado Astudillo, 1985).

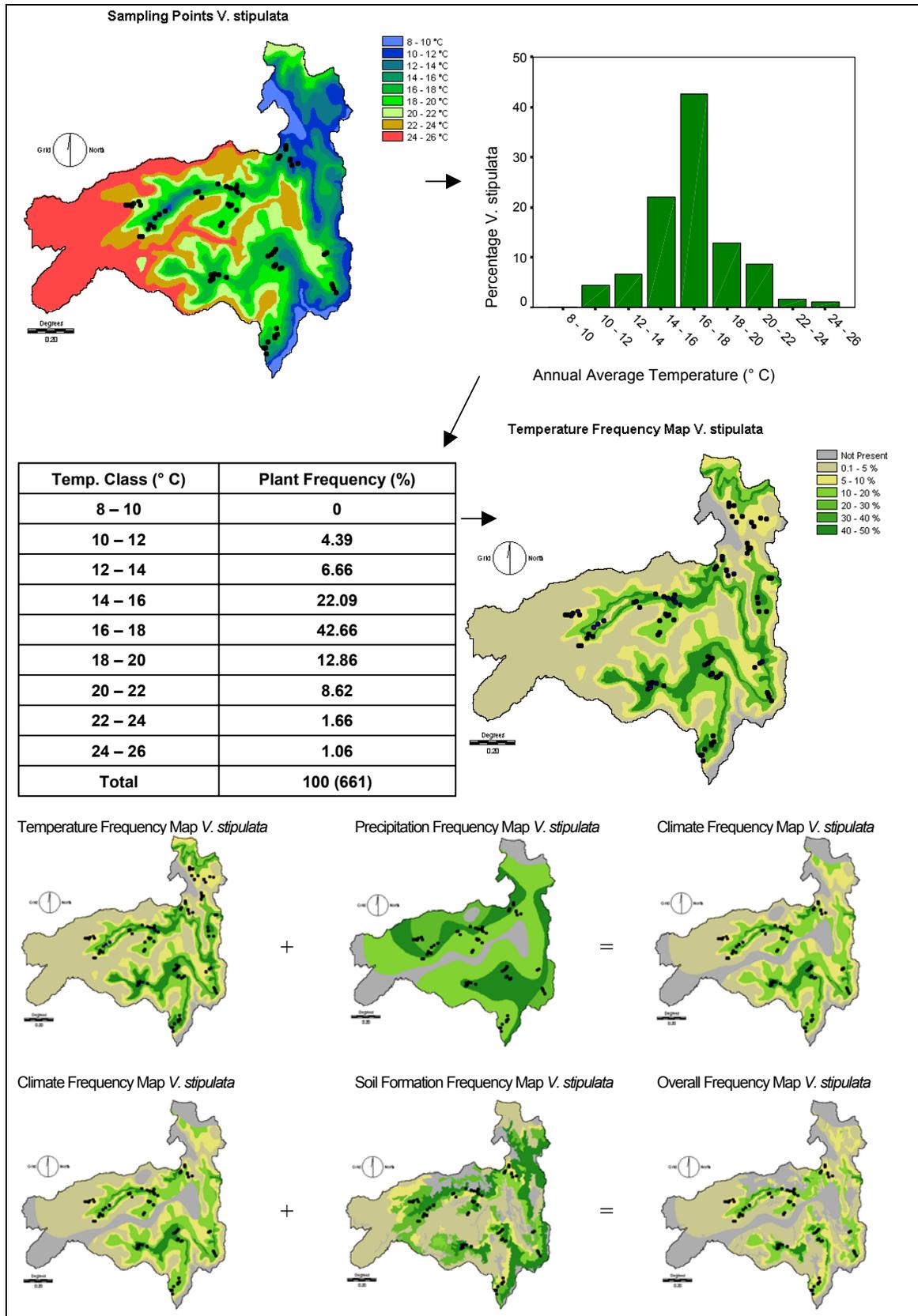


Figure 4.6. Schematic overview of the methodology used for the zonation: example of *V. stipulata*

Maximum and minimum temperatures were obtained using a relation between minimum and maximum temperature and altitude as determined for the local situation by Bydekerke *et al.* (1998) (Max. Temp. (°C) = 34.924 + 4.2 Altitude (km) - 4 Altitude (km)² / Min. Temp. (°C) = 16.37 + 1.7 Altitude (km) – 0.8 Altitude (km)²). Soil sampling was realised on 52 sampling points from 0 - 100 cm or less in case of the presence of a root-restricting layer. Texture and coarse fragments were recalculated, using weight factors (Table 4.1), over the rooting depth, considered to be 100 cm in optimal conditions. Values of organic matter and pH were averaged over the upper 25 cm of the mineral soil.

Highland Papayas (*Vasconcellea* spp.)

Field sampling for the edaphoclimatic study of highland papayas took place in January - April 1999 in different municipalities where during earlier highland papaya germplasm collection wild, tolerated or cultivated plants had been found. A total of 21 municipalities in 10 counties (Figure 4.7) were selected and in each municipality 20 farms were randomly selected. Of all 420 surveyed farms, 104 farms (Figure 4.7) owned one or more *Vasconcellea* plants, resulting in a total of 1,588 plants. Edaphoclimatic characteristics, combined with plant frequencies of each species, found at these farms were used to determine the local preferences. Soil sampling (0 - 60 cm) was realised at each of these 104 sites. All soil characteristics were recalculated using weight factors (Table 4.1).

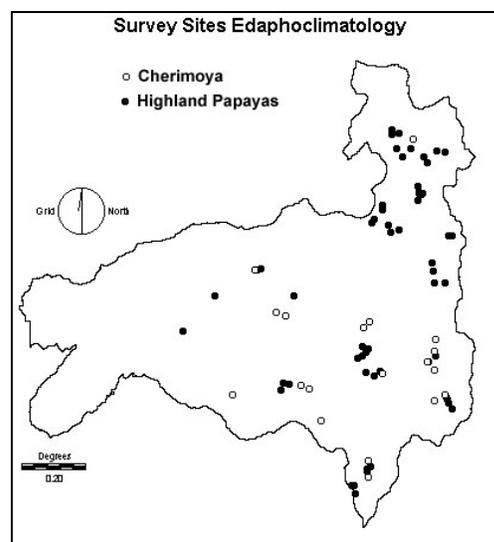


Figure 4.7. Sampling sites for the edaphoclimatic study

Table 4.1. Sections and weight factors used to recalculate the soil parameters of different depths to one value per sampling point

| Species | Section (cm) | Weight Factor |
|------------------|--------------|---------------|
| Cherimoya | 0 – 25 | 1.75 |
| | 25 – 50 | 1.25 |
| | 50 – 75 | 0.75 |
| | 75 – 100 | 0.25 |
| Highland papayas | 0 – 20 | 1.66 |
| | 20 – 60 | 1.33 |

4.3 GERmplasm COLLECTION AND CHARACTERISATION

A germplasm collection and characterisation in a species' centre of origin gives valuable information on morphologic variability, which can be used in selection and

breeding programmes.

Due to lack of space and time, germplasm characterisation was realised *in situ*. Characterisation of germplasm in collection fields (*ex situ*) would require several years. This is especially the case for cherimoya, which requires at least four years before fructification starts. As *in situ* characterisation will yield data that are the result of a combination of environmental factors, such as soil, climate and management, which differ according to the area where the material is collected, characterisation results must be considered as preliminary. In this study they are mainly used to indicate the existing potential and thus serve as a first indicative step in selection.

Germplasm characterisation is based on crop descriptors, which indicate the most important morphologic characteristics, often based on a multiple-choice system (IPGRI, 1980; IPGRI, 1988). In the case of perennial fruit crops, each tree is considered as a different accession (morphotype) and is characterised on an individual basis. The methodology of germplasm collection and characterisation consisted in a visit to different municipalities, known for their presence of the targeted fruit species. In each site visited, fruit bearing trees were located in native forests, or in farmers' fields and gardens where they are maintained and receive little management care. Each plant was marked and altitude and geographic location were determined, using GPS (Garmin® GPS45) and altimeter (Thommen® Altitronic Traveler), for possible future investigations and further collection of vegetative material. A maximum number of ripe fruits was taken from each plant for characterisation.

Characterisation was mainly realised on fruits, although the different *Vasconcellea* species were characterised more in detail (leaf, flower and inflorescence). Due to the fact that characterisation was realised on different moments throughout the plant cycle and done only once for each specimen encountered, no data on yields could be obtained. Fruit characteristics measured (per fruit basis) included fruit weight (using an Ohaus® Portable Plus balance, absolute error ± 0.1 g), seed weight, number of seeds, soluble solids (measured at physiological ripeness as evidenced by fruit colouring and softening, using a hand 0 – 32 ° Brix refractometer, absolute error ± 0.2 ° Brix), longest length from base to top, without peduncle (using a calliper, absolute error ± 0.1 mm), biggest diameter (using a calliper, absolute error ± 0.1 mm) and fruit colour (using Royal Horticultural Society Colour Chart). Calculated values included seed index (number of seeds/100 g fruit) and length/diameter ratios which gives an indication of fruit form. Characteristics of the separate fruits were averaged for each plant. All data were stored in a computerised database for further treatment.

Cherimoya (*Annona cherimola*)

Selection of cherimoya collection sites was based on earlier studies by González Lituma & González Lituma (1980) and Farré Massip & Hermoso González (1987) and on personal communications from local botanists and agronomists. Germplasm collection and characterisation took place between January 1996 and March 1998 on

32 sites, situated in 16 municipalities (Figure 4.8). All in all, 137 trees were marked and sampled, and 448 fruits collected and characterised.

Collection and characterisation was realised during the cherimoya fruit production season, which in Loja province starts in January and lasts till May with some annual variation due to rainfall. In each site visited, fruit-bearing trees were located in native forests or in farmers' fields and gardens where they are tolerated. As cherimoya fruits on any given tree ripen over a period covering more than two months, and as some trees do not bear many fruits, the number of mature fruits collected was usually low and varied between two and ten fruits per tree.

The descriptor used for fruit characterisation was based on a general descriptor for tropical fruits (IPGRI, 1980) which was adapted for cherimoya. Fruit characteristics added included fruit skin weight, thalamus weight, botanical form (Popenoe, 1974; Figure 1.14) and calculated pulp weight (fruit weight minus skin weight minus thalamus weight minus seed weight).

Preliminary selection of the most promising accessions was done using the characterisation data from the germplasm collection. Characterisation was only based on some important fruit characteristics but did not include all data necessary for a definitive market selection. Especially data on yield and susceptibility to pests and diseases, which need a closer follow up on the trees, could not be gathered.

Final selection criteria were based on criteria from selection programmes in Spain and Australia, which coincide with consumer preferences in Loja (Marissens, 1998). For consumption, the most important fruit characteristic is the amount of pulp per fruit. This can be expressed by seed index, i.e. number of seeds in 100 g of fruit, used in Spanish selection programmes (Farré Massip & Hermoso González, 1985), by number of seeds per 100 g pulp, used in New Zealand selection (Richardson & Anderson, 1993a), by percentage flesh recovery indicating pulp weight percentage, used in Australian selection programmes (Nissen & George, 1992) or by seed weight percentage. Other important fruit characteristics are weight, skin type (using botanical forms defined by Popenoe, 1974) and soluble solids. Selection criteria used in Spain and Australia and the final criteria used in the present selection approach can be found in Table 4.2.

Accessions with less than three fruits characterised were eliminated from the database, resulting in 83 accessions with 357 fruits characterised. These remaining accessions were ranked using the final set of selection criteria as presented in Table 4.2.

The best selections of Loja Province were compared to 5 important cultivars taken from cherimoya producing countries with characterisation data as presented in literature for cultivars '*Fino de Jete*', '*Bays*', '*White*', '*Bronceada*' and '*Concha Lisa*' (data from Spain (Estación Experimental 'La Mayora', Malaga; Morales, 1993), New Zealand (Kerikeri Research Centre, Kerikeri; Richardson & Anderson, 1993a) and

Chile (Pavez, 1985)). Characterisation results of the different countries did not always include all varieties, nor all fruit characteristics. As a consequence only fruit weight and number of seeds in 100 g of pulp, the New Zealand criterion, were used and applied to the five commercial cultivars retained. Characterisation data can be very variable within one cultivar, confirming the existence of genotype-by-environment interactions. It should be noted that characterisation data from Spain (Estación Experimental 'La Mayora') were obtained from trees growing in collection fields that did not receive much management practices and may therefore give the best comparison with the Ecuadorian accessions.

Table 4.2. Selection criteria for cherimoya used in breeding programmes in Spain and Australia, and final criteria used in the present selection approach (Farré Massip & Hermoso González, 1985; Nissen & George, 1992)

| Fruit characteristic | Spain | Australia | Final criteria |
|------------------------------------|--------------------------|---------------|--------------------------|
| Seed percentage (%) | - | 3 - 5 | < 5 |
| Flesh recovery (percentage pulp) | - | > 60 | > 60 |
| Seed index (no. seeds/100 g fruit) | < 6 | < 10 | < 6 |
| Weight (g) | > 300 | 300 - 600 | > 300 |
| Skin type | <i>laevis - impressa</i> | <i>laevis</i> | <i>laevis - impressa</i> |
| Soluble solids (° Brix) | > 20 | 18 - 20 | > 20 |

Highland Papayas (*Vasconcellea* spp.)

Vasconcellea germplasm collection was based on earlier collection trips (Castro Cardénas, 1990) and local knowledge on areas with high incidence of *Vasconcellea* material. Germplasm collection and characterisation took place from August 1997 to August 1998. Although the project also collected accessions of *V. candicans*, *v. microcarpa*, *V. monoica*, *V. palandensis*, *V. parviflora* and *V. weberbaueri*, and even some unidentified accessions, this study only targets *V. stipulata*, *V. cundinamarcensis* and *V. × heilbornii*.

The *Vasconcellea* descriptor was based on IPGRI's *Carica papaya* descriptor (IPGRI, 1988) and was adjusted for *Vasconcellea* plants. In contrast to cherimoya characterisation where only fruits were considered, *Vasconcellea* characterisation was also performed on infrutescences, leaves, inflorescences and flowers. Additional characteristics include leaf pubescence, length of stipules, length of petals, number of flowers per inflorescence, length of inflorescence (maximal length of inflorescence stalk), length of peduncle, number of fruits per infrutescence and pulp weight (fruit weight minus seed weight). All characteristics were, if possible, measured five times, and averaged per accession. As plants were only visited once, not all accessions were completely characterised due to absence of fruits, flowers or leaves.

Initially, a total of 211 accessions, found in 70 sites of 31 municipalities (Fig 4.9) were collected and characterised.

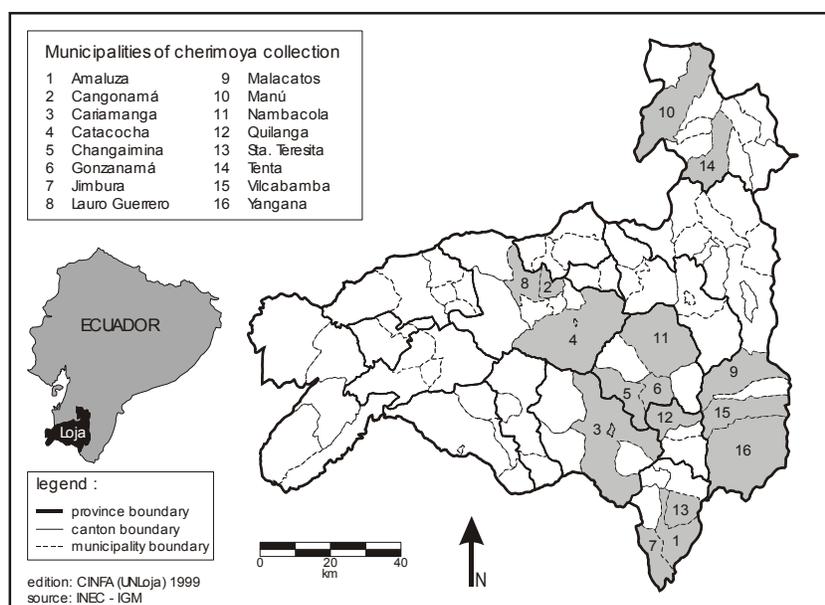


Figure 4.8. Map of municipalities in Loja Province where the germplasm collection and *in situ* characterisation of cherimoya was realised

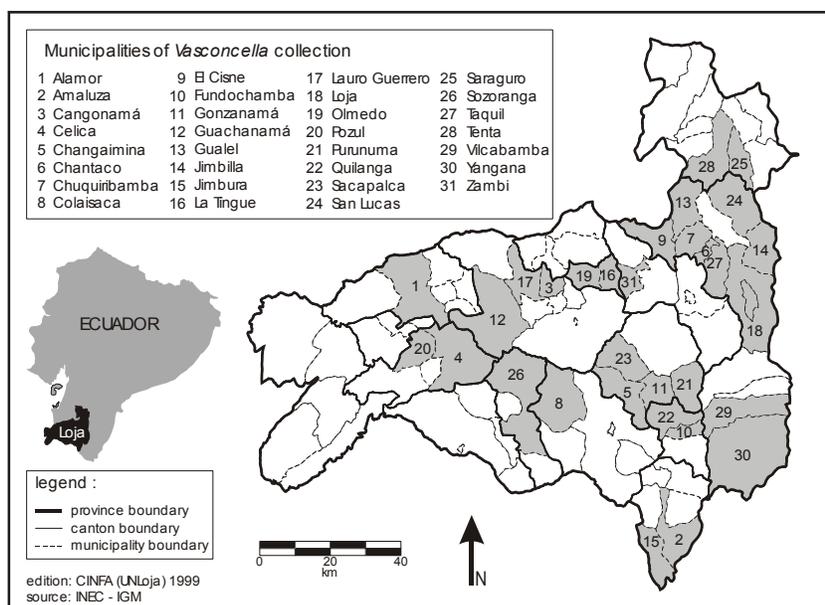


Figure 4.9. Map of municipalities in Loja Province where the germplasm collection and *in situ* characterisation of highland papayas was realised

A first data analysis and interpretation lead to the elimination of 71 accessions. This elimination was mostly due to a lack of data (fruit characteristics) level but also due to plant sex, as male plants could not be withheld in the analysis, and due to plant species, eliminating other *Vasconcellea* species not included in this study. Finally, 140 female accessions with complete fruit characterisations were withheld for future analysis. Data description was realised using the statistical package SPSS 9.0.

Cluster analysis and principal component analysis were only performed on data of accessions that consisted of a complete dataset of fruits, leaves and flowers. This dataset included 99 female accessions. Principal component analysis was performed using the statistical package SPSS 9.0 applying factor analysis, analysing the principal components of the correlation matrix. Cluster analysis was realised through the statistical package NTSYSpc 2.10L applying UPGMA cluster analyses on Euclidean distance between standardised values of the significant factors (eigenvalues superior to 1.0) obtained in the PCA.

4.4 GENERATIVE PROPAGATION

In germplasm conservation and management, knowledge of germination dynamics and seed structure are essential tools. Defining whether seeds show an orthodox or recalcitrant behaviour can help determining appropriate strategies to conserve the seeds and thus the genetic resources. Seed structure was examined on un-germinated seeds, imbibed for 48 hours in water at room temperature and on germinating seeds, using a binocular stereomicroscope (Leica M28). Germination was studied on filter paper substrate, normal germinating seeds of genus *Taraxacum* proving the non-toxicity of this paper (Ellis *et al.*, 1985a). The filter paper was placed in paper bags for *A. cherimola* and petri dishes for *Vasoncella* spp. following the methodology recommended by the International Plant Genetic Resources Institute (IPGRI) (Hong & Ellis, 1996). It was kept at an optimal constant humidity level as described by Ellis *et al.* (1985a) using distilled water. Due to the long evaluation period and the lack of a sterile environment, problems with fungi occurred. Fungicide (active components: carboxin 20 %, captan 20 %) with concentration 0.025 g/l was applied when necessary. Evaluation data were calculated into germination percentage, defined as percentage of initial seeds that had germinated at a certain time, and in case of cherimoya into germination rate. Germination rate was defined as germination percentage during one day, obtained by dividing the total germination during 30 days by the number of days, in this case 30. Data were processed using a One-Way ANOVA, using Tukey's Honestly Significant Difference at 5 % level to detect significant differences using SPSS 9.0. Evaluation of germination took place until there were few or no seeds germinating, leading to long evaluation periods as described by Hong & Ellis (1996).

Cherimoya (*Annona cherimola*)

The germination test was carried out on a homogeneous mix of seeds, obtained from different accessions from Loja Province. After manually extracting the seeds from the ripe fruits (as indicated by fruit softening) and cleaning them, seeds were dried at room temperature for 14 days. Afterwards, they were stored in cold room (3 °C) for three months, considering that embryos are often immature when fruit is harvested and that storage for some months may improve germination (Hayat, 1963; Ellis *et al.*, 1985b; Sanewski, 1991). Abnormal seeds (aberrations concerning colour, weight, shape, pests), counting for a weight percentage of 5.2 %, were removed and on the

remaining normal seeds twelve pre-application treatments were tested (Table 4.3). These pre-applied treatments were selected based on earlier positive results in germination tests, particularly in Annonaceae (Venkataratnam & Satyanaranaswamy, 1956; Ibar Albiñana, 1986; George & Nissen, 1987; Sanewski, 1991; Franciosi Tijero, 1992; Ellis *et al.*, 1985b).

Table 4.3. Pre-applied treatments used in cherimoya germination test

| | |
|-----|--|
| 1: | Soaking 48 h in 500 ppm gibberellic acid (GA ₃) solution |
| 2: | Soaking 24 h in 500 ppm gibberellic acid (GA ₃) solution |
| 3: | Soaking 24 h in 1,000 ppm gibberellic acid (GA ₃) solution |
| 4: | Soaking 24 h in 5,000 ppm gibberellic acid (GA ₃) solution |
| 5: | Soaking 24 h in 10,000 ppm gibberellic acid (GA ₃) solution |
| 6: | Soaking 72 h in distilled water |
| 7: | Soaking 48 h in distilled water |
| 8: | Soaking 24 h in distilled water |
| 9: | Soaking 12 h in distilled water |
| 10: | Soaking 12 h in distilled water with initial temperature of 92 °C, gradually cooling |
| 11: | Soaking 10 min. in sulphuric acid (H ₂ SO ₄ , 75 %) |
| 12: | No treatment (control) |

Each treatment consisted of four replications of 50 seeds. For each replication, seeds were wrapped in thick filter paper (25 x 50 cm). Four replications of each treatment were placed together in an open plastic bag.

The germination test was carried out in a darkened glass chamber in a greenhouse. During the first year minimum and maximum temperatures inside the glass chamber were measured. Temperatures varied from 19 °C to 32 °C with a more or less stable average temperature of 25.5 °C. A non-recurrent insect infection on the filter paper was treated with insecticide (active components: cypermethrin 0.25 %, tetramethrin 0.40 %). Filter paper was changed about every 200 days when paper conditions of strength and absorption were judged unsatisfactory.

Evaluation started 20 days after seeds were put on filter paper and continued for 900 days. This long evaluation period was decided by the observation of a continuing germination. For determination of the best seed treatments applicable by local farmers, evaluation results after 98 days were used. For the first 60 days, evaluation was carried out every 2 days, and then weekly for the following 500 days. Afterwards the test was evaluated every 14 days. Seeds were evaluated as 'germinated' when the root was about 2 cm long. Rotten seeds were removed when clearly dead.

Initial viability of seeds was examined with a tetrazoliumchloride test. As no procedures for this test are described for Annonaceae, standard procedures were used (Mac Kay *et al.*, 1976; Dickie *et al.*, 1984; Ellis *et al.*, 1985a). Seeds, soaked in water for 24 h, were cut longitudinally. The halves exposing the embryo were soaked in a 1 % tetrazoliumchloride solution at 30 °C. This test was carried out with 12 replications of 50 seeds.

Highland Papayas (*Vasconcellea* spp.)

Germination tests in highland papayas consisted of three experiments. All experiments were conducted in plastic petri dishes (diameter 80 mm) on two layers of filter paper, kept at optimal humidity using distilled water. Each treatment consisted of 4 replications of 25 seeds. The petri dishes were placed randomly in an incubator (Boekel® 132000 and New Line® 100 A) at 25 °C. A seed was evaluated as germinated when root emergence was observed. Rotten seeds were removed.

Sarcotesta Removal

A first test aimed at elaborating a methodology, requiring minimal time and labour, to remove the mucilaginous sarcotesta, the latter being a constraint in germination tests and seed conservation. Fresh seeds of one ripe (as indicated by fruit colour and aroma) *V. cundinamarcensis* accession were submitted to different treatments (Table 4.4) as described by Moriera de Carvalho & Nakawama (1982). After each treatment, seeds were washed and dried for 24 h.

Table 4.4. Treatments used to remove sarcotesta in *Vasconcellea cundinamarcensis* seeds

| | |
|-----|---|
| 1 : | Manual removal of sarcotesta (control) |
| 2 : | Fermentation 21 days (room temperature) |
| 3 : | Soaking 24 h in 10 % Na ₂ CO ₃ solution (ratio seeds/solution: 1/1) |
| 4 : | Soaking 30 min. in 26 % H ₂ SO ₄ solution (ratio seeds/solution: 1/2.5) |
| 5 : | Soaking 30 min. in 38 % HCl solution (ratio seeds/solution: 1/10) |

After sarcotesta removal, seeds were submitted to a standard germination test. Evaluation started after 14 days and continued during 253 days, with a check every two days during the first 100 days and weekly during the rest of the germination test.

A topographic tetrazolium test, to asses viability after each treatment, was conducted using a 1 % tetrazoliumchloride solution at 30 °C for 24 h hours. Results, however, could not be related with germination results and were discarded as not useful. Due to the lack of a standardised methodology for *Vasconcellea* it was difficult to correctly evaluate embryo colouration.

Improving Germination Using Pre-applied Treatments

A second experiment aimed at evaluating the effect of different pre-applied treatments to develop methodologies to improve and homogenize the often difficult germination of *Vasconcellea* species. A mixture of seeds from different *V. cundinamarcensis* accessions was submitted to different pre-applications (Table 4.5) known to promote or improve germination of *Carica papaya* (Ellis *et al.*, 1985b; Andreoli & Khan, 1993; Sippel & Claassens, 1993; Bertocci *et al.*, 1997). Seeds were extracted from a mixture of *V. cundinamarcensis* accessions and the sarcotesta was removed manually.

Table 4.5. Pre-applied treatments used in *Vasconcellea* germination test

| | |
|------|--|
| 1 : | Soaking 24 h in 10 ppm gibberellic acid (GA ₃) solution |
| 2 : | Soaking 24 h in 100 ppm gibberellic acid (GA ₃) solution |
| 3 : | Soaking 24 h in 1,000 ppm gibberellic acid (GA ₃) solution |
| 4 : | Soaking 24 h in 10,000 ppm gibberellic acid (GA ₃) solution |
| 5 : | Soaking 24 h in 0.2 % KNO ₃ solution |
| 6 : | Soaking 24 h in distilled water |
| 7 : | Pre-chill 3 days at 5 °C |
| 8 : | Pre-chill 3 days at 5 °C + soaking 24 h in distilled water |
| 9 : | Partly removal of sclerotesta |
| 10 : | Chemical scarification: soaking 30 min. in sulphuric acid (H ₂ SO ₄ , 95-98 %) |
| 11 : | No treatment (control) |

Evaluation started after 14 days and continued for 234 days, checking every two days during the first 100 days and weekly during the rest of the germination test.

Germination of Different Species and Accessions

A third experiment compared differences in germination behaviour between species and within any given species. Fruits of different *Vasconcellea* species and accessions, dioecious and monoecious plants in case of *V. cundinamercensis*, together with two *C. papaya* accessions were collected in Loja Province. Seeds were extracted and sarcotestas removed using the Na₂CO₃ method. Seeds of earlier gemplasm collections were included as well in order to assess conservation potential. These seeds had been conserved at 3 °C during 2 years. Half of the seeds were subjected to a 1,000 ppm GA₃ pre-application treatment for 24 hours while the other half was used as control. In case of *V. × heilbornii* and *C. papaya*, 4 replications of 20 seeds, instead of the usual 25 seeds were used. Table 4.6 gives an overview of the different species and accessions with their characteristics. Evaluation started after 7 days and was continued for 174 days, every three days during the first 60 days and weekly till the end of the germination test.

Table 4.6. Overview of species and accessions used in germination test

| Species | Accession code | Plant sex | Conservation |
|----------------------------|----------------|---------------|--------------|
| <i>V. cundinamarcensis</i> | fem. 1 | Female | fresh |
| <i>V. cundinamarcensis</i> | fem. 2 | Female | fresh |
| <i>V. cundinamarcensis</i> | fem. 3 | Female | 2 years |
| <i>V. cundinamarcensis</i> | mon. 1 | Monoecious | fresh |
| <i>V. cundinamarcensis</i> | mon. 2 | Monoecious | fresh |
| <i>V. cundinamarcensis</i> | mon. 3 | Monoecious | 2 years |
| <i>V. stipulata</i> | acc. 1 | Female | fresh |
| <i>V. stipulata</i> | acc. 2 | Female | fresh |
| <i>V. stipulata</i> | acc. 3 | Female | fresh |
| <i>V. stipulata</i> | acc. 4 | Female | 2 years |
| <i>V. × heilbornii</i> | acc. 1 | Female | fresh |
| <i>V. × heilbornii</i> | acc. 2 | Female | fresh |
| <i>V. × heilbornii</i> | acc. 3 | Female | fresh |
| <i>V. × heilbornii</i> | acc. 4 | Female | 2 years |
| <i>C. papaya</i> | fem. 1 | Female | fresh |
| <i>C. papaya</i> | her. 1 | Hermaphrodite | fresh |

4.5 ALTERNATIVE USES

Preliminary phytochemical enzymatic analysis of green *Vasconcellea* fruits was performed after experiencing severe irritation symptoms on hands during collections, indicating a high papain content. Latex was extracted, in October 2000, on green fully-grown fruits of different accessions (Table 4.7). At each location, geographic coordinates were determined using GPS (Garmin® GPS45). Fresh latex was extracted through 2 mm deep vertical cuts as described by Skelton (1969). The latex flowing from the cuts was collected in plastic petri dishes. From every accession fruit size (using a calliper, absolute error ± 0.1 mm) and weight (using an Ohaus® Portable Plus balance, absolute error ± 0.1 g) of fresh and dried latex was determined. Estimation of fruit volume was realised assuming an ellipsoid shape. Latex was dried at 55 °C for 24 h and conserved at -18 °C, until analysis in January 2001. Analysis of the proteolytic activity was carried out by Blue Star Chemicals, a Belgian papain importing and processing company. Proteolytic activity was determined using BAPNA method, which measures the amount of N- α -benzoyl-DL-arginine-p-nitroanilide (BAPNA) that is hydrolysed by the proteolytic enzymes (Dubois *et al.*, 1988).

Table 4.7. Species and accessions, and their location, used in the analysis of proteolytic activity

| Species | Site | County | Latitude | Longitude |
|---|---------------|----------|-----------|------------|
| <i>Carica papaya</i> | | | | |
| Accession 1 | San José | Catamayo | 3°59.7' S | 79°21.1' W |
| Accession 2 | San José | Catamayo | 3°59.7' S | 79°21.1' W |
| <i>V. cundinamarcensis</i> | | | | |
| Accession 1 (fem.) | San Lucas | Loja | 3°43.9' S | 79°15.8' W |
| Accession 2 (fem.) | Capur | Loja | 3°49.7' S | 79°13.7' W |
| Accession 3 (fem.) | Las Lagunas | Loja | 3°38.0' S | 79°14.1' W |
| Accession 4 (fem.) | Pichig | Loja | 3°42.7' S | 79°15.9' W |
| Accession 5 (mon.) | San Lucas | Loja | 3°43.9' S | 79°15.8' W |
| Accession 6 (mon.) | Pichig | Loja | 3°42.7' S | 79°15.9' W |
| <i>V. stipulata</i> | | | | |
| Accession 1 | Chuquiribamba | Loja | 3°50.6' S | 79°20.6' W |
| Accession 2 | Chuquiribamba | Loja | 3°50.6' S | 79°20.6' W |
| Accession 3 | Gualel | Loja | 3°46.1' S | 79°22.6' W |
| Accession 4 | La Nona | Loja | 3°48.6' S | 79°24.1' W |
| Accession 5 | Sasanama | Celica | 4°05.1' S | 79°56.2' W |
| Accession 6 | Sasanama | Celica | 4°05.1' S | 79°56.2' W |
| Accession 7 | El Coposo | Paltas | 4°03.7' S | 79°54.7' W |
| Accession 8 | El Coposo | Paltas | 4°03.7' S | 79°54.7' W |
| <i>V. × heilbornii</i> | | | | |
| <i>V. × heilbornii</i> 'Babacó' | | | | |
| Accession 1 | La Argelia | Loja | 4°02.2' S | 79°11.9' W |
| Accession 2 | San Lucas | Loja | 3°43.9' S | 79°15.8' W |
| Accession 3 | La Argelia | Loja | 4°02.2' S | 79°11.9' W |
| <i>V. × heilbornii</i> var. <i>chrysopetala</i> | | | | |
| Accession 1 | San Lucas | Loja | 3°43.9' S | 79°15.8' W |
| <i>V. × heilbornii</i> unclass. variety | | | | |
| Accession 1 | Capur | Loja | 3°49.7' S | 79°13.7' W |
| Accession 2 | Chantaco | Loja | 3°52.6' S | 79°19.8' W |
| Accession 3 | Las Lagunas | Loja | 3°38.0' S | 79°14.1' W |
| Accession 4 | Pichig | Loja | 3°38.0' S | 79°15.9' W |
| Accession 5 | Anganuma | Quilanga | 4°16.6' S | 79°25.4' W |
| Accession 6 | Gonzanamá | Loja | 4°14.5' S | 79°26.9' W |

5. RESULTS

5.1 CHERIMOYA (*ANNONA CHERIMOLA* MILL.)

5.1.1 ETHNOBOTANICAL SURVEY

5.1.1.1 NOMENCLATURE AND USE

All surveyed farmers use the vernacular name 'chirimoya' for naming the cherimoya tree. '*Chirimoya*' is the officially accepted Spanish name for the plant, although sometimes a difference is made between '*chirimoya*', referring to the fruit and '*chirimoyo*' being applied to the tree. As mentioned earlier, the word '*chirimoya*' has a Quechua origin and is apparently used throughout the province as the only vernacular name.

In Loja Province, cherimoya fruits have no other use than direct fresh consumption, although internationally, consumption in processed products as juice or ice cream has been reported. Some farmers mention the use of bad quality fruits, especially fruit fly infested, as feed for pigs. Cherimoya leaves are used by a minority, 8 % of surveyed farmers, for their medicinal characteristics. Leaves are cooked and the extract obtained is applied on the head to reduce sunstroke related headaches. Cherimoya wood has no specific use, although 59 % of the surveyed farmers uses the wood, as most other wood, for fences or firewood. One farmer in Quilanga reports using cherimoya trees as shadow trees for coffee plants.

5.1.1.2 AGRICULTURAL PRACTICES

Although commercial cherimoya plantations always use grafted seedlings, belonging to one or several cultivars, in order to obtain a homogeneous stand and subsequently high-quality yield, farmers in Loja Province do not use this practice. Moreover, 83 % of the surveyed farmers did not actively plant their cherimoya plants. Trees developed as a result of seed dispersion by humans or animals and were only protected by the farmer from the seedling stage onwards. Of the farmers that did actively sow their trees, almost half did not apply any selection of the seeds used. In only 10 % of cases some positive selection towards fruit quality was applied. This interesting group of farmers actively selecting cherimoya seeds could not be related to age, nor origin and the selecting process must be considered as an individual initiative. None of the farmers surveyed possess grafted trees.

Despite the low number of trees that are actively propagated, most farmers possess a considerable number of trees. An average of 55 trees per farmer was found to occur. Discarding some doubtful numbers of over 1000 trees per farmer as some claimed they owned, an average of 37 trees per farmer still indicates the abundance of cherimoya trees in the farming system in Loja Province. It is clear that cherimoya is not only present in backyard gardens but also, tolerated, along or in the farmers' fields. As farmers do not often have an exact knowledge of the actual number of

trees they have, results should only be used as an indication. The majority, 64 %, of farmers state that cherimoyas were more abundant in their region in earlier times.

Fertilisation is, as for the majority of fruit crops, an important practice to obtain and maintain good yields. Nevertheless, 86 % of the surveyed farmers do not apply any form of fertilisation. A total of 11 % use organic fertilisation, mostly manure or organic waste from kitchen and gardens. This type of fertilisation is a general application to most perennial crops and is not specifically aimed at cherimoya trees. Only 4 % use chemical fertilisation, mostly urea or composite NPK-fertilisers.

Use of phytosanitary products such as herbicides, insecticides or fungicides is even less common than the use of fertilisation. None of the surveyed farmers use any chemical to control weeds, pests or diseases. This is a typical feature of the basic low-input farming system common in Loja Province.

Pruning, necessary to obtain optimal tree shape and to improve fructification, is another commonly applied practice in commercial cherimoya plantations but is rarely seen in Loja Province. Only 35 % of surveyed farmers confirm they prune, but closer examination of pruning techniques reveals that it deals mostly with eliminating dry wood, removing obstructing branches or taking out suckers. Sometimes farmers perform a severe renovation pruning on old trees but in all cases the pruning is not part of the cultivation practice *sensu stricto*.

The same remark can be made for the use of irrigation. Most farmers, 67 % of the total, do not irrigate. The ones who claim they do, do it in an indirect way. They irrigate their backyard or field, using gravitation, when weather conditions require it.

In Loja Province, cherimoya harvest starts in November and lasts till August, beginning soonest in warmer zones. Generally the main harvest period is from March till June, which coincides with the end of the rainy season (Figure 5.1). As trees are not pruned, tree height can easily exceed 5 m, considerably complicating harvest activities. Harvesting is realised by climbing in the trees, especially done by children, or by using a forked stick and whisking out fruits one by one. As cherimoya is a delicate fruit, fruits are often damaged before leaving the farm. The number of harvested fruits per tree varies between 10 and 200 with an average of 66, comparable to or slightly higher than yields found in literature, but obtained on considerably more voluminous trees.

As the presence of cherimoya trees in Loja Province is the result of natural dispersion, without additional farmer intervention, it can be concluded that agricultural practices are very limited. Most practices are part of the general management of the backyard or fields and are not aimed directly nor specifically at the cherimoya trees. This is a typical feature for the cultivation system of the area, which is characterised by very diverse backyard systems and all-purpose agricultural practices. Furthermore, this system discriminates native plant species as no costs or propagation practices are needed to obtain the plants.

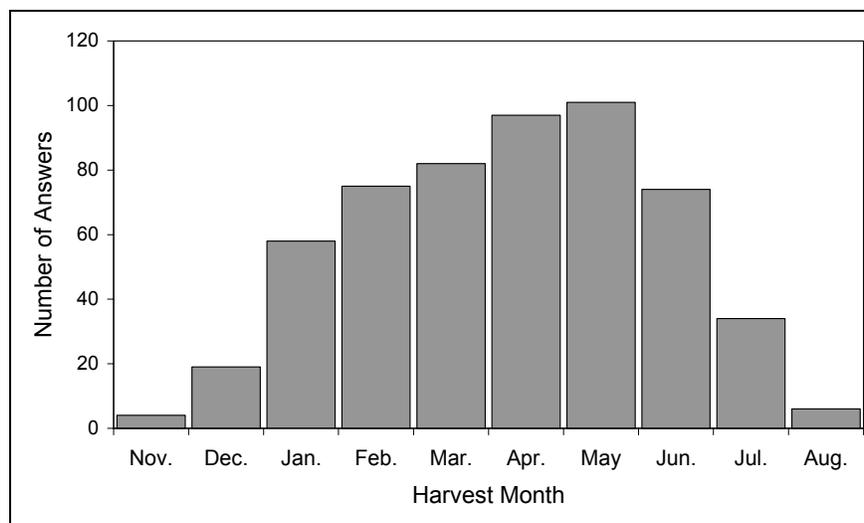


Figure 5.1. Cherimoya harvest months in Loja Province according to 162 local farmers

5.1.1.3 COMMERCIALISATION

A total of 61 % of the surveyed farmers do sell all or part of the harvest, averagely 50 % of the harvested fruits are sold, with only small differences between different zones. Most of these farmers sell to a middleman, who visits the farm or buys fruits at a meeting point. Only 3 % of the cherimoya-selling farmers go to the market to sell directly to the consumer. Fruits are transported from the harvesting place to the farmer's house on a man's shoulder or by mule. Transport to the middleman, if selling occurs at a meeting point, is done by mule or by car. Some farmers need up to six hours by mule from their cherimoya plants, often wild stands, to their farm, indicating the extensiveness of cherimoya cultivation. In 93 % of cases, cherimoyas are packed in bags or 'alforjas', a local type of woollen woven bag, leaving the fruits much space to move and get damaged even more. The ideal packing tool, a wooden crate, is only used by 2 % of the farmers.

The price received for cherimoya fruits varies, depending on season and availability, between 1 and 5 euro for 100 well-sized fruits (75 % percentile). The middleman sells to retailers for 7.5 euro. Finally, cherimoya fruits are sold to consumers at an average of 0.25 euro per fruit, or 25 euro for 100 fruits. As cherimoya fruits have a short shelf life and are often infested with fruit fly (*Anastrepha* spp.) losses for the retailer can amount to 50 %, resulting in an estimated income of 12.5 euro for 100 fruits. Considering the farmer's income, with an average yield of 66 fruits per tree and half of them suitable for sale, each tree results in an estimated income of 0.33 to 1.5 euro per year. This gives an estimated total yearly income ranging 20 - 80 euro for an average farmer, owing 55 cherimoya trees, illustrating that cherimoya is not a cash crop for local farmers.

5.1.1.4 MAJOR CROP CONSTRAINTS

The survey reveals that the major crop problem, reported by 64 % of the surveyed farmers, comes from phytopathological problems. Minor difficulties are the lack of water (9 %) and difficult commercialisation (9 %), due to bad road conditions, especially at harvest time which coincides with the end of the rainy season, and lack of market. Three farmers mention lack of cultivation knowledge. Upon closer inspection, 93 % mentioned the fruit fly (*Anastrepha* spp.) as the main phytopathological problem, adding that the problem seems to worsen every year. Fungal fruit diseases, locally called 'hielo' and 'mancha negra', and probably belonging to a complex of *Fusarium* sp., *Cladosporium* sp., *Phomopsis* sp. and *Centrospora* sp., are reported as minor problems.

5.1.1.5 CONCLUSIONS

The survey gives a clear image about the actual status of extensive cherimoya cultivation in Loja Province. Most farmers are tolerating naturally dispersed seedlings in their backyards or fields, resulting in several spontaneously generated cherimoya trees, but do not apply any special agricultural practices to their trees. Some farmers however include cherimoya trees in their general backyard managing systems and do apply organic fertilisation, gravitational irrigation or some pruning. Although phytosanitary problems are mentioned as the major crop constraint no farmer attempts to control fruit fly. This lack of agricultural practices leads to big trees, which render harvests difficult, and results in heterogeneous and infested harvests and an overall low fruit quality. As a result of incorrect packing and long transport distances to markets fruit quality is even more deteriorating. Consequences of bad fruit quality are low fruit prices and a small yearly income obtained through cherimoya cultivation. This small income does not stimulate the local farmer to invest in the cultivation of cherimoya as a source of income and closes the vicious circle. This low-input cultivation system found in Loja Province is confirmed by the description of cherimoya cultivation, and its related danger of biodiversity loss, in the Andes by other authors (Farré Massip & Hermoso González, 1987; National Research Council, 1989)

5.1.2 CROP ECOLOGY

5.1.2.1 *IN SITU* EVALUATION OF GROWING CONDITIONS

Edaphoclimatic conditions at 20 locations with abundant wild cherimoya stands were evaluated and used as an indication of cherimoya growth preferences. All sites were located between 1,500 and 2,000 masl, corresponding to the findings of Guzman (1951) who confines cherimoya occurrence to this altitude range. At lower and higher elevations, cherimoya trees can still be found, although in much smaller quantities and in dispersed stands.

Mean annual temperatures, derived from an isotherm map, at these sites are all situated in the range between 14 and 24 °C. Figure 5.2 shows the distribution of the mean annual temperatures for the 20 sites. One can conclude that 18 - 20 °C is the most frequent temperature range, accounting for 55 % of sites. Average minimum and maximum temperatures, based on relation between altitude and temperature, vary respectively between 10 and 12 °C, and 26.5 and 30 °C.

Annual precipitation ranges from 600 to 1,400 mm. A high proportion of the total territory of Loja province shows similar precipitation values but has no wild cherimoya stands, suggesting that precipitation is less limiting than temperature (altitude) for cherimoya to occur. Figure 5.3 shows that annual precipitation between 800 - 1,000 mm is the most common range in places where wild cherimoya occurs. Annual relative humidity values are high and range between 75 and 85 %.

Recalculated values for the 52 different sampling points at the 20 sites give valuable information about soil conditions prevailing in wild cherimoya stands. Cherimoya clearly prefers soils with high sand content. Predominate soil textures are sandy loam, loam and sandy clay loam (Figure 5.4). This coincides with results found in commercial cherimoya orchards in Australia and Spain (George *et al.*, 1987; Sanewski, 1991; Morales, 1993). High amounts of coarse materials (Figure 5.5), typical for the shallow rock soils in Loja Province, do not seem to cause any problem for cherimoya plant development. Soil depth also varies a lot. Shallow soils (10 - 15 cm) can support cherimoya, but most sites have soils of 50 cm or deeper.

Organic matter content and pH, recalculated for the upper 25 cm, are also variable but most soils show slightly acidic properties with pH between 5 and 6.5 (Fig. 5.6). Organic matter content is moderate with common values ranging 1 to 5 % (Fig. 5.7).

5.1.2.2 PREDICTION OF SUITABLE CULTIVATION ZONES

Applying the modified simple limitation method on some basic edaphoclimatic characteristics of Loja Province gives a preliminary understanding of potential cultivation zones. Applying the site frequencies for each class of temperature and precipitation, retaining the lowest frequency, results in a Climate Frequency Map where as the site frequencies for each class of the soil map yields the Soil Frequency Map (Figure 5.8). As the soil map of Loja Province is not very detailed, the Soil Frequency Map of Loja Province shows large areas with relatively high frequencies. Finally, a combination of both frequencies, again retaining the lowest frequency, results in the Overall Frequency Map (Figure 5.8). This map also shows that climate, more specifically temperature, is the most important factor in defining the final zones.

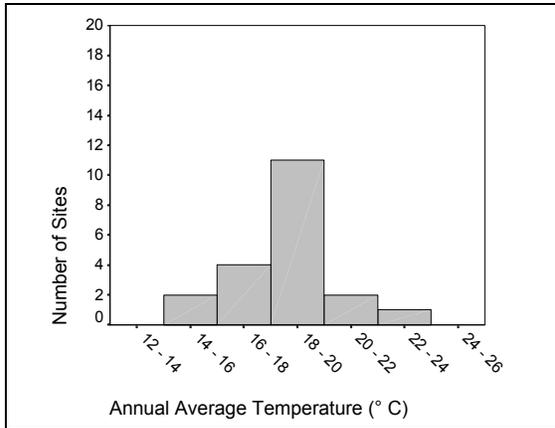


Figure 5.2. Annual average temperature at 20 sites in Loja Province with wild cherimoya stands

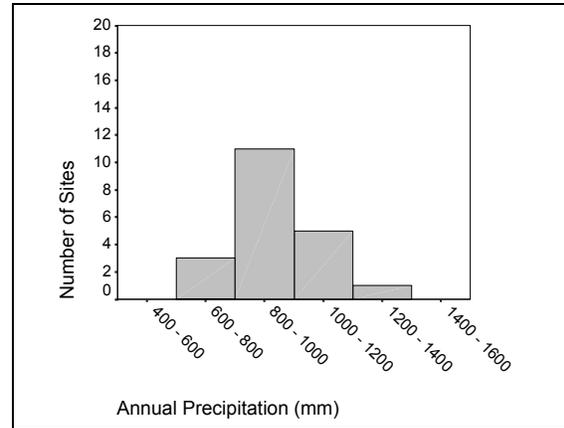


Figure 5.3. Annual precipitation at 20 sites in Loja Province with wild cherimoya stands

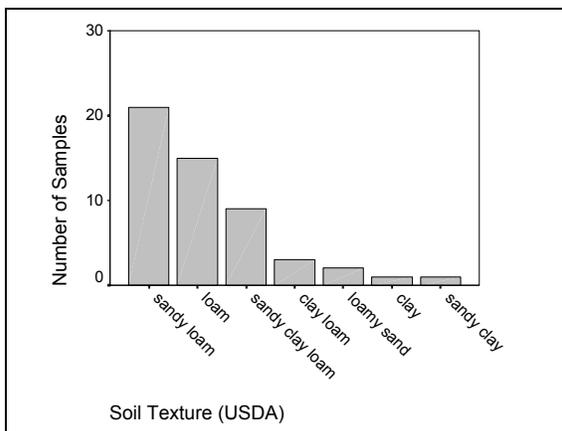


Figure 5.4. Soil texture at 52 sampling points in Loja Province with wild cherimoya stands

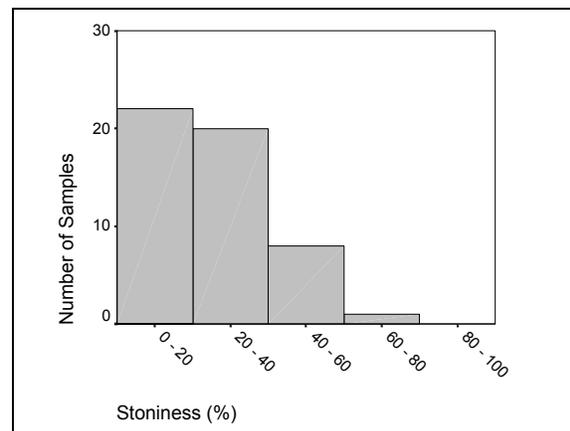


Figure 5.5. Stoniness at 52 sampling points in Loja Province with wild cherimoya stands

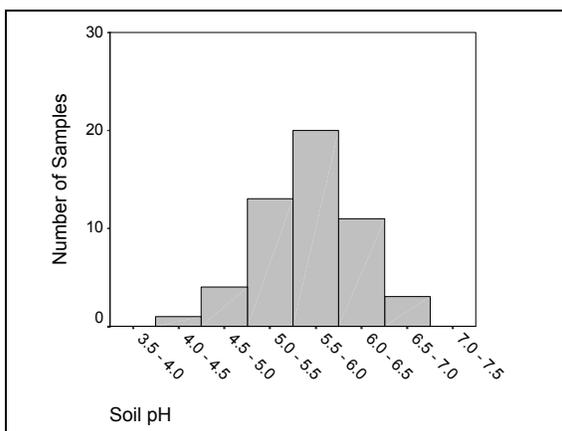


Figure 5.6. Soil pH at 52 sampling points in Loja Province with wild cherimoya stands

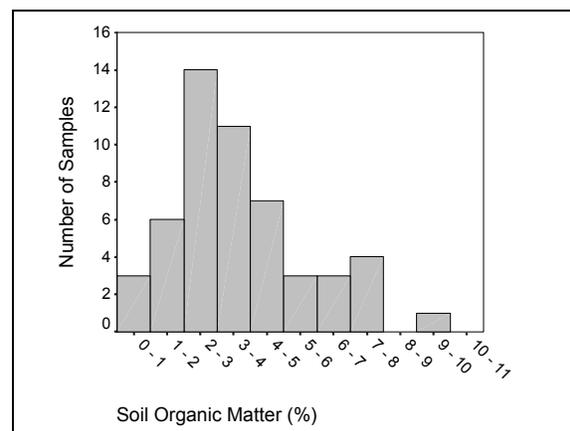


Figure 5.7. Soil organic matter content at 52 sampling points in Loja Province with wild cherimoya stands

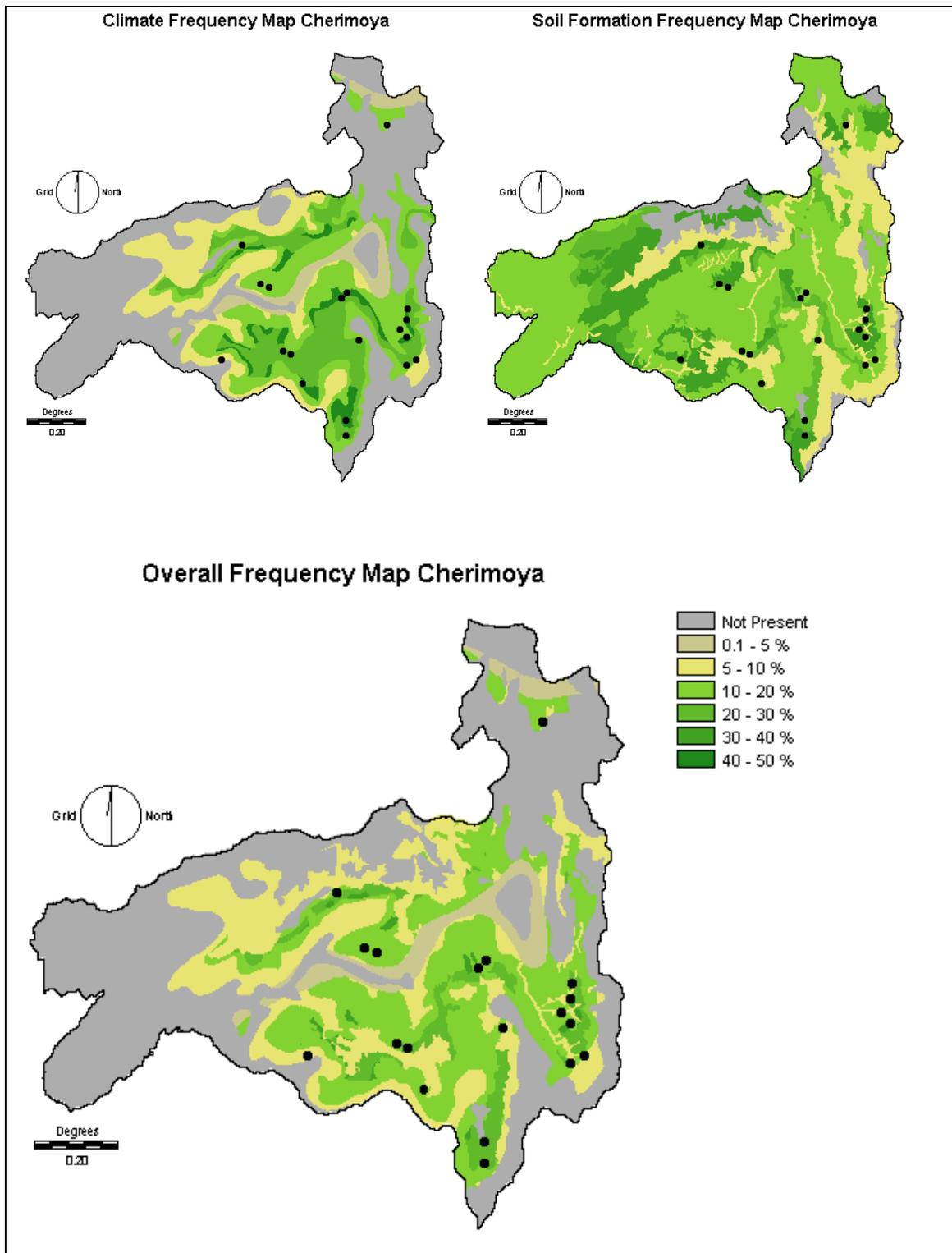


Figure 5.8. Climate Frequency Map, Soil Frequency Map and Overall Frequency Map for cherimoya in Loja Province, with indication of sampling sites

The typical cherimoya growing zones in Loja Province, concentrated in the south-eastern part of the province, around the villages of Cariamanga, Gonzanamá, Amaluza and Vilcabamba, as evidenced through field visits and number of farmers, are clearly reflected in the Overall Frequency Map. A total of 27 % of the province, or 2,913 km², shows a frequency of over 20 % and may be considered as suited. Very well suited areas, with a frequency of over 30 %, are confined to 5 % (523 km²) of Loja Province. These areas must be considered as the most suited zones for cherimoya cultivation as they show edaphoclimatic conditions where a high number of wild cherimoya forest can be found. A total of 48 % of Loja Province is evaluated as not suited for cherimoya, mainly due to climatic (temperature) conditions.

5.1.2.3 CONCLUSIONS

Given the limited area where wild cherimoyas occur worldwide, presence of wild cherimoya stands in Loja Province offers the unique opportunity to study environmental preferences of cherimoya trees in their natural habitat.

Mean optimal annual temperature for cherimoya is clearly located in the range between 18 and 20 °C, figures confirmed by Hermoso González *et al.* (1999) who found in Ecuador an optimal temperature between 17 and 20 °C and by Franciosi Tijero (1992) who states 18 – 20 °C being optimal for cherimoya cultivation in Peru.

The world's main producing zone, located in the Malaga area in southern Spain, has a mean annual temperature of 18.5 °C (Morales, 1993). Typical for southern Ecuador however are the small annual variations, less than 2.4 °C for Loja Province. Mean optimal minimum temperature in Loja Province ranges 10 – 12 °C and mean maximum temperature 26.5 – 30 °C, indicating the preference of cherimoya trees for moderate minimum and maximum temperatures. Optimal annual precipitation is 800 – 1,000 mm, similar to intra-annual precipitation of 700 – 1,400 mm cited by Hermoso González *et al.* (1999) and included in the 250 - 1,000 mm precipitation range described by Franciosi Tijero (1992) in Peru. Southern Spain has an annual precipitation of 400 – 500 mm. It is clear that precipitation is less limiting than temperature. Moreover, a lack of precipitation can be overcome by irrigation in commercial plantations or by use of natural water sources, often in small valleys, in the case of wild cherimoya stands. An important factor in pollination is air humidity that ranges 75 - 85 % in the sampling sites, similar to figures mentioned by George *et al.* (1987).

Soil characteristics at sampling sites show a preference for slightly acid well-drained textures, with presence of coarse materials, and moderate organic material contents. In Loja Province, cherimoyas are often found on Entisols and Inceptisols. Most authors (George *et al.*, 1987; Sanewski, 1991; Morales, 1993) agree on soil textures of sand to sandy loam emphasising susceptibility of cherimoya for water logging. Farré Massip *et al.* (1999) however, mentioned that cherimoya develops well in Spain

on soils with a pH of 7.5 – 8.5. This illustrates that beside soil texture, other edaphic characteristics seem not to play a major role in cherimoya development.

Given the small number of sampling points, only vast cherimoya stands were used in the study, and the limited edaphoclimatic characteristics, due to sparse data availability, results must be considered as a first indication for possible cultivation zones. Moreover, due to lack of exact production data in Loja province, resulting from the absence of commercial production, this zonal classification presents only indicative values and shows zones where adequate cherimoya growth and yields could be obtained. Nevertheless, the Overall Frequency Map, obtained using the simple limitation method, clearly includes the major cherimoya zones in Loja Province and can thus be used as a first tool in selecting adequate cherimoya cultivation zones. Once preliminary zones are selected, a more detailed study of soil characteristics is necessary in order to refine the zonal qualification.

Due to the pressure that exists on fertile land in Loja province, the question is, however, whether the best cherimoya soils have not been exposed to deforestation leaving only some marginal plots, unsuited for annual crop cultivation, covered with wild cherimoya trees. This hypothesis was confirmed by the fact that during the ethnobotanical survey, farmers confirmed that the number of cherimoya trees is diminishing.

Although lack of data and the problems resulting from using wild cherimoya stands as an indication for optimal characteristics warrant some precaution in interpreting results, the zones which were found by the analysis, and which are based on the prevailing soil and climate conditions, are zones in Loja province known by local people as 'the land of cherimoya' a fact also confirmed by germplasm collections from the region. In this respect, it is probable that the areas that have been defined through this study really coincide with the optimal areas for cherimoya growing in Loja Province.

5.1.3 GERMPASM COLLECTION AND CHARACTERISATION

5.1.3.1 COLLECTION AND *IN SITU* POMOLOGICAL CHARACTERISATION

A total of 137 trees, covering 32 sites, were selected and 448 fruits from these trees were collected and characterised. To describe the fruits, six important fruit characteristics: fruit weight, seed percentage (ratio of seed weight/total weight), seed index (number of seeds per 100 g fruit), pulp percentage (ratio of pulp weight/total weight), soluble solids and skin type were taken into account. These are the best characteristics to describe fruit quality in general, and fruit quality per tree more specifically. The histograms of each of these characteristics illustrate the existing variability (Figures 5.9 – 5.14), illustrating the existing pomological variability (Figure 5.15).

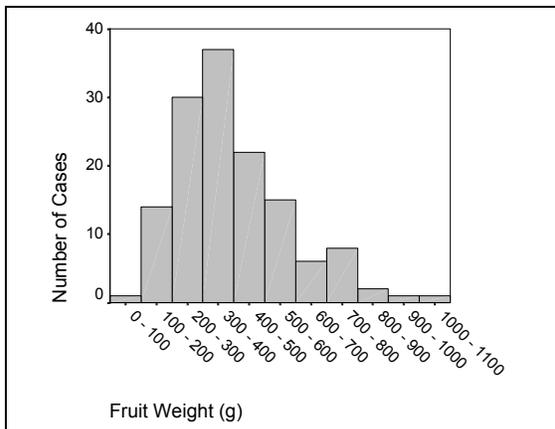


Figure 5.9. Histogram of fruit weight for 137 collected accessions in Loja Province

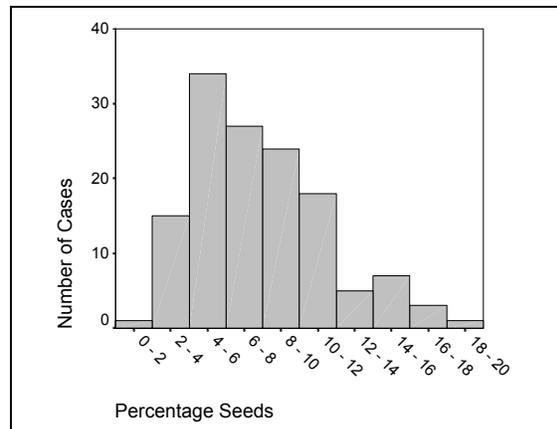


Figure 5.10. Histogram of seed weight percentage for 137 collected accessions in Loja Province

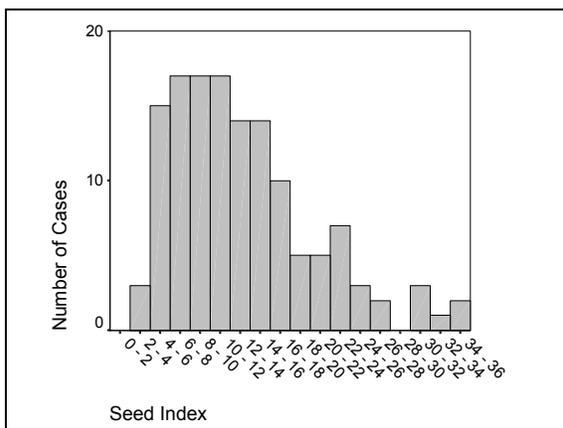


Figure 5.11. Histogram of seed index for 137 collected accessions in Loja Province

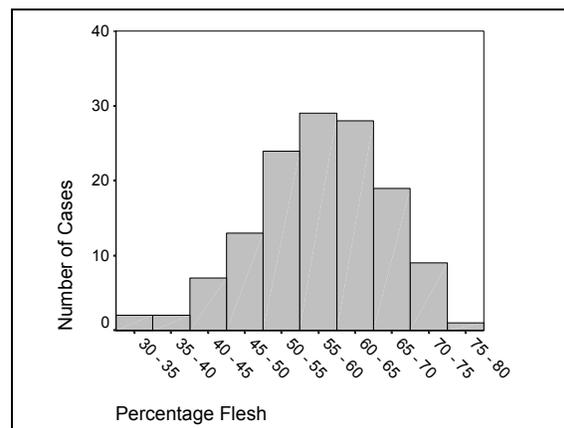


Figure 5.12. Histogram of percentage for 137 collected accessions in Loja Province

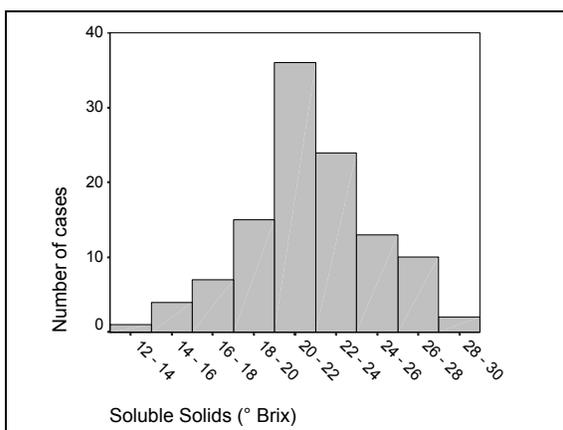


Figure 5.13. Histogram of soluble solids for 137 collected accessions in Loja Province

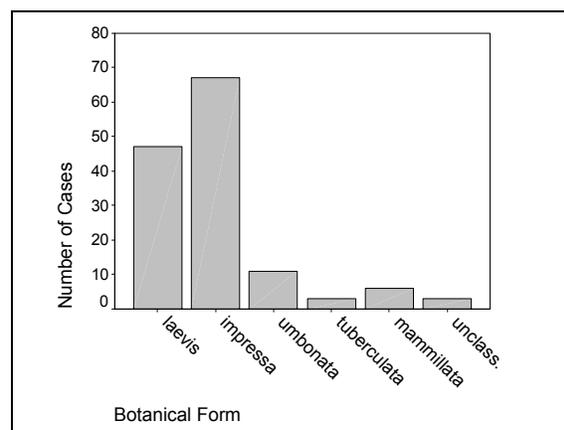


Figure 5.14. Histogram of botanical forms for 137 collected accessions in Loja Province

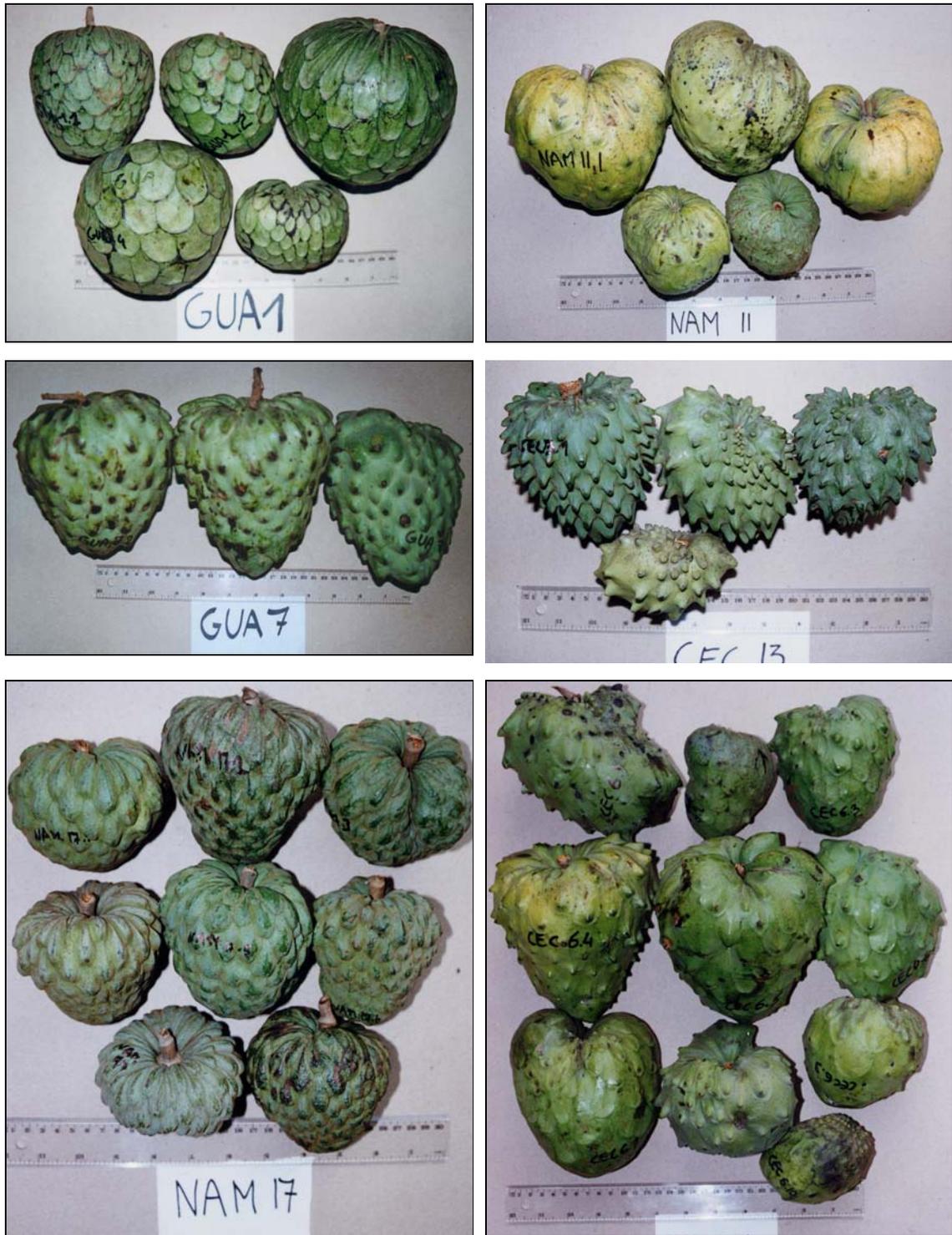


Figure 5.15. Examples of different cherimoya accessions collected in Loja Province

From the histograms, it can be observed that quantitative fruit characteristics show a wide variability with normal to log normal distributions. This wide variability is a typical feature for plant species in or near their centre of origin and offers good possibilities for selection of new varieties. The average cherimoya tree in Loja Province has fruits with a weight of 400 g, a seed index of 13 with 8 % seeds on a seed weight/total weight basis, and 22 °Brix of soluble solids. Most fruits show the *laevis* or *impressa* botanical form, although few unclassified botanical forms, showing a skin type resembling to *A. squamosa*, were found.

As fruits were collected in different sites, part of this variability can probably be explained by differences in soil quality, climate and even management. However, management applied to cherimoya trees is very low to non-existent due to the very poor prices farmers receive for their fruits.

Statistical analysis, using one-way ANOVA, of the characteristics shows that means of sampled trees are significantly different at the 95 % level for each quantitative characteristic. As a low seed content is, as in many other fruit crops, the main selection factor in cherimoya breeding programmes worldwide, this characteristic was analysed in more detail. Regression analysis shows that there is no significant correlation between fruit weight and number of seeds (Figure 5.16), indicating that number of seeds, or number of pollinated carpels, is not depending on fruit size but is genetically defined. In order to compare different accessions, the seed index, which indicates the number of seeds for 100 g of fruit, was used.

This seed index does significantly depend on the fruit weight as is illustrated by a power regression analysis (Figure 5.17). This indicates that larger fruits tend to accumulate more fruit pulp around one carpel and/or that more seedless carpels can be found. In fact, two factors influence seed index: the minimum number of pollinated carpels necessary to initiate fruit development and the ability to accumulate considerable amounts of pulp in each carpel. This last factor probably consists of both a genotypical and an environmental factor, which should be further evaluated in different collection fields.

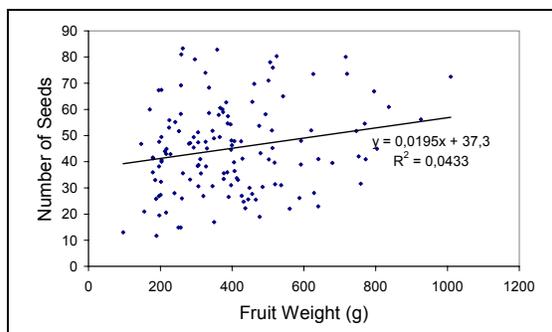


Figure 5.16. Correlation and regression between number of seeds and fruit weight for collected cherimoya accessions

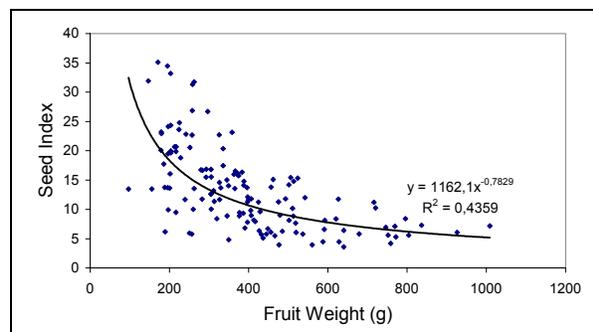


Figure 5.17. Correlation and regression between seed index and fruit weight for collected cherimoya accessions

A common maxim with local people is that botanical form or skin type gives an indication of other fruit characteristics. Taking into account the two most common skin types, i.e. *laevis* and *impressa*, and comparing them for the two most important characteristics, i.e. seed index and fruit weight, a t-test for independent samples shows that no significant differences exist at 95 % level between the two botanical forms. Although not significant at 95 % level, *laevis* accessions tend to have a slightly lower seed index than *impressa* accessions. The boxplots show that both botanical forms show a wide variability for the two characteristics mentioned but do not differ between each other (Figure 5.18 and 5.19).

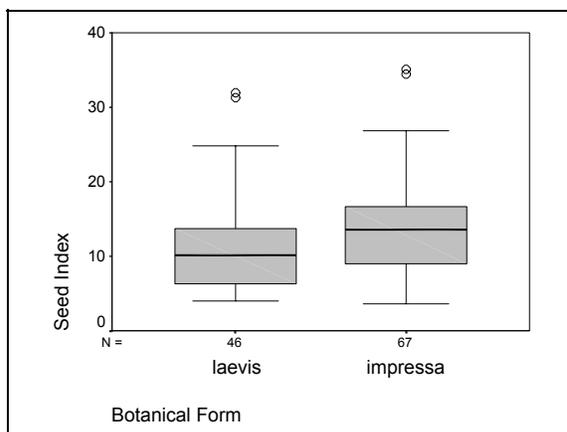


Figure 5.18. Boxplot of cherimoya seed index for collected *laevis* and *impressa* accessions

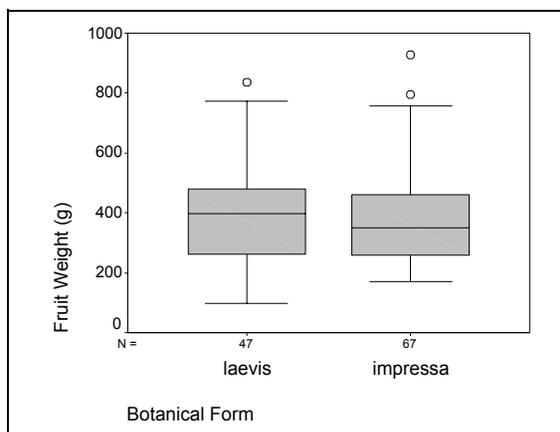


Figure 5.19. Boxplot of cherimoya fruit weight for collected *laevis* and *impressa* accessions

5.1.3.2 SELECTION OF PROMISING ACCESSIONS

Accessions were classified with seed index being the ranking criterion. From the 83 retained accessions, the 15 best are shown in Table 5.1.

It is clear that selected accessions show excellent fruit characteristics. Important characteristics as fruit weight, varying between 431 g and 926 g, seed index, ranging 3.9 - 7.1, and soluble solids, varying between 20.0 and 27.5 °Brix, indicate that fruits are rather big, pulpy and sweet.

5.1.3.3 PRELIMINARY COMPARISON WITH COMMERCIAL CULTIVARS

Comparison between the five best accessions from Loja Province (TIO1, GUA3, NAM11, GUA4 and GUA5) and five important commercial cultivars ('Fino de Jete', 'Bays', 'White', 'Bronceada' and 'Concha Lisa') for fruit weight and number of seeds per 100 g fruit flesh are shown in Figures 5.20 and 5.21. From these graphs, it is clear that some of the selected accession trees found in Loja Province can easily compete with commercial cultivars from cherimoya exporting countries.

Table 5.1. Classification and characteristics (and number of fruits characterised) of the 15 best accessions of the cherimoya collection realised in Loja Province

| Code | County | Municipality | Seed index | Fruit weight (g) | Percentage seed (%) | Percentage pulp (%) | Soluble solids (° Brix) | Botanical form | Fruit fly infestation (%) | No. of fruits charact. |
|-------|-----------|----------------|------------|------------------|---------------------|---------------------|-------------------------|---------------------|---------------------------|------------------------|
| TIO1 | Espíndola | Sta. Teresita | 3.9 | 560.6 | 2.8 | 79.6 | 20.6 | <i>impressa</i> | 0 | 4 |
| GUA3 | Espíndola | Jimbura | 4.5 | 587.7 | 4.2 | 67.1 | 26.5 | <i>laevis</i> | 66.6 | 3 |
| NAM11 | Gonzanamá | Nambacola | 4.5 | 628.4 | 4.3 | 64.6 | 21.5 | <i>laevis</i> | 80 | 5 |
| GUA4 | Espíndola | Guarinja | 5.1 | 436.7 | 1.7 | 67.8 | 22.1 | <i>laevis</i> | 33.3 | 3 |
| GUA5 | Espíndola | Guarinja | 5.3 | 771.9 | 4.7 | 63.3 | 22.7 | <i>laevis</i> | 40.0 | 5 |
| CEC6 | Paltas | Sta. Cecilia | 5.6 | 752.5 | 3.1 | 67.5 | 20.0 | <i>tuberculata</i> | 50.0 | 4 |
| CEC1 | Paltas | Sta. Cecilia | 5.6 | 804.1 | 4.4 | 65.5 | 20.0 | <i>unclassified</i> | 0 | 5 |
| MAR1 | Loja | Marcobamba | 5.7 | 431.6 | 5.2 | 69.4 | 26.3 | <i>laevis</i> | 66.6 | 3 |
| JUA5 | Calvas | San Juan | 5.8 | 444.7 | 3.4 | 66.4 | 20.4 | <i>impressa</i> | 66.6 | 3 |
| YUR1 | Quilanga | Quilanga | 5.8 | 679.3 | 3.1 | 68.4 | 20.8 | <i>laevis</i> | 0 | 4 |
| GUA1 | Espíndola | Guarinja | 6.1 | 926.0 | 4.9 | 72.9 | 22.9 | <i>impressa</i> | 25 | 4 |
| MAR3 | Loja | Marcobamba | 6.1 | 456.2 | 4.3 | 65.7 | 24.1 | <i>impressa</i> | 0 | 4 |
| LAU1 | Paltas | Lauro Guerrero | 6.4 | 640.4 | 4.9 | 71.8 | 27.5 | <i>laevis</i> | 0 | 5 |
| JUA3 | Calvas | San Juan | 6.9 | 746.1 | 5.7 | 62.7 | 22.6 | <i>impressa</i> | 25 | 4 |
| JUA4 | Calvas | San Juan | 7.1 | 769.6 | 6.7 | 58.2 | 20.4 | <i>umbonata</i> | 0 | 5 |

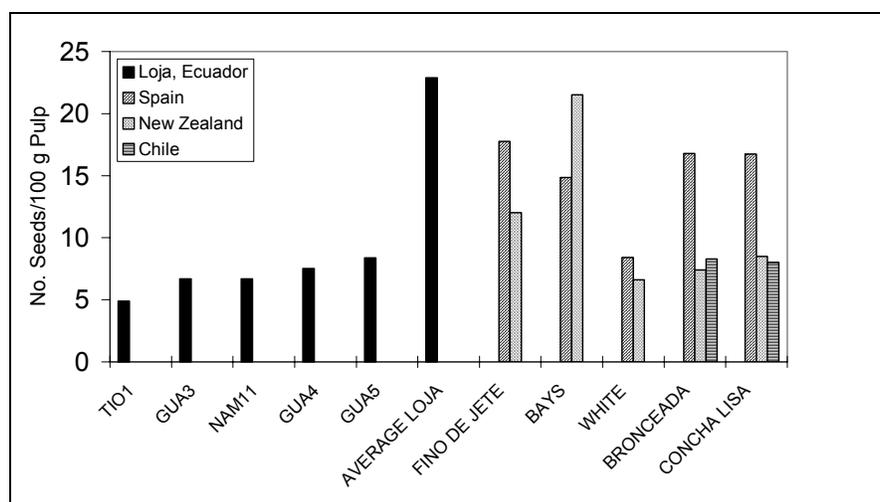


Figure 5.20. Comparison of number of seeds/100 g flesh between locally selected cherimoya accessions and commercial cultivars, characterised in different countries

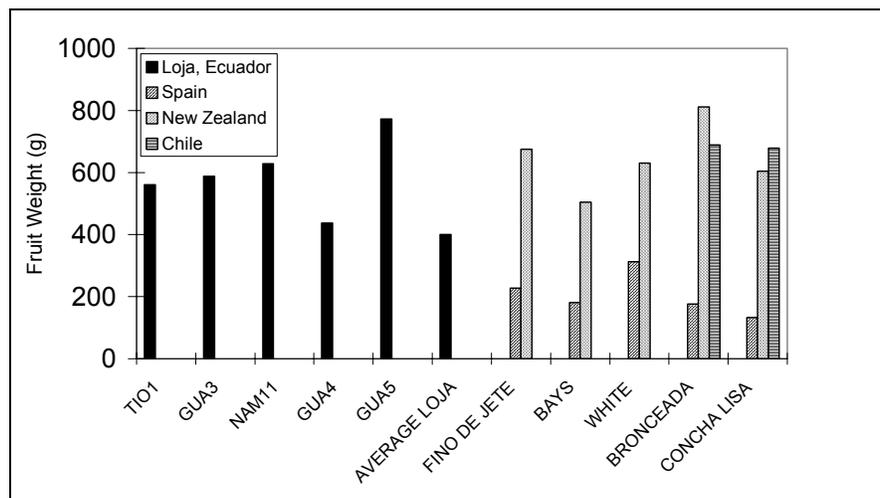


Figure 5.21. Comparison of fruit weight between local selected cherimoya accessions and commercial cultivars, characterised in different countries

A considerable difference in the selected fruit characteristics within each commercial cultivar, according to country of cultivation, can also be noted. The same cultivar has smaller fruits with more seeds when it is cropped in Spain than when cultivated in other countries. As explained earlier, this is probably due to the fact that characterisation in Spain was realised on a germplasm collection field with minimal cultural practices, while the characterisation data from other countries were obtained from commercial plantations under optimal conditions. This again indicates the existence of an influence of environment and management on fruit development. Data of wild cherimoya trees in Loja Province are therefore best compared to results of the Spanish cultivar characterisation, which uses minimal cultivation practices.

It should be noted that fruit characteristics for local accessions were calculated for only three to five fruits per accession, which could be interpreted as a weak basis of

comparison with commercial cultivars for which extensive data are available. However, when taking the average of Loja Province, local trees still show fruit characteristics that are almost comparable to these of commercial cultivars. Applying optimal management could only raise fruit weight and decrease number of seeds. As fruits were collected from trees occurring in the wild, no data about yields are known, another important characteristic, however, for tree selection.

5.1.3.4 CONCLUSIONS

The wide variability found in fruit characteristics, combined with the large numbers of wild cherimoya trees still present in the area, would clearly favour the hypothesis of Loja Province being located in the centre of origin of this tree species. This wide variability is an ideal base for future selection and breeding programmes. Seed index seems to be one of the best selecting parameters for preliminary *in situ* characterisation, while the botanical form should not be used as an indicator of fruit quality. Due to transport problems for cherimoyas that lack a smooth skin surface, this criterion can, however, be used as an additional selection criterion.

Within the described morphological variation, a number of promising accessions with excellent fruit characteristics, which can easily compete with commercial cultivars from cherimoya exporting countries, can be found. This does not mean that cherimoya fruit quality in Loja province is good. The ethnobotanical survey, indicating poor harvesting and handling techniques, and the large proportion of poor quality fruits, showing high seed numbers, indicate that on average fruit quality is low. Nevertheless, there exists an important, but clearly under-utilised, potential of very interesting cherimoya accessions, that could considerably improve cherimoya quality in Loja Province (and elsewhere).

The cherimoya germplasm collection and characterisation realised in Loja Province needs to be considered as a preliminary basis for future research. There is a need to increase the germplasm collection (e.g. Espindola county where four out of the five best accessions were found), and thus the genepool from which material can be taken, and to eliminate external factors such as climate and soil characteristics encountered *in situ*. It is necessary that characterisation continues *ex situ* in multi-location field collections where all accessions are grown under the same environmental and edaphic conditions. These collections should form the basis for Ecuador and more specifically Loja Province to use existing biodiversity to develop some excellent, new cherimoya cultivars, which is clearly a challenge for the future.

Presently, local farmers consider cherimoya trees to have limited value, often resulting in the clearing of cherimoya areas. Moreover, the fact that trees are rarely planted, increases the risk of genetic erosion with concomitant loss of valuable material. Considering this risk, acute due to increase of demographic pressure, the challenge will be to rescue these valuable resources and use them in future national and international breeding programmes.

5.1.4 GENERATIVE PROPAGATION

5.1.4.1 SEED STRUCTURE

The imbibed seed shows the seed structure before germination processes start. Figure 5.22 shows a longitudinal, nearly median section of a cherimoya seed with the different structures that can be observed. The dark brown testa consists of an impermeable woody sclerenchym layer, meaning that water can only enter the seed at the hilum, through the micropyle. The embryo is very small (3 - 4 mm) and rudimentary, located with its radicle directed towards the micropyle. The white endosperm consists of two clearly distinguishable parts, the inner central unlobed endosperm and the outer lobed endosperm. Protrusions of the tegmen, a membrane-like cover of the endosperm, can be observed as lamellas between the lobed endosperm. The external structure of a germinating seed, and its internal structure, in a longitudinal, median section, after removing one cotyledon, are shown in Figure 5.23.

During germination, cotyledons grow in the median plane of the unlobed endosperm and stay flat without developing, between the reserve tissue. Typical for epigeal germination is the initial strong development of the root system compared to the weak development of the cotyledons.

5.1.4.2 GERMINATION

The germination test was carried out for 900 days. Daily and cumulative germination results for the 12 pre-application treatments are given in the germination curves (Figure 5.24). Germination after 98 and 900 days of evaluation and germination rate in the initial 90 days of the germination are given in Table 5.2.

Table 5.2. Cherimoya germination percentages after 98 and 900 days, with significant differences (95% probability) according to Tukey-test, and germination rate in the three initial 30-day time-intervals

| Pre-applied treatment | Germination (%) | | Germination rate (% germ./day) | | |
|--|--------------------|--------------------|--------------------------------|-----------|-----------|
| | 98 Days | 900 Days | Day 0-30 | Day 31-60 | Day 61-90 |
| 24 h 1000 ppm GA ₃ | 74.5 ^e | 77.0 ^b | 0.93 | 1.41 | 0.13 |
| 24 h 5000 ppm GA ₃ | 69.5 ^e | 72.5 ^{ab} | 0.23 | 1.74 | 0.35 |
| 24 h 10.000 ppm GA ₃ | 65.0 ^e | 67.0 ^{ab} | 0.40 | 1.53 | 0.22 |
| 24 h 500 ppm GA ₃ | 58.0 ^e | 65.5 ^{ab} | 0.81 | 0.93 | 0.20 |
| 48 h 500 ppm GA ₃ | 47.5 ^{de} | 49.5 ^a | 0.66 | 0.81 | 0.10 |
| 72 h H ₂ O | 29.5 ^{cd} | 76.0 ^b | 0.03 | 0.48 | 0.43 |
| 12 h H ₂ O (92 °C), cooling | 29.0 ^{cd} | 70.5 ^{ab} | 0.44 | 0.38 | 0.15 |
| 48 h H ₂ O | 22.0 ^{bc} | 70.0 ^{ab} | 0.21 | 0.31 | 0.18 |
| 12 h H ₂ O | 15.0 ^{ab} | 64.5 ^{ab} | 0.12 | 0.20 | 0.17 |
| 24 h H ₂ O | 15.0 ^{ab} | 61.5 ^{ab} | 0.13 | 0.21 | 0.15 |
| 10' sulphuric acid | 12.5 ^{ab} | 64.5 ^{ab} | 0.05 | 0.25 | 0.08 |
| Control | 10.0 ^a | 59.0 ^{ab} | 0.08 | 0.16 | 0.10 |

Results - Cherimoya

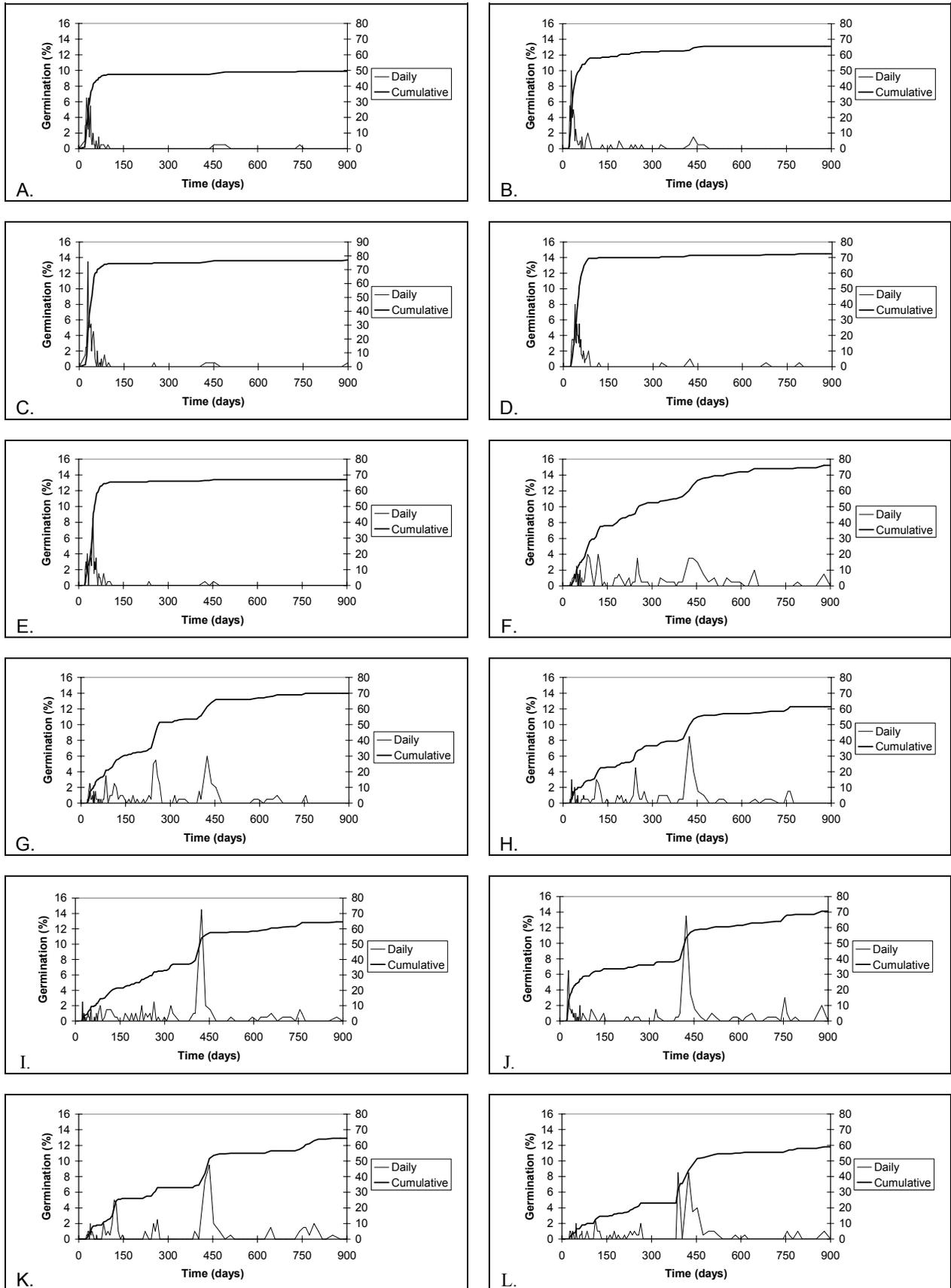


Figure 5.24. Germination of cherimoya seeds under different pre-applied treatments: A. soaking 48 h in 500 ppm GA_3 ; B. soaking 24 h in 500 ppm GA_3 ; C. soaking 24 h in 1,000 ppm GA_3 ; D. soaking 24 h in 5,000 ppm GA_3 ; E. soaking 24 h in 10,000 ppm GA_3 ; F. soaking 72 h in distilled water; G. soaking 48 h in distilled water; H. soaking 24 h in distilled water; I. soaking 12 h in distilled water; J. soaking 12 h in cooling distilled water; K. soaking 10' in H_2SO_4 (75 %); L. control

Germination curves and evaluation data clearly show that pre-application of the phytohormone gibberellic acid (GA_3) has a positive effect on germination percentage and also on germination rate, although no significant differences can be shown between the different concentrations. Germination is high and regular during the first 90 days, slowing down to a very low level. This results in typical germination curves. Soaking in distilled water also has a germination promoting effect, especially when soaking is maintained between 48 to 72 hours or when water is heated before soaking. However, in these cases germination is much slower and more irregular resulting in more continuous germination curves than with the GA_3 -treatments. Chemical scarification with sulphuric acid has no significant effect on stimulating germination.

A sizeable germination promoting effect, most evident in the irregular germination curves, is noted around 400 days. This may possibly be due to the uncontrolled environmental conditions of temperature and suggests that fluctuating temperatures could also be a good treatment to stimulate germination in cherimoya.

In general, germination started after 20 days but germination rate is highest around 30 - 61 days after sowing. After 900 days, seeds were still germinating indicating that cherimoya seeds can be dormant for a very long time, even when brought in an optimal germination environment. This long germination time proves that some cherimoya seeds can keep their germinating capacity for a long time, even under humid conditions. None of the pre-application treatments reached the level of viability as initially determined by the tetrazoliumchloride test, which was 89.8 % (error \pm 5.4 %), indicating that some of the seeds lost their viability, before germinating, during the 900 days that the test was carried out.

5.1.4.3 CONCLUSIONS

Before proceeding to the germination test, the structure of cherimoya seeds was examined, showing a very small rudimentary embryo (3 - 4 mm) located in the lower part of the median plane of the white unlobed endosperm, and surrounded by a lobed endosperm which is surrounded by an impermeable testa, leaving only the micropyle as an entry for water. During germination, the radicle shows strong initial development compared with cotyledons that grow in the median plane of the unlobed endosperm.

Cherimoya seed germination went on for 900 days, at which point germination levels became very low. Germination was very heterogeneous, typical for seeds showing dormancy. To avoid this and in order to have a rapid and high germination, seeds should be treated before sowing. The germination test compared different pre-application treatments, looking for the highest and fastest germination. Pre-application of the phytohormone gibberellic acid (GA_3) proved to give the most rapid and highest germination in a short time (98 days), without significant differences between different concentrations. A concentration of 500 - 1,000 ppm of gibberellic acid gives a large stimulation to germination and, with regard to the economic factor,

should be considered as the best pre-application treatment. This is confirmed by other scientists (Hayat, 1963; Campbell & Popenoe, 1968; Vidal Hernández, 1993) who found similar results in cherimoya and other Annonaceae.

No evaluation of seedling development was carried out. Literature (Campbell & Popenoe, 1968) suggests that treatments with concentrations of 3,500 ppm caused abnormal seedling development, whereas concentrations of 350 ppm did not. Remarkable is that a soaking time of 48 hours with a concentration of 500 ppm has the lowest germination percentage after 900 days. Soaking for 48 hours in gibberellic acid seems to give an overexposure of the seeds to the hormone, although provoking the typical stimulation in the initial phase, but final germination percentage remains low as compared to the other GA₃-treatments.

Gibberellic acid is a costly phytohormone, unaffordable for most resource-poor cherimoya growers, especially in developing countries. An alternative, which also resulted in rapid and high germination, could be soaking for 48-72 hours in, preferably, distilled water, or for a shorter time in heated (92 °C), gradually cooling, water. This treatment results in high, although still irregular, germination, with significantly better values than the control. Scientists (Hayat, 1963; Ellis *et al.*, 1985b) do not consider pre-applied soaking in water as a successful dormancy-breaking treatment. Our experiments however, show that these pre-application treatments improved germination considerably compared with the control. Significant differences between control, and gibberellic acid and water treatments suggest the presence of dormancy in cherimoya seeds. It is probably a combination of morphological (undeveloped embryo) and physical (seed coat) dormancy although exact determination of dormancy type requires further research.

The fact that some seeds did not germinate in more than two years, even under optimal, i.e. humid conditions, indicates that cherimoya seeds do not lose their germinating power very rapidly, as suggested by some authors. Another germination test, using seeds conserved for different times, under different environmental conditions, could give valuable information about the conservation potential of cherimoya seeds.

During the course of the experiment, similar germination rates occurred at the same time, for different treatments, indicating that an external factor, probably temperature, also stimulated germination. A further investigation of germination under fluctuating temperature regimes could also give useful information on how to improve and regularise cherimoya germination.

5.1.5 OVERALL DISCUSSION AND CONCLUSIONS

Despite the lack of selection and optimal cultivation practices, promising cherimoya accessions, that could withstand a preliminary comparison with commercial cultivars, were found among the large pomological diversity in Loja Province. Using optimal

cropping zones for cherimoya, as revealed by the edaphoclimatic study, combined with cultivation of selected accessions could form the basis for future commercial cherimoya production. Loja Province offers the ideal biological environment for this activity.

Nevertheless, production constraints at farmer and commercialisation level exist and need to be overcome to obtain profitable yields. Obviously, both levels are linked by the crop profitability and should be addressed simultaneously.

The production constraints or lack of cultivation practices consist mainly of a lack of access to selected material and a lack of interest for a low-profit crop, as is currently the case with cherimoya. In order to break the vicious circle of 'poor quality harvest – low prices' it is necessary to raise awareness with the farmers of the economic potential of cherimoya fruits. Local subsistence farmers are more interested in economic potential than in pomological potential, and are reluctant to risk unprofitable investments. Once convinced of the economic potential, farmers may be prepared to invest money and time in the cultivation of cherimoya.

Commercialisation constraints consist mainly of the existing commercialisation channels that traditionally pass through a middleman and this at a high cost for the local farmer.

Given their poor economic conditions, local farmers are not capable of overcoming this complex of difficulties on their own. Intervention of institutional help is necessary to obtain viable commercial cultivation of cherimoya. This institutional help, governmental or non-governmental, must address both the production and the commercialisation constraints. Production constraints could be overcome successfully through the following actions: (i) of supplying grafted selected plants; (ii) of providing information on specific cropping techniques as grafting and harvesting with regular follow-ups; and (iii) of offering micro-credit programmes to finance the cultivation. The commercial component should consist of looking for ways to sell fruits, or if possible processed products, through farmer cooperatives (Van Damme & Scheldeman, 1999b).

5.2 HIGHLAND PAPAYAS (*VASCONCELLEA* SPP.)

5.2.1 ETHNOBOTANICAL SURVEY

5.2.1.1 KNOWLEDGE, NOMENCLATURE AND USE

Of the 660 interviewees, a sample of the market population in different county capitals, 81 % easily recognises babaco (*V. × heilbornii* 'Babacó') fruits (Figure 5.25). The majority (96 %) use the vernacular name babaco for this type of fruit. Remarkably is the difference of familiarity with babaco in the different counties. In Loja and Saraguro county, babaco is known by respectively 99 % and 97 % of the interviewees, while in Celica county it is only known by 66 %. Age or education level do not influence familiarity with babaco.

The survey also included fruits of *V. × heilbornii* var. *chrysopetala* (Figure 5.26) and five unclassified varieties that can be found sporadically at higher elevations. Only 18 % of the interviewees recognise the fruits of *V. × heilbornii* var. *chrysopetala*. These fruits are best known in Gonzanama county (43 %) whereas nearly a quarter of the market population of Loja and Saraguro counties is familiar with the fruits. Nomenclature for this type is very variable. A total of 46 % calls the fruit 'toronche', sometimes 'toronche de castilla', while 34 % name it babaco or 'babaco pequeño' (small babaco). Other less common names include 'chamburo' (5 %) and 'siglalón' (3 %). The other, rarer, *V. × heilbornii* varieties are only recognised by a minority of the interviewees, varying between 2 % and 11 %. The nomenclature used by the people recognising these fruits indicate a general use, on average 67 %, of the name 'toronche', sometimes more detailed as 'toronche pequeño' or 'toronche de cerro'. Other often-used names are 'chamburo' (average 8 %) and babaco or 'babaco pequeño' (average 20 %).

V. cundinamarcensis (Figure 5.27) fruits are recognised by 24 % of interviewees. Remarkable again are the differences between the counties. In Saraguro county, where *V. cundinamarcensis* is abundant, 81 % is familiar with the fruit. In Calvas, Catamayo and Celica counties less than 5 % know the fruits (Figure 5.27). The name given to this fruit is, again, 'toronche' (73 %) while other vernacular names include 'chamburo' (15 %) and 'siglalón' (5 %). It is clear that *V. cundinamarcensis*, although being one of the most common *Vasconcellea* species in Latin America, is only locally known, especially in the eastern part of Loja Province.

Two types of *V. stipulata* were included in the survey, one showing typical fruits and another one with particularly long slender fruits. The typical fruit is known by 50 % of interviewees, making it the second most known *Vasconcellea* species of Loja province (Figure 5.28). This knowledge is distributed mainly in Celica and Calvas counties where 95 % and 78 % respectively of the surveyed persons is familiar with the fruits. In Saraguro county only 2 % recognise the fruits. The pattern of *V. stipulata* is opposed to that of *V. cundinamarcensis*, which is well-known in Saraguro county

and relatively unknown in Celica and Calvas counties (Figures 5.27 – 5.28). The rare form of *V. stipulata* is recognised by only 4 % of interviewees. Local names for *V. stipulata* are ‘toronche’ in 88 % of the cases and ‘chamburo’ (4 %).

‘Toronche’ and to a lesser degree ‘chamburo’ are names given to all *Vasconcellea* species besides *V. × heilbornii* ‘Babacó’. Local people seemingly do not make any difference in nomenclature between *V. cundinamarcensis*, *V. stipulata* and *V. × heilbornii* varieties other than babaco.

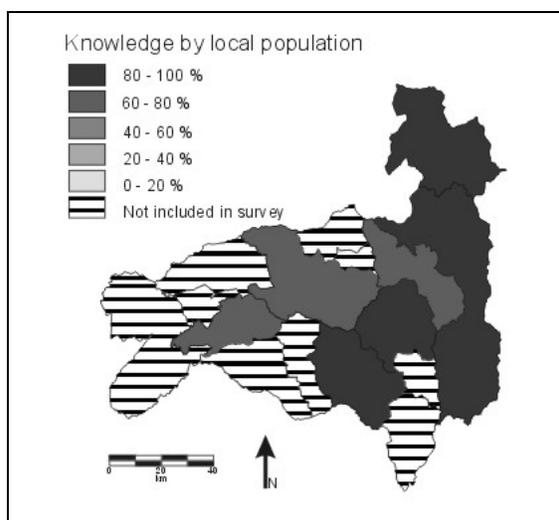


Figure 5.25. Knowledge of 660 interviewees on *V. × heilbornii* ‘Babacó’ in different counties of Loja Province

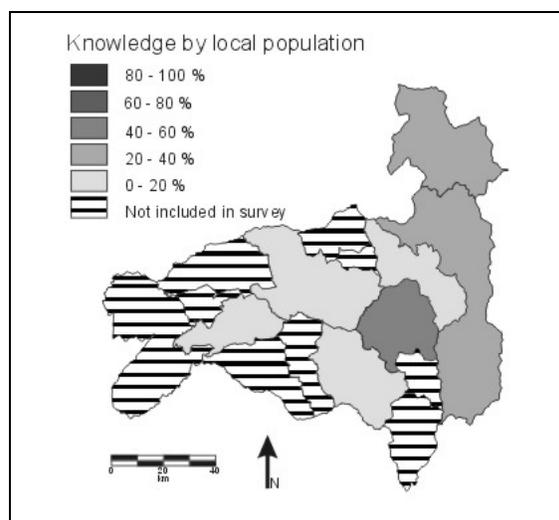


Figure 5.26. Knowledge of 660 interviewees on *V. × heilbornii* var. *chrysopetala* in different counties of Loja Province

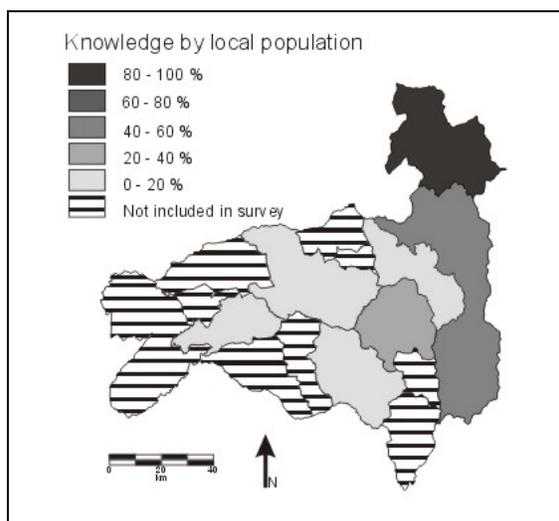


Figure 5.27. Knowledge of 660 interviewees on *V. cundinamarcensis* in different counties of Loja Province

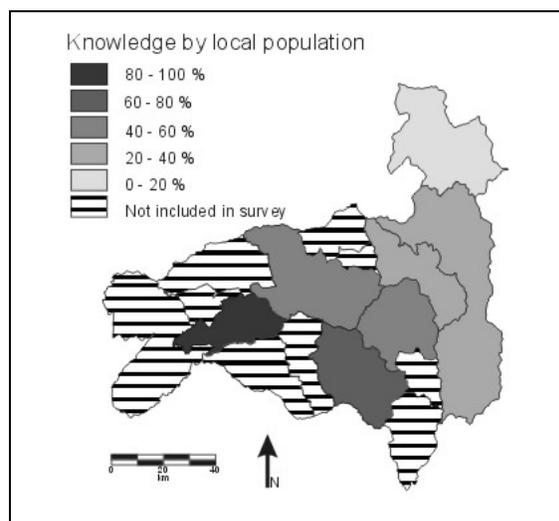


Figure 5.28. Knowledge of 660 interviewees on *V. stipulata* in different counties of Loja Province

A comparison between knowledge of the interviewees on local *Vasconcellea* species and their age shows a decrease of knowledge with younger people. The total number

of recognised *Vasconcellea* samples, out of the ten shown types, decreases by decreasing age (Figure 5.29) while especially for the lesser known species *V. × heilbornii* var. *chrysopetala* and *V. stipulata* the percentage of familiarity with the fruits also decreases with decreasing age (Figure 5.30). This illustrates the actual situation, where beside babaco, the local people are losing interest and subsequently knowledge of other *Vasconcellea* species.

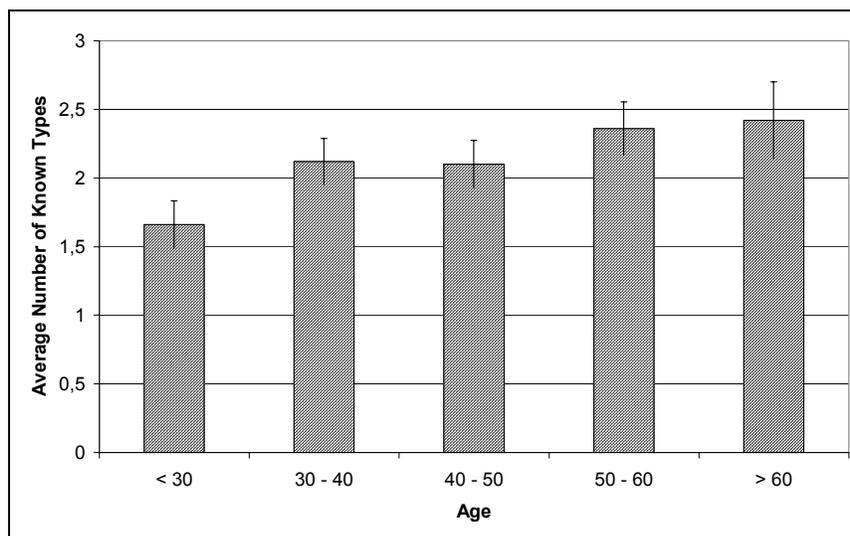


Figure 5.29. Average number of recognised *Vasconcellea* samples of 660 interviewees in Loja Province, according to age

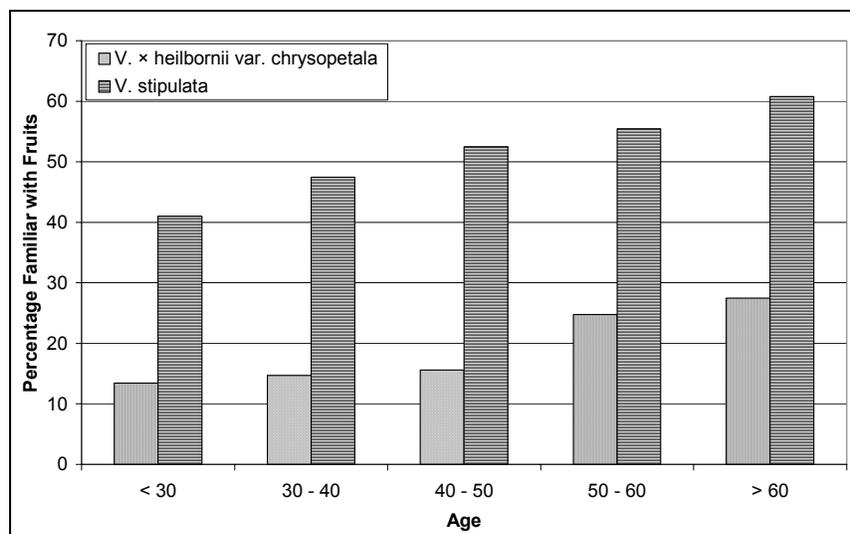


Figure 5.30. Familiarity with different *Vasconcellea* species of 660 interviewees in Loja Province, according to age

When asked which *Vasconcellea* sample they prefer, *V. × heilbornii* ‘Babacó’ is almost everywhere the most mentioned highland papaya with 59 % of the interviewees indicating it as their preference; the only exception is Celica county. *V. stipulata* ranks second with 27 %. In Celica, where *V. stipulata* is well-known, this

highland papaya is considered superior to babaco with 59 % indicating *V. stipulata* as their preference with only 27 % for babaco. In Calvas and Paltas counties, where *V. stipulata* is also known, this species has almost equal appreciation than babaco. This clearly indicates that in places where *V. stipulata* is known it is highly appreciated.

Vasconcellea species are generally consumed after processing, although a few interviewees (3 %) also claimed they consume the fruits fresh. Main uses can be divided into two classes: drinks and cooked products. Drinks comprise juices ('*jugo*') made of water and sugar mixed with high fruit content; refreshments ('*refresco*'), similar drinks with a lower fruit content; '*coladas*', a typical local beverage prepared by cooking fruit, water and spices (Van den Eynden *et al.*, 1999); infusions ('*aguas aromaticas*') and milkshakes ('*batidas*'). As this local terminology is not well determined and often misused, e.g. the difference between juice and refreshment, delimitations between liquid products are not very strict. Liquid products always form the main use of the common *Vasconcellea* species. A total of 87 % of interviewees that consumed babaco stated they process babaco into drinks, mainly juices. *V. stipulata* is the least used in liquid products with only 62 % of consuming interviewees using it in drinks, mainly in lighter refreshments. This can be due to the higher papain content that makes it too strong to use in beverages, especially concentrated juices. Generally, the use in coladas, infusions and milkshakes is rather uncommon. The use as an additive to aromatise alcoholic beverages was mentioned by two interviewees.

Beside the use in drinks, *Vasconcellea* species are frequently used in preparation of sweet cooked products. Again, some confusion and overlap in terminology exists, complicating data processing. All products are prepared by boiling down fruits with sugar and water, sometimes by adding spices. Typical products include '*dulce*', '*passado*', '*miel*', '*conserva*' and marmalade, all local homemade products obtained by cooking till fruits decompose. Slightly different are conserves ('*almibar*') where fruits are cooked but maintain their aspect. *V. stipulata* is most used in this type of preparation (86 %) while only 56 % of *V. cundinamarcensis* consumers process their fruits in this way. Other, rarer, ways of consumption of *Vasconcellea* species include roasting (especially for *V. stipulata*) and processing into ice cream. A few interviewees report the medicinal use of *Vasconcellea* fruits against nervousness.

5.2.1.2 AGRICULTURAL PRACTICES

Vasconcellea species are often cultivated in Loja Province. Cultivation of different species shows a similar pattern as the knowledge figures given above, only with lower numbers. Babaco is the most popular species with 29 % of interviewees cultivating babaco plants. Plant numbers reach up to 1,000 plants in case of commercial plantations, but 80 % of babaco cultivators possess 10 or fewer plants. The second most cultivated *Vasconcellea* species is *V. stipulata* with 17 % of interviewees cultivating this species. *V. cundinamarcensis* is cultivated by only 7 % of the interviewed market population.

Striking differences in cultivation (Fig. 5.31 – 5.34) can be found between different counties. Babaco cultivation is common in Saraguro (61 %), Gonzanamá (47 %) and Loja counties (44 %) and almost absent in Celica county (6 %). Cultivation of other *V. × heilbornii* varieties is rarer, all below 20 % of interviewees and only important in the case of *V. × heilbornii* var. *chrysopetala* in Gonzanamá (16 %), Loja (14 %) and Saraguro (13 %) counties. *V. stipulata* cultivation is very common in Celica county (42 %), less common in Calvas (24 %) and Paltas (21 %) counties and almost inexistent in Saraguro (2 %). At odds with the latter results is the cultivation of *V. cundinamarcensis* which is most important in Saraguro (30 %) and Loja (15 %) counties, very low in Paltas county (2 %) and inexistent in the other ones.

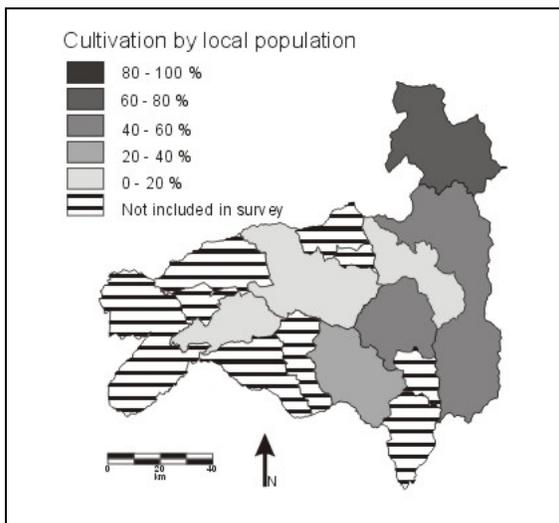


Figure 5.31. Cultivation of *V. × heilbornii* 'Babacó' in different counties according to 660 interviewees in Loja Province

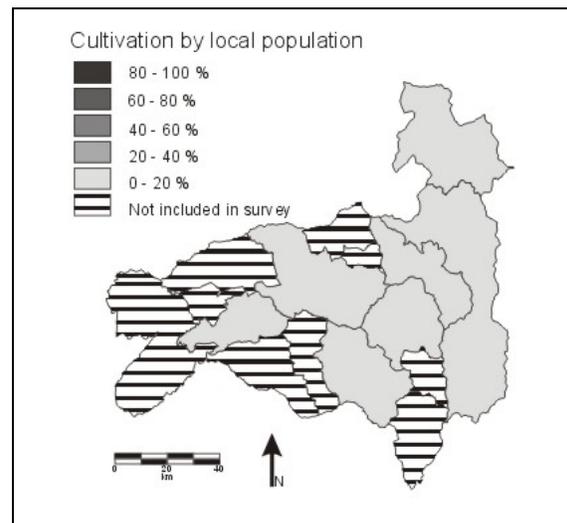


Figure 5.32. Cultivation of *V. × heilbornii* var. *chrysopetala* in different counties according to 660 interviewees of Loja Province

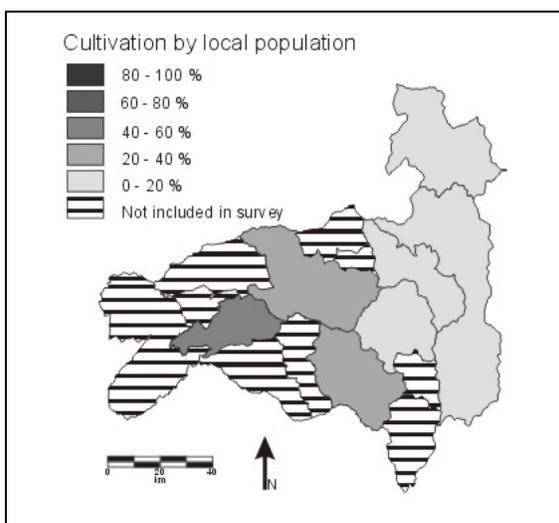


Figure 5.33. Cultivation of *V. × cundinamarcensis* in different counties according to 660 interviewees of Loja Province

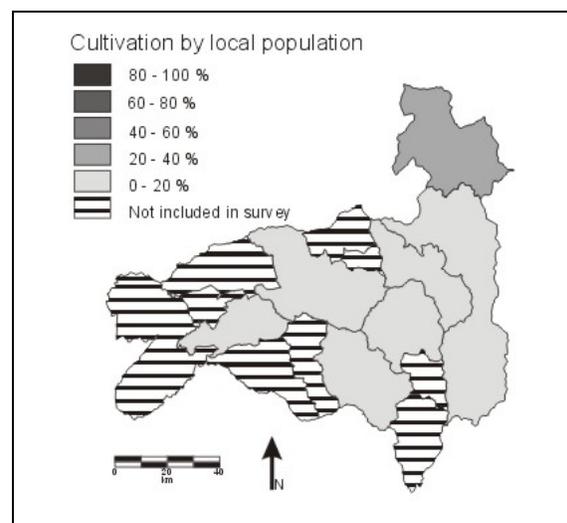


Figure 5.34. Cultivation of *V. × stipulata* in different counties according to 660 interviewees of Loja Province

Vasconcellea species are cultivated at different levels of intensity. Babaco plants do receive most agricultural practices. Logically, the seedless hybrid is propagated by cuttings, 91 % of babaco cultivators, although a minority claims to propagate babaco by seeds. This false answer is probably due to a confusion in the use of the word 'semilla' (seed), that is sometimes applied for cuttings as well. *V. stipulata* is mostly propagated by seed (76 %), often spontaneously as claimed by a lot of farmers in Celica county. Finally, *V. cundinamarcensis* is propagated in 52 % of cases by cuttings, again with possible slight differences due to the misuse of terminology.

Fertilisation is a common practice in highland papaya cultivation, with 76 % of babaco cultivators applying organic fertilisation to their plants, often organic waste or manure. Only 15 % apply chemical fertilisers to babaco plants. Other *Vasconcellea* species receive less fertilisers, 67 % for *V. cundinamarcensis* and only 33 % for *V. stipulata*, which is often only harvested, without any input. Use of pesticides is limited and is only important in babaco cultivation in the case of 19 % of babaco farmers, especially those who cultivate for sale. The use of irrigation varies between 60 % for babaco to 35 % for *V. stipulata* and is often included in a general irrigation scheme for the backyard garden.

5.2.1.3 COMMERCIALISATION

Vasconcellea fruits are sold by a limited number of interviewees. Of the people that own babaco plants only 20 % sell their fruits. This is even less for the other highland papayas: 17 % in case of *V. stipulata* and 9 % in case of *V. cundinamarcensis*. Places with abundant cultivation are logically the places where most fruits are sold, e.g. Loja and Saraguro counties in case of babaco. Generally (90 % of the babaco sale), fruits are directly sold by the farmers at the market, without intervention of a middleman. Babaco fruits are sold at 0.36 euro/fruit and monthly volumes can amount to 2,000 fruits in the case of commercial cultivation, although 79 % of babaco sellers sell 100 or less fruits/month. Fruits of *V. stipulata* are sold on average at 0.07 euro/fruit with volumes up to 700 fruits/month, but 72 % of the farmers sell less than 200 fruits/month. In the case of *V. cundinamarcensis* fruit sale is rare and price is 0.06 euro/fruit.

Of the 660 interviewees, 39 % claim to buy babaco fruits. This is much higher in Loja and Saraguro counties, with respectively 61 and 47 %. *V. stipulata* fruits are bought by 18 % with the highest number in Celica county (67 %). Only 4 % buy *V. cundinamarcensis* fruits. *Vasconcellea* availability in local markets is thus highly correlated to cultivation and knowledge and shows a similar pattern among the different counties. Prices and volumes differ only slightly between counties with an average buying price for babaco of 0.57 euro/fruit with 95 % of prices situated between 0.2 - 1.0 euro/fruit. Monthly buying volumes amount to 80 fruits/month, especially in case of small retailer stores, but generally, in 70 % of the cases, volumes are lower than 10 fruits/month. In case of *V. stipulata* and *V.*

cundinamarcensis, average prices are 0.08 euro/fruit and 0.06 euro/fruit respectively with buying volumes of up to 40 fruits/month.

5.2.1.4 CONCLUSIONS

Of the huge variety of highland papayas found in Loja Province, only babaco (*V. × heilbornii* 'Babacó') is well known throughout the whole province. It is especially cultivated and commercialised in Loja and Saraguro counties. Cultivation is sometimes well developed with use of chemical fertilisers and pesticides, especially in commercial plantations. Even in cultivation for self-consumption, farmers do care for their plants, often using organic fertiliser and irrigation. The fact that plants have to be acquired, by propagating cuttings or by buying, in contrast to the other spontaneous propagating highland papayas, and the relatively high market value of the fruit make babaco a well appreciated and well cared for highland papaya.

Other varieties of *V. × heilbornii*, also propagated by cuttings, are lesser known and cultivated. They are sometimes seen as smaller versions of babaco fruits and less appreciated. Only *V. × heilbornii* var. *chrysoptala* is known and cultivated, although on a small scale, in some counties. Other, more rare, varieties are almost unknown, illustrating ongoing genetic erosion and probably uniformisation towards monoculture of babaco. This is even more accentuated in the younger generation where knowledge of highland papayas is clearly diminishing.

V. stipulata is the only highland papaya, besides babaco, that still has considerable appreciation, especially in the western part of the Lojan highlands. Wherever this species is well known, its fruits are appreciated as much or even more than babaco fruits. Its cultivation however, is much less intensified than that of *V. × heilbornii*, probably due to its hardiness, its often natural multiplication by seeds and the lower commercialisation possibilities.

V. cundinamarcensis is only well known in the north-eastern part of the province, especially Saraguro county, where it is very common. It is however less appreciated and less commercialised than babaco, which has its main cultivation zone in the same county, and is also in danger of being lost.

The use of vernacular names for the highland papayas in Loja Province does not discriminate between different *Vasconcellea* species. Only babaco is used for the larger seedless fruits whereas all other smaller *Vasconcellea* species are grouped under 'toronche' or to a lesser degree 'chamburo'. Local people do not consider the different species as separate units but regard them as one very variable species. In one region different species are often given the same name while the same species can be given a different name in different regions.

Local uses of the present *Vasconcellea* species only show minor differences. They are very seldom consumed as fresh fruit, but are mostly processed into local drinks

or sweetened cooked products. The highly aromatic fruits show low sugar content making addition of sugar almost indispensable.

5.2.2 CROP ECOLOGY

5.2.2.1 *IN SITU* EVALUATION OF GROWING CONDITIONS

Edaphoclimatic conditions at 104 sites were used to assess the growing range of naturally occurring highland papayas. All sites were located between 993 and 2,900 masl. *V. stipulata* was found in the entire altitude range, thus considerably widening the altitudes at which the species occurs according to Badillo (1993) who states that *V. stipulata* is limited at a zone ranging 1,600 – 2,450 masl. Altitude range for *V. cundinamarcensis* varies from 1,950 to 2,678 masl, a slightly more confined zone than the one described by Badillo (1,500 – 3,000 masl). *V. × heilbornii* occurs from 1,572 up to 2,900 masl, corresponding to the findings of Badillo who situates *V. × heilbornii* between 1,600 and 2,800 masl. Babaco is present between 1,572 and 2,750 masl.

In our survey, a total of 1,656 highland papayas were counted. A total of 908 plants (55 %) were *V. × heilbornii* plants, of which 448 (27 %) babaco plants. 702 plants (42 %) were *V. stipulata* plants and only 46 plants (3 %) belonged to *V. cundinamarcensis*.

Temperature ranges differ according to the species. Especially the optimal mean annual temperature range is slightly different for each species (Table 5.3). *V. stipulata* can be found in a very wide temperature range, with a maximum up to 26 °C where it grows together with *C. papaya*. This contrasts with *V. cundinamarcensis* that shows a very limited temperature range: 75 % of all plants are found at relatively low temperatures (12 - 14 °C) and no *V. cundinamarcensis* plant is found at temperatures above 18 °C. *V. × heilbornii* has a temperature interval, which lies between the wide temperature range of *V. stipulata* and the narrow margin of *V. cundinamarcensis*. Its optimum seems to coincide with the combination of its two presumed progenitors. Areas with an mean annual temperature below 10 °C do not grow any *Vasconcellea* species. Annual precipitation ranges indicate a slight preference of *V. stipulata* for wetter zones while *V. cundinamarcensis* is more found in relatively drier areas although the former again shows a broader precipitation range (Table 5.3). The wide precipitation ranges, found for the different *Vasconcellea* species, seem to indicate that precipitation is a less limiting factor than temperature.

Histograms (Annex) of the recalculated values of soil parameters indicate preferences and ranges (Table 5.4) of the different highland papayas. There is a striking preference of *V. cundinamarcensis* for light soil types, with a lower nutrient status, when compared to the other species. Generally, highland papayas prefer acid soils with moderate organic matter content, variable nutrient status and a low amount of coarse materials.

Of all soil formations, formation F (Inceptisol) is the only one that is well-suited for all *Vasconcellea* species. Especially *V. cundinamarcensis* (91 %) and *V. stipulata* (52 %) occur very frequently in this soil formation. This soil formation covers 18 % of Loja Province and is restricted to zones above 2,000 masl. Remarkably is the high frequency of *V. × heilbornii* plants (47 %) in formation G. This is a soil formation that combines Alfisols and Inceptisols with a high base saturation, covering only 6 % of Loja Province. Only a limited number of *V. stipulata* (10 %) plants are found in this formation unit while *V. cundinamarcensis* was completely absent.

Table 5.3. Optimal climatic characteristics and their range for different highland papayas in Loja Province

| | <i>V. stipulata</i> (702 plants) | <i>V.</i> <i>cundinamarcensis</i> (46 plants) | <i>V. × heilbornii</i> (908 plants) | <i>V. × heilbornii</i> 'Babacó' (448 plants) |
|------------------------------|-------------------------------------|---|--|--|
| Mean annual Temp. (° C) | 14 – 18 (10 – 26) | 12 – 14 (10 – 18) | 12 – 18 (10 – 22) | 12 – 18 (10 – 22) |
| Annual precipitation (mm) | 1,000 – 1,300 (600 – 1,300) | 800 – 1,000 (700 – 1,200) | 800 – 1,200 (700- 1,300) | 700 – 1,200 (700 – 1,300) |

Table 5.4. Optimal edaphic characteristics and their range for different highland papayas in Loja Province

| | <i>V. stipulata</i> (702 plants) | <i>V.</i> <i>cundinamarcensis</i> (46 plants) | <i>V. × heilbornii</i> (908 plants) | <i>V. × heilbornii</i> 'Babacó' (448 plants) |
|-------------------------|-------------------------------------|---|---|--|
| Soil texture (USDA)* | C - CL (C - SaL) | SaCL – SL (C - SaL) | C - L (C - SaL) | C - L (C – SaL) |
| PH | 4.0 – 6.5 (3.6 – 7.1) | 4.7 – 6.0 (4.7 – 7.2) | 5.0 – 7.0 (3.6 – 7.3) | 5.0 – 7.0 (3.6 – 7.2) |
| Organic matter (%) | 4.0- 8.0 (1.7 - 11.3) | 3.0 – 6.0 (1.5 - 6.8) | 3.0 – 7.0 (1.5 - 11.3) | 3.0 – 7.0 (1.5 - 11.3) |
| Stoniness (%) | 0.0 – 15.0 (0.3 – 44.7) | 0.0 – 5.0 (1.5 - 23.4) | 0.0 – 15.0 (0.3 - 44.7) | 0.0 – 15.0 (0.3 - 44.7) |
| CEC (meq/100 g soil) | 15.0 – 40.0 (13.3 - 67.4) | 10.0 – 25.0 (13.1 - 30.7) | 15.0 – 35.0 (10.2 - 45.1) | 15.0 – 30.0 (10.2 – 45.1) |
| Available N (µg/ml) | 40.0 – 100.0 (20.7 – 140.9) | 40.0 – 80.0 (19.2 – 84.8) | 40.0 – 80.0 (19.2 – 140.9) | 40.0 – 80.0 (19.2 – 140.9) |
| Available P (µg/ml) | 1.0 – 50.0 (1.0 - 225.1) | 25.0 – 125.0 (1.0 - 120.3) | 0.0 – 225.0 (1.0 - 225.1) | 1.0 – 225.0 (1.0 - 225.1) |
| Available K (µg/ml) | 75.0 – 200.0 (39.4 - 237.8) | 25.0 – 100.0 (49.6 - 178.3) | 75.0 – 200.0 (39.4 - 215.6) | 75.0 – 200.0 (49.6 - 215.6) |
| Soil formation** | F, Ass. (S, F, K, G, R) | F (S, F, N, Ass.) | S, F, G, Ass. (S, F, K, G, T, V, N, Ass.) | S, F, G, Ass. (S, F, K, G, T, V, N, Ass.) |

* C : Clay; CL : Clay Loam; SiCL : Silty Clay Loam; SaCL: Sandy Clay Loam; SaL: Sandy Loam; L: Loam

** See 1.2.3 (Table 1.3) for explanation on soil formation units

Comparing to literature data, the observed optimal mean temperature range for babaco, 12 – 18 °C, is slightly lower than the 15 – 20 °C range described by Merino

Merino (1989). The whole temperature range found in Loja Province, 10 – 22 °C, is similar to the 13 – 22 °C range described by Cossio (1988) for Ecuador. The optimal temperature range for *V. cundinamarcensis* found in Loja province, is slightly lower than the 14 – 18 °C range found by CAF (1992). Our experiences confirm the wide precipitation range found in literature (Cossio, 1988; Soria & Viteri, 1999; CTIFL, 1992). Preferences for slightly acid, light textured soils as described by Soria & Viteri (1999) and Merino Merino (1989) for babaco are confirmed as well.

5.2.2.2 PREDICTION OF SUITABLE CULTIVATION ZONES

Applying the modified simple limitation method, using plant frequencies instead of yields, can give a preliminary idea about potential cultivation zones for different *Vasconcellea* species in Loja Province. Figure 5.35 gives the Overall Frequency Maps for four different highland papayas. Table 5.5 gives the percentage and their corresponding absolute surface areas in Loja Province suited for *Vasconcellea* growth.

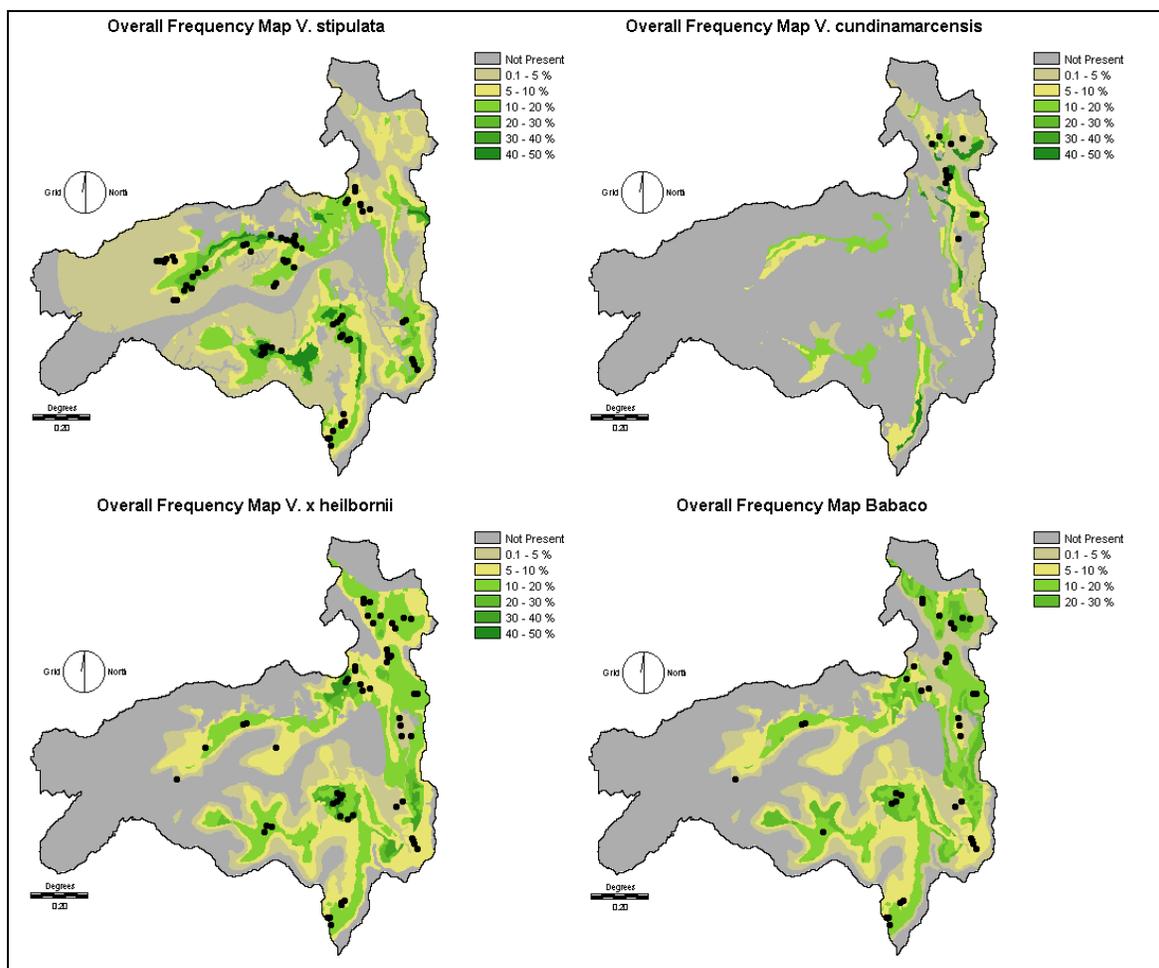


Figure 5.35. Overall Frequency Map for *V. cundinamarcensis*, *V. stipulata*, *V. × heilbornii* and babaco (*V. × heilbornii* 'Babaco') in Loja Province with indication of sampling sites

Table 5.5. Percentage of Loja Province and the corresponding absolute surface areas for different frequency classes for the different *Vasconcellea* species

| | <i>V. stipulata</i> | <i>V. cundinamarcensis</i> | <i>V. × heilbornii</i> | <i>V. × heilbornii</i> 'Babacó' |
|--------------------------|---------------------------------|---------------------------------|---------------------------------|------------------------------------|
| Frequent (%) (> 10 %) | 16.7 % 1,803 km ² | 6.3 % 685 km ² | 19.3 % 2,084 km ² | 19.3 % 2,084 km ² |
| Not Present | 29.4 % 3,174 km ² | 79.9 % 8,621 km ² | 48.1 % 5,195 km ² | 48.1 % 5,195 km ² |

Although *V. cundinamarcensis* is the most common highland papaya in South America, the map clearly shows that its distribution range in Loja Province is restricted to the northern part of the province with exception of the extreme north which is too dry. The western part of the province, with temperatures above 18 °C, is basically too warm. Nevertheless, similar suitable edaphoclimatic conditions as those coinciding with the present distribution range, and thus cultivation possibilities, can be found in other parts of the province. An important part of Loja Province, 79.9 %, is considered as not suitable for *V. cundinamarcensis* (Table 5.5), making it the less-suited highland papaya for Loja Province.

V. stipulata, on the contrary, has a very broad distribution range, although it often occurs with low plant frequencies. Only 29.4 % of the province is considered unsuited to *V. stipulata*, whereas 16.7 % of the area shows edaphoclimatic characteristics that are often found with *V. stipulata* plants. The central part of the province is not suited due to lack of precipitation while the northern part is too cold and too dry. The absence of overlap in the zones of *V. stipulata* and *V. cundinamarcensis* is remarkable. Both species occur in different parts of Loja Province. In the north-eastern part of the province a small zone seems to hold conditions suited both for *V. stipulata* and *V. cundinamarcensis* but only *V. cundinamarcensis* has been recorded there so far.

V. × heilbornii and more specifically *V. × heilbornii* 'Babacó' show intermediate characteristics and do overlap with *V. stipulata* and *V. cundinamarcensis*. These highland papayas can be found all over the province at higher altitudes, with the notable exception of the northern part which is too dry.

All frequency maps clearly show the influence of temperature (altitude), making this, with exception of the extreme north, the most important limiting factor for growth and production.

5.2.2.3 CONCLUSIONS

Due to the lack on information regarding cultivation requirements, especially with regard to *V. stipulata* and *V. cundinamarcensis*, edaphoclimatic conditions on sites with mostly wild or tolerated plants were assessed in order to get an idea about soil and climate characteristics useful for future cultivation.

V. stipulata, although restricted to southern Ecuador and northern Peru, is clearly the most widely distributed highland papaya in Loja province. It can be found from 1,000 up to 2,900 masl, a much wider altitude range than described by Badillo (1993), at mean annual temperatures from 10 to 26 °C in all types of soils. The species occurs very frequently in heavy acid soils at temperatures between 14 and 18 °C and annual precipitation between 1,000 and 1,300 mm. Only 29.4 % of Loja province is not suited for *V. stipulata* although a limited area (4.5 %) is considered as well suited.

V. cundinamarcensis, widely spread at higher altitudes on the South American continent, is limited to the northern part of the province, although some small scattered patches with similar edaphoclimatic conditions can be found throughout the province. In contrast to *V. stipulata*, the range of these conditions is quite small with an optimum in sandy acid soils at temperatures between 12 - 14 °C and annual precipitation between 800 - 1,000 mm. Almost 80 % of Loja Province is evaluated as not suited for *V. cundinamarcensis*. No overlap exists in the distribution zone of *V. stipulata* and *V. cundinamarcensis*. This may be due to different soil preferences.

V. × heilbornii and its specific cultivar *V. × heilbornii* 'Babacó' show a behaviour which lies intermediate between their supposed progenitors *V. stipulata* and *V. cundinamarcensis*. The distribution range overlaps that of both species and plants are often found together with or *V. stipulata* or *V. cundinamarcensis*. This wide distribution range results in a broad edaphoclimatic adaptability. Although maximum range values are less extreme than in the case of *V. stipulata*, occurrence frequencies are more evenly distributed over the different edaphoclimatic classes. In 19.3 % of Loja Province *V. × heilbornii* is found frequently.

Comparing the Overall Frequency Maps with the relief map (Figure 1.3) it is clear that the areas with high frequencies are mainly limited by contour lines. Edaphoclimatic conditions, especially temperature, in Loja Province are strongly correlated to relief. In terms of the climatic classification of Köppen (Figure 1.6) it can be concluded that the highland papayas *V. cundinamarcensis*, *V. stipulata* and *V. × heilbornii* are mainly found in the temperate climates.

5.2.3 GERMPLASM COLLECTION AND CHARACTERISATION

5.2.3.1 COLLECTION AND *IN SITU* CHARACTERISATION

In situ germplasm characterisation revealed large variability exists at the level of the different accessions, together with some possible differentiating characteristics amongst them. A data exploration, using important characteristics of fruits, flowers and leaves, gives an idea about the variability that exists at the morphological level for the different *Vasconcellea* species. Table 5.6 gives the data range for the different highland papayas.

V. cundinamarcensis can clearly be distinguished from all other species by its pubescence on the abaxial leaf side. This species has an almost round, small fruit that contains numerous seeds. Leaves do not possess stipules. Fruiting *V. cundinamarcensis* can be female or monoecious. A clear difference in flowering, and thus fruiting, can be found between both forms. Female plants tend to have a small flower and fruit number, with flowers and fruits occurring on a short peduncle, while monoecious plants have numerous flowers (with different proportions of male flowers) resulting in long peduncles with numerous fruits.

Table 5.6. Variability of morphological characteristics of *Vasconcellea* species in Loja Province, as observed in the data exploration

| | <i>V. stipulata</i> | <i>V. cundinamarcensis</i> | <i>V. × heilbornii</i> * | <i>V. × heilbornii</i> 'Babacó' |
|--|---------------------------------------|-----------------------------|--|---|
| Fruit Weight (g) | 52.7 – 187.4 | 45.2 – 216.3 | 46.2 – 492.5 | 383.4 – 1269.5 |
| Seed Index | 31 – 114 | 62 – 138 | 0 – 40 | 0 – 1 |
| Relation. Length/Diameter | 1.29 – 3.04 | 1.35 – 1.76 | 1.63 – 2.95 | 2.32 – 3.21 |
| No. of Fruits per Infrutescence | 1.0 – 2.4 | 1.0 – 5.2 | 1.0 – 4.4 | 1.0 |
| Length Fruit Peduncle (cm) | 3.4 – 15.3 | 1.8 – 14.0 | 5.1 – 15.4 | 8.0 – 14.9 |
| Percentage Pulp | 57.4 – 89.3 | 62.4 – 74.8 | 65.5 – 99.7 | 85.3 – 96.7 |
| Soluble Solids (°Brix) | 3.6 – 10.4 | 2.6 – 8.2 | 3.0 – 9.7 | 4.4 – 7.8 |
| Length Stipules (cm) | 0.18 – 1.36 | 0.00 | 0.00 – 0.64 | 0.00 |
| Leaf Pubescence | NO | YES | NO | NO |
| No. of Flowers per Inflorescence | 1.0 – 6.2 | 1.6 – 25.8 | 1.0 – 7.3 | 1.0 |
| Length Inflorescence (cm) | 0.65 – 5.12 | 0.96 – 12.36 | 2.12 – 7.10 | 4.36 – 6.64 |
| Length Flower Petal (cm) | 2.57 – 4.12 | 3.16 – 3.95 | 2.74 – 5.16 | 2.61 – 3.85 |
| Petal Colour | pale yellow – orange (6A – 22A) | pale green (144C – 145C) | pale green – orange (4B – 16A or 144C - 145A) | pale green – yellow (4A – 12A or 145A - 151C) |
| Plant Sex | Male, Female | Male, Female, Monoecious | Female, Monoecious | Female |

* with exception of *V. × heilbornii* 'Babacó'

V. stipulata shows similar fruit characteristics as *V. cundinamarcensis*, i.e. small fruits with high seed content, but fruits tend to be more variable in form. Some plants produce elongated fruits while others yield rounded ones. *V. stipulata* differs largely from *V. cundinamarcensis* at leaf level. *V. stipulata* leaves are not pubescent but all

have small to large stipules. These stipules change into spines as leaves fall. *V. stipulata* shows a strict dioecism.

Babaco (*V. × heilbornii* 'Babacó') is also relatively easily distinguished by its elongated, large seedless fruits and single-flowered inflorescences. Other *V. × heilbornii* species, *V. × heilbornii* var. *chrysopetala* and unclassified varieties, are more difficult to categorise. They tend to be very variable in fruit, flower and leaf characteristics, with intermediate characteristics between *V. cundinamarcensis*, *V. stipulata* and babaco. Leaves sometimes possess stipules but are never pubescent. Fruits vary from small to relatively big, with seed content of 0 up to 40 seeds/100 g fruit. As can be seen on Figures 5.36 and 5.37 some overlap exists between characteristics of *V. stipulata* and *V. × heilbornii* plants. Especially low-seeded fruits of *V. stipulata* with leaves having small stipules cannot be distinguished from *V. × heilbornii* with a relatively high seed content and long stipules.

All *Vasconcellea* accessions show relatively low contents of soluble solids, generally lower than 10 °Brix. This low sugar content forms a constraint for their consumption as fresh fruit and implies a use of the fruits in processed products as juices, jams and conserves, as is generally the case in Loja Province. This use in processed products was one of the reasons for the failure of babaco introduction in other countries as Italy and New Zealand. Consumption of freshly prepared juices, widespread in Ecuador, is not a habit in most countries that could adapt *Vasconcellea* cultivation or could form a target for exporting fruits.

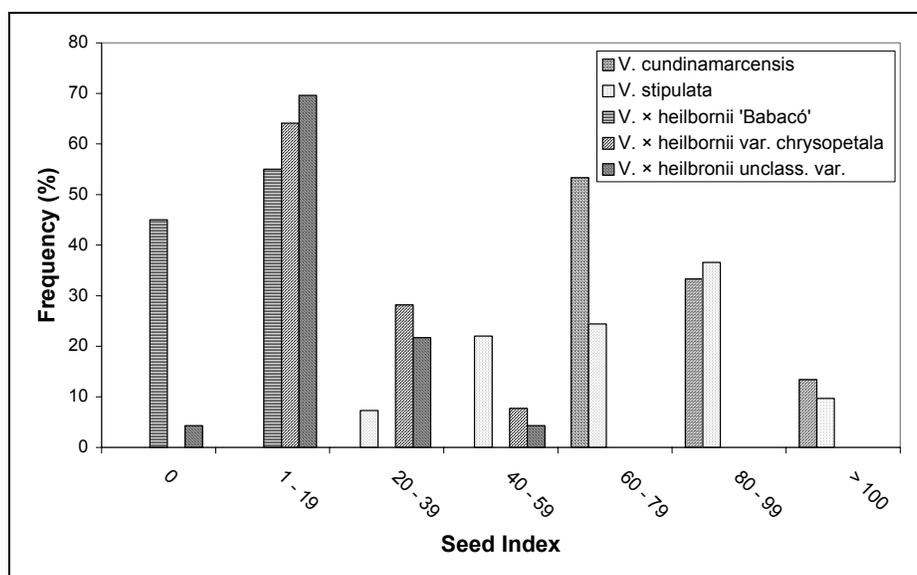


Figure 5.36. Clustered histogram for seed index (no. seeds/100 g fruit) for different *Vasconcellea* species based on characterisation data of 140 accessions from Loja Province

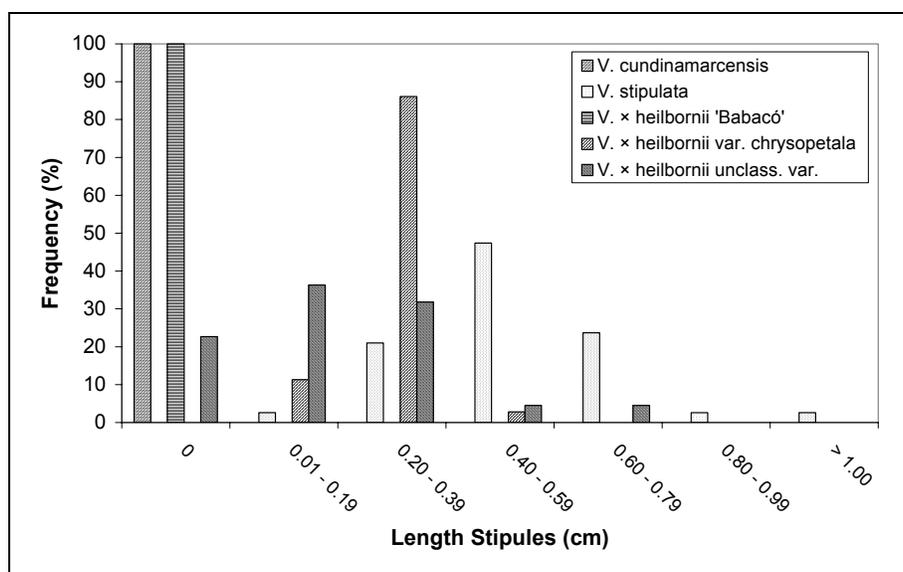


Figure 5.37. Clustered histogram for length of stipules for different *Vasconcellea* species based on characterisation data of 140 accessions from Loja Province

A principal component analysis using the quantitative characterisation data of completely characterised female *Vasconcellea* accessions shows similar results. The first factor explains 40 % (Table 5.7) of variance and consists of characteristics related to seed index, fruit form and weight, pubescence and length of inflorescence and peduncle (Table 5.8). The second factor mainly consists of stipule length and number of flowers and fruits per inflorescence. The PCA plot (Figures 5.38 – 5.39) shows that the first axis differentiates between the smaller, rounder, high-seeded fruits of *V. stipulata* and *V. cundinamarcensis* and the heavier elongated low-seeded fruits of all *V. x heilbornii* accessions. The second axis completely separates the *V. cundinamarcensis* group without stipules, with pubescence and often high flower and fruit numbers per inflorescence. The *V. stipulata* group is also separated by the often large stipules. Again there is overlap between the *V. x heilbornii* and *V. stipulata* that can not be distinguished by the three first components.

Table 5.7. Percentage of variance explained by each factor of the PCA analysis on *Vasconcellea*

| | Variance (%) | Eigenvalue |
|-----------|--------------|------------|
| Factor 1 | 41.7 | 4.582 |
| Factor 2 | 21.3 | 2.343 |
| Factor 3 | 10.7 | 1.179 |
| Factor 4 | 8.4 | 0.926 |
| Factor 5 | 4.8 | 0.533 |
| Factor 6 | 4.0 | 0.438 |
| Factor 7 | 2.7 | 0.299 |
| Factor 8 | 2.3 | 0.255 |
| Factor 9 | 2.0 | 0.227 |
| Factor 10 | 1.3 | 0.140 |

Table 5.8. Contribution of each plant characteristic to each factor of the PCA analysis on *Vasconcellea*

| Characteristic | Factor 1 | Factor 2 | Factor 3 |
|------------------------|----------|----------|----------|
| Seed Index | -0.889 | -0.208 | -0.170 |
| Perc. Pulp | 0.886 | 0.179 | -0.140 |
| Rel. Length/Breadth | 0.850 | -0.099 | -0.166 |
| Fruit Weight | 0.701 | 0.132 | -0.550 |
| Pubescence | -0.644 | 0.490 | -0.425 |
| Length Fruit Peduncle | 0.642 | -0.074 | 0.195 |
| Length Inflorescence | 0.597 | 0.503 | 0.269 |
| No. Flowers/Infloresc. | 0.543 | 0.639 | -0.039 |
| No. Fruits/Infloresc. | -0.483 | 0.641 | 0.214 |
| Length Stipules | -0.205 | -0.769 | 0.414 |
| Length Petal | 0.198 | 0.576 | 0.539 |

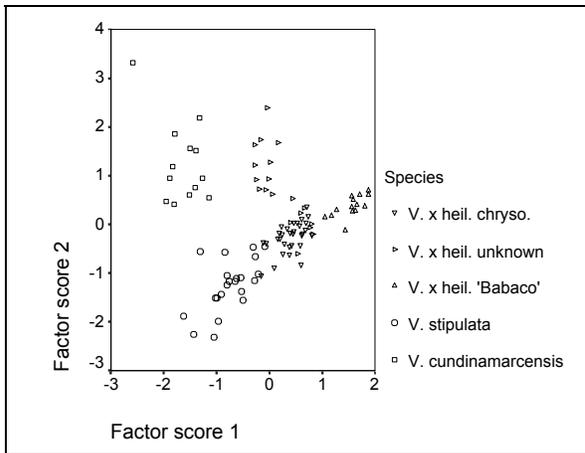


Figure 5.38. PCA of different *Vasconcellea* species according to fruit, leaf and flower characteristics: factors 1 and 2

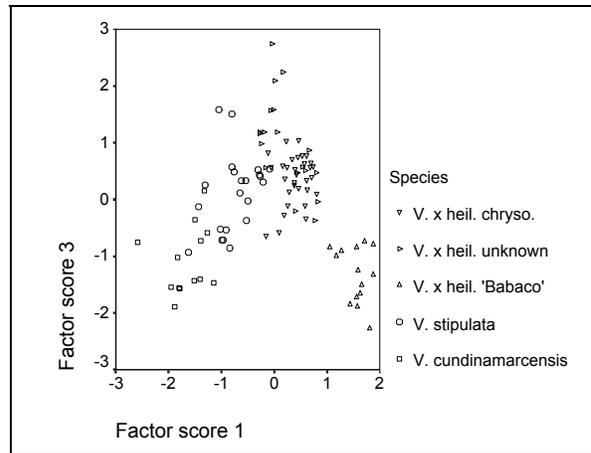


Figure 5.39. PCA of different *Vasconcellea* species according to fruit, leaf and flower characteristics: factors 1 and 3

Finally, a cluster analysis (UPGMA, with Euclidean distances) is used to try to classify the female *Vasconcellea* accessions according to morphological characteristics (Figure 5.40). *V. cundinamarcensis* accessions form a first cluster, with the presence of pubescence separating it from the other *Vasconcellea* accessions.

A second clear cluster is formed by the babaco accessions, which, without any exception, form a separate group, based on the large fruit weight. All other *V. × heilbornii* accessions cluster together with *V. stipulata* accessions. Some small unclassified *V. × heilbornii* form a separate cluster caused by their low fruit weight. The rest of the *V. × heilbornii* and *V. stipulata* accessions do not form well-definable clusters with some *V. × heilbornii* accessions scattered between the *V. stipulata* accessions and vice versa, indicating once more the tight relationship between both species and the difficulties to distinguish them.

5.2.3.2 PRELIMINARY SELECTION

As the main aim of the study is to determine the different species' cultivation potential, an exact taxonomical identification of *Vasconcellea* species was not an objective per se, although it could considerably ease future breeding programmes. Generally, the large genetic and morphologic variability combined with easy hybridisation possibilities offer some promising breeding possibilities. As no selection criteria for *Vasconcellea* species could be found in literature, no detailed germplasm selection could be realised. Nevertheless, some preliminary directions that selection should take, can already be established. It is clear that seedless *V. × heilbornii* accessions will be preferred, especially for future fruit processing. The ethnobotanical study revealed that processed fruit is the main form of consumption of highland papayas in southern Ecuador.

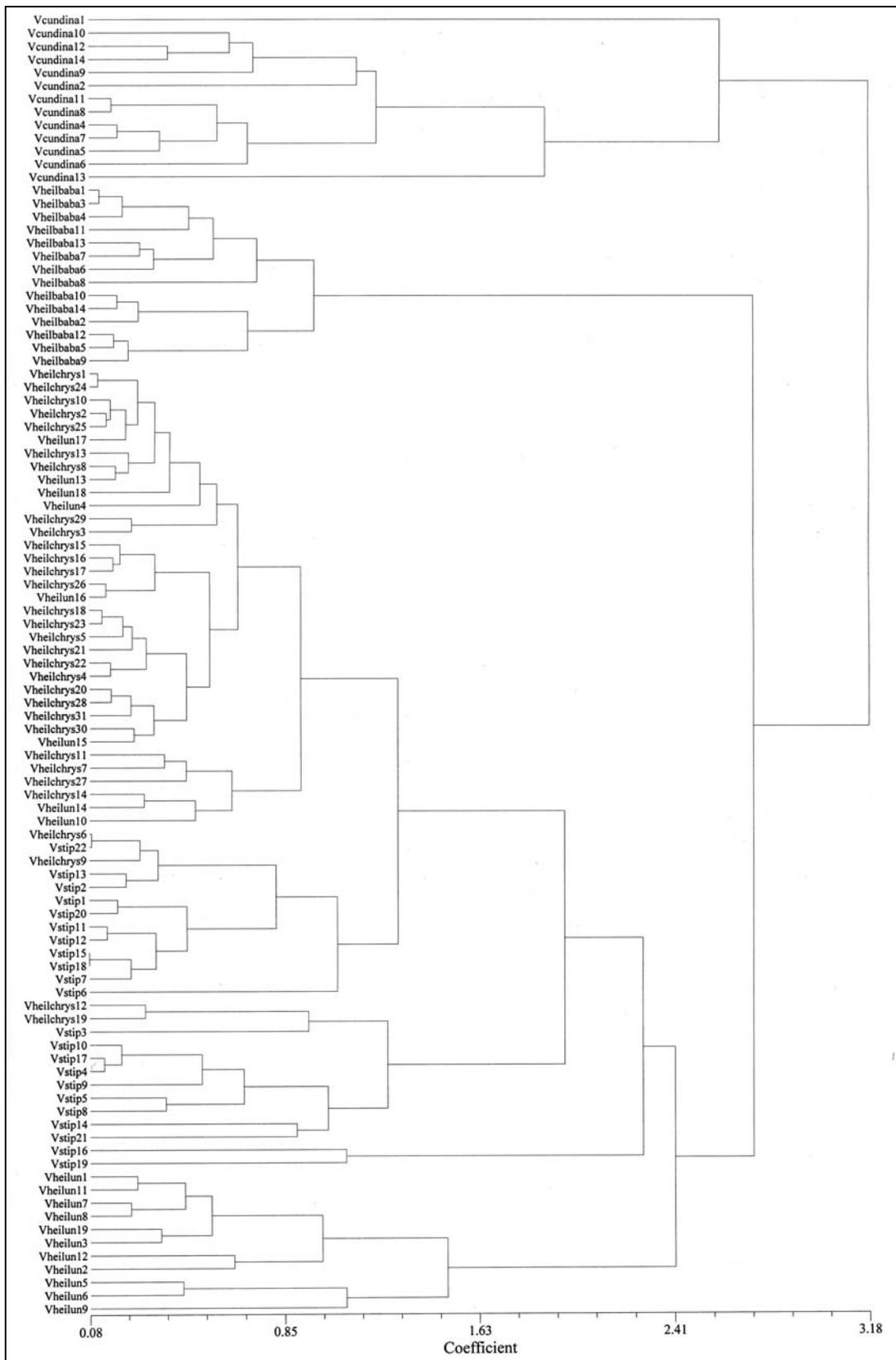


Figure 5.40. Dendrogram, using UPGMA with Euclidean distances, of the 99 *Vasconcellea* accessions using morphological characteristics of fruit, leaf and flower

Soria & Viteri (1999) mention that at this moment the Ecuadorian market prefers large babaco fruits of over one kilogram while international markets demand smaller fruits. Large fruit size was established by other authors as one of the main factors for the failure of babaco introduction in Europe (Ferarra *et al.*, 1993; Hewett, 1993). Small and low-seeded yet unclassified *V. × heilbornii* varieties, as found during the germplasm collection, could form a good alternative for the oversized babaco (Table 5.9 & Figure 5.41). The ethnobotanical study also revealed that in zones where *V. stipulata* is common, e.g. Celica county, it is the preferred *Vasconcellea* species due to its pleasant aroma. As aroma is not a quantifiable characteristic, and very dependent on environmental conditions, it could not be taken into account in the classification. Future selection should be aimed at high performing *V. stipulata* accessions, greatly appreciated locally, whereas small, low-seeded *V. × heilbornii* varieties could be selected for eventual export purposes.

5.2.3.3 CONCLUSIONS

Lack of detailed taxonomical studies and occurrence of continuous natural hybridisation (Horovitz & Jiménez, 1967) make germplasm identification very difficult. Especially the difference between some *V. × heilbornii* and *V. stipulata* accessions remains very difficult. The used identification keys reflect this difficulty. The identification key of 1983 (Badillo, 1983) uses a floristic characteristic. To differentiate both species, the position of corolla lobes in relation to calyx lobes should be opposite in *V. stipulata* and alternate in *V. × heilbornii*. Our flower characterisation showed corolla lobes generally alternate in most *Vasconcellea* species. Only one *V. stipulata* and four *V. × heilbornii* accessions showed an opposite calyx.

In the identification key of 1993 (Badillo, 1993), this floristic characteristic has been eliminated and the only real distinguishing characteristic remaining is the status, wild for *V. stipulata* and cultivated, protected or spontaneous for *V. × heilbornii*.

Genetic studies, using AFLP techniques (Van Droogenbroek *et al.*, 2002) revealed the same difficulties. This study, the most comprehensive of DNA for *Vasconcellea* so far, illustrates and supports the genetic relationships between *V. cundinamarcensis*, *V. stipulata* and their putative hybrid progeny *V. × heilbornii*. *V. × heilbornii* displays a very wide genetic variability. In contrast to the results obtained by the PCA and cluster analysis based on morphological characteristics, analysis based on genetic properties separates *V. × heilbornii* accessions and group them either with *V. cundinamarcensis* or with *V. stipulata*, indicating a continuous process of introgression. A principal coordinate analysis shows some accessions identified as *V. stipulata* grouping close to some accessions of *V. × heilbornii*.

The difficulties in identification and classification can be due to two reasons. The first one is the difficult exact taxonomical identification due to a lack of good differentiating botanical characteristics. The second one deals with the vague border between the two species due to natural hybridisation and repeated backcrossing.

Table 5.9. Preliminary selection of small low-seeded *V. × heilbornii* varieties found in Loja Province that could form an alternative for babaco

| Species | Code | Fruit weight (g) | Seed index |
|---|-----------|------------------|------------|
| <i>V. × heilbornii</i> unknown variety | 1.17(2) | 81.2 | 0.98 |
| <i>V. × heilbornii</i> unclassified variety | CAR65 | 80.4 | 1.49 |
| <i>V. × heilbornii</i> unclassified variety | CAR93 | 424.6 | 5.95 |
| <i>V. × heilbornii</i> unclassified variety | G2.15 | 232.2 | 6.12 |
| <i>V. × heilbornii</i> unclassified variety | CAR105 | 492.5 | 8.26 |
| <i>V. × heilbornii</i> unclassified variety | CAR27 | 278.3 | 8.84 |
| <i>V. × heilbornii</i> unclassified variety | CAR82 | 74.1 | 10.25 |
| <i>V. × heilbornii</i> unclassified variety | CAH5(5) | 73.6 | 17.38 |
| <i>V. × heilbornii</i> unclassified variety | VEERLE | 140.5 | 21.35 |
| <i>V. × heilbornii</i> unclassified variety | 1.17(1) | 110.9 | 40.39 |
| <i>V. × heilbornii</i> var. <i>chrysopetala</i> | CAR48 | 147.0 | 1.36 |
| <i>V. × heilbornii</i> var. <i>chrysopetala</i> | SL2(2) | 122.0 | 6.97 |
| <i>V. × heilbornii</i> var. <i>chrysopetala</i> | CAR53 | 46.2 | 7.94 |
| <i>V. × heilbornii</i> var. <i>chrysopetala</i> | CERA26(1) | 143.9 | 9.73 |
| <i>V. × heilbornii</i> var. <i>chrysopetala</i> | S4#3.2(1) | 224.1 | 8.48 |

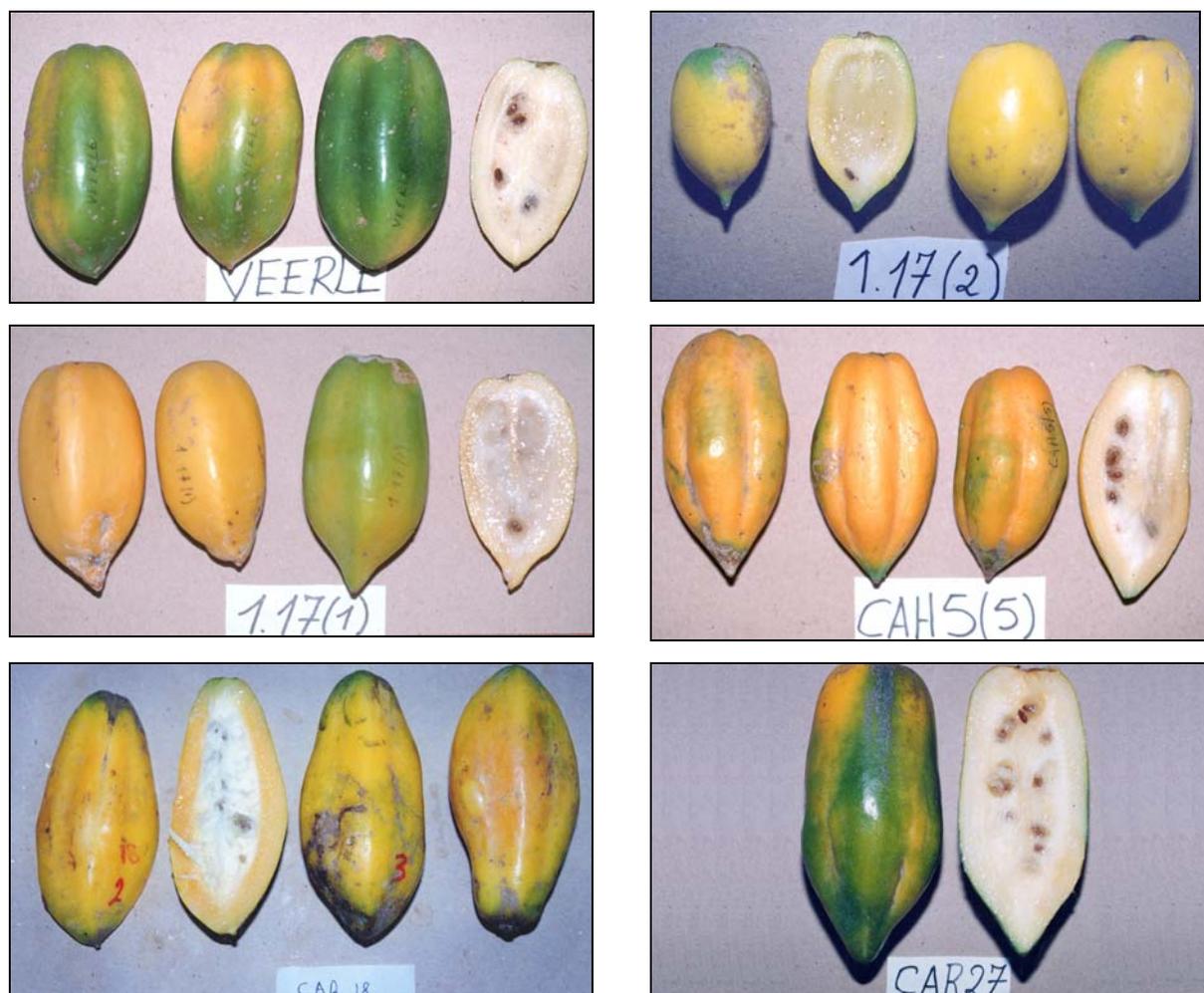


Figure 5.41. Examples of small low-seeded unclassified *V. × heilbornii* varieties found in Loja Province that could form an alternative for *V. × heilbornii* 'Babacó'

The huge variability, especially in the species which are currently preferred by consumers, i.e. *V. stipulata* and *V. × heilbornii*, make it relatively easy to select specific accessions. Hybridisation may even enlarge this variability and make selection of highland papayas relatively easy. Currently, the lack of selection criteria is the main constraint in local breeding programmes. A thorough consumer survey should be realised in order to establish these selection criteria. Furthermore yields and pest and disease susceptibility of promising accessions should be compared under different environmental conditions.

5.2.4 GENERATIVE PROPAGATION

As most research on seed structure and germination of Caricaceae is concentrated on *Carica papaya*, little is known about seed structure and germination dynamics of *Vasconcellea* species. In this chapter the structure of a germinating *Vasconcellea* seed is presented. Germination is studied in detail in order to find methods to improve germination and seed conservation.

5.2.4.1 SEED STRUCTURE

The structure of a germinating *Vasconcellea* seed is very similar to that of a *Carica papaya* seed. Only the sclerotesta is different between both genera. The sclerotesta in *Carica papaya* shows lamelliform protuberances in parallel rows whereas in *Vasconcellea* it is smooth or mostly showing broad irregular protuberances. This is one of the morphologic characteristics used in the distinction between both genera (Badillo, 2000). Besides differences in sclerotesta, all *Vasconcellea* species have a similar structure.

Figures 5.42 and 5.43 show a longitudinal section in two different planes of a *Vasconcellea* seed. The sarcotesta, a mucilaginous tissue covering the complete seed, was removed and is not shown on the figures. The embryo consists of broad flattened cotyledons, which divide the oily endosperm into two parts. The radicle and hypocotyl to the contrary are very small. The seed testa can clearly be divided into a sclerotesta, mesotesta and endotesta. The endotesta is light brown and consists of several compact cell layers. The mesotesta is built up of aerenchymatic and sclerenchymatic cell layers whereas the sclerotesta consists of a cork-like tissue built up from cells that contain cellulose in the cell walls. The vascular bundle and chalaza, which is the attachment site of the integuments and nucellus tissue, can clearly be distinguished at the opposite site of the hilum, indicating an anatropous ovulum. The micropyle could not be distinguished. Figures 5.44 and 5.45 show a germinating seed and a small seedling. Germination of a *Vasconcellea* seed starts with the radicle piercing through the weakened micropyle. At the same time, the seed is swelling through water absorption, and the sclerotesta bursts open through two or three splits. At the base of the hypocotyls, adventive roots are formed and at the end of the main root hair roots are abundantly present.

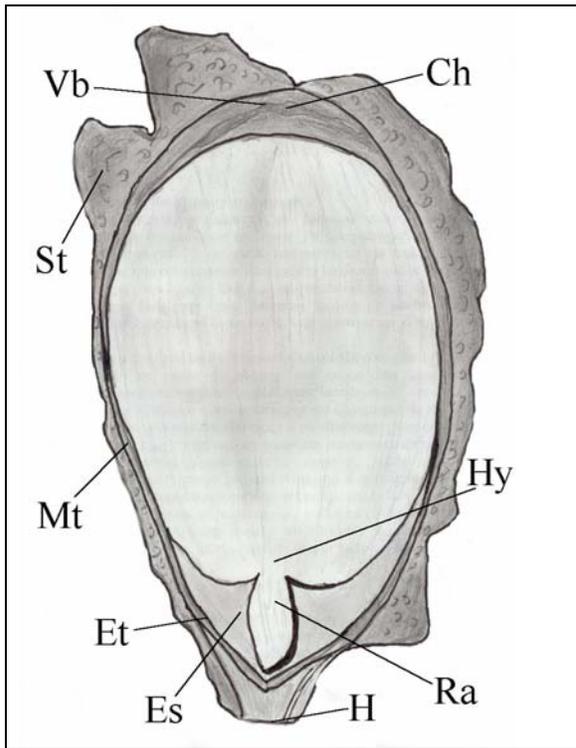


Figure 5.42. Longitudinal, median section of an imbibed *Vasconcellea* seed (parallel to cotyledon plane). Structures: mesotesta (Mt), endotesta (Et), sclerotesta (St), endosperm (Es), hilum (H), radicle (Ra), hypocotyl (Hy), vascular bundle (Vb), chalaza (Ch)

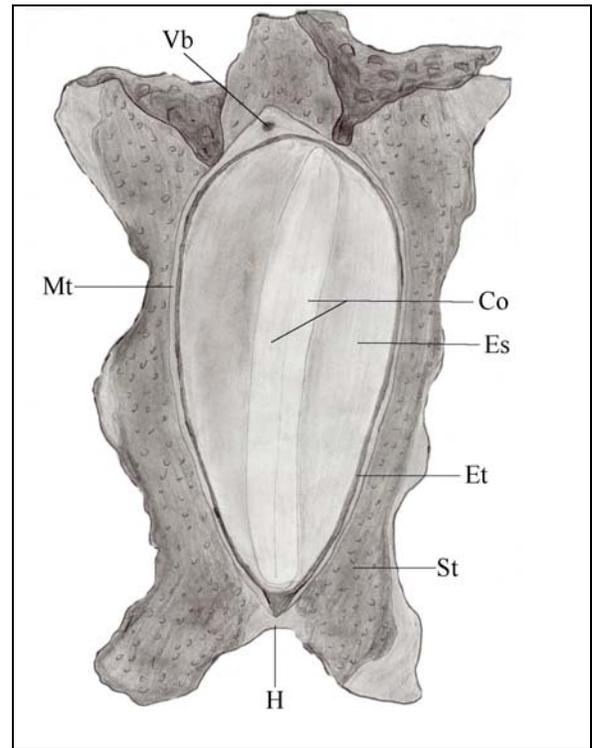


Figure 5.43. Longitudinal, median section of an imbibed *Vasconcellea* seed (transverse on cotyledon plane). Structures: mesotesta (Mt), endotesta (Et), sclerotesta (St), endosperm (Es), hilum (H), cotyledons (Co), vascular bundle (Vb)

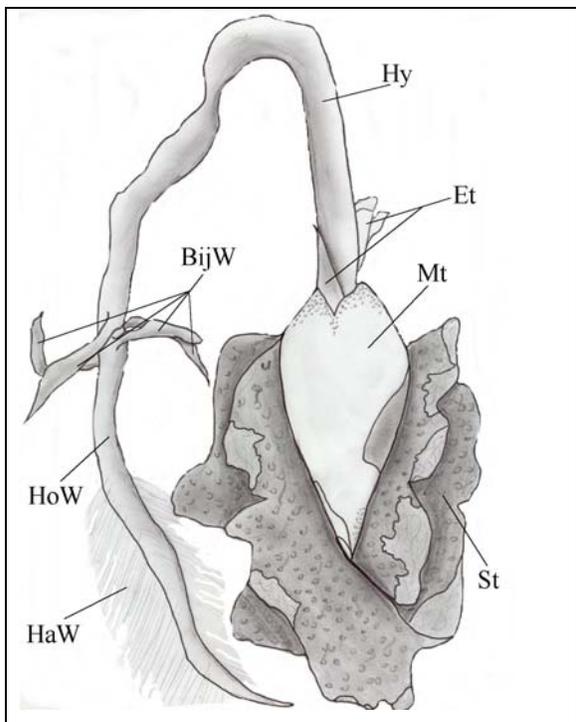


Figure 5.44. External structure of a germinating *Vasconcellea* seed. Structures: mesotesta (Mt), endotesta (Et), sclerotesta (St), main root (HoW), adventive roots (BijW), hairroot (HaW), hypocotyl (Hy)

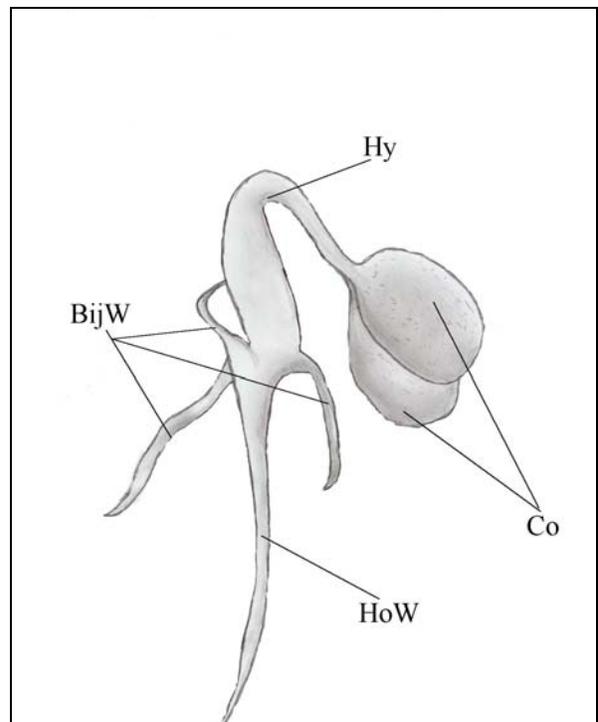


Figure 5.45. External structure of a *Vasconcellea* seedling. Structures: cotyledons (Co), main root (HoW), adventive roots (BijW), hypocotyl (Hy)

5.2.4.2 GERMINATION

Sarcotesta Removal

The mucilaginous sarcotesta forms an obstacle in germination tests and seed conservation. Although this gelatinous layer does not form a physical barrier, proved by the fact that seeds do germinate in natural conditions, removal of this layer has been reported as a method to improve germination in *Carica papaya* (Lange, 1961). Different methods to remove the sarcotesta were applied on *V. cundinamarcensis* seeds. Subsequently, seeds were submitted to a germination test to evaluate the effect on germination of the method applied. Table 5.10 shows the results of the experiment.

Table 5.10. Effect of different sarcotesta removal methods on germination of *V. cundinamarcensis*

| Removal method | Total germination percentage* | |
|---|-------------------------------|------------------|
| | After 51 days | After 253 days |
| Fermentation (21 days) | 56 ^a | 65 ^a |
| 10 % Na ₂ CO ₃ (24 h) | 49 ^a | 64 ^a |
| Control (manual removal) | 40 ^{ab} | 58 ^a |
| 38 % HCl (30 min.) | 24 ^{bc} | 39 ^{ab} |
| 26 % H ₂ SO ₄ (30 min.) | 10 ^c | 21 ^b |

* significant differences (95 % probability) according to Tukey test

Results clearly indicate the negative effect of HCl and H₂SO₄. No significant differences between fermentation, sodium carbonate (Na₂CO₃) and manual removal were found. Taking into account factor time (excluding fermentation) and labour (excluding manual removal) application for 24 hours of a 10 % sodium carbonate solution is considered the best method to remove the sarcotesta, although farmers can easily use the fermentation method.

Improve Germination Using Pre-applied Treatments

Different germination-enhancing pre-applications were applied to seeds of *V. cundinamarcensis*. Results are shown in Table 5.11 and Figure 5.46.

Table 5.11. Effect of different pre-applications on germination of *V. cundinamarcensis*

| Pre-application | Total germination percentage* | |
|---|-------------------------------|-------------------|
| | After 51 days | After 234 days |
| 10,000 ppm GA ₃ (24 h) | 55 ^a | 63 ^a |
| 1,000 ppm GA ₃ (24 h) | 51 ^a | 57 ^{ab} |
| 100 ppm GA ₃ (24 h) | 27 ^b | 44 ^{ab} |
| 0.2 % KNO ₃ (24 h) | 22 ^{bc} | 41 ^{ab} |
| 5 °C (3 days) + H ₂ O (24 h) | 20 ^{bcd} | 33 ^{abc} |
| 10 ppm GA ₃ (24 h) | 12 ^{bcd} | 28 ^{bcd} |
| H ₂ O (24 h) | 11 ^{bcd} | 28 ^{bcd} |
| Partly removal of sclerotesta | 7 ^{cde} | 9 ^{cd} |
| 5 °C (3 days) | 5 ^{cde} | 8 ^{cd} |
| Control | 2 ^{de} | 5 ^{cd} |
| 96 % H ₂ SO ₄ (30') | 0 ^e | 0 ^d |

* significant differences (95 % probability) according to Tukey test

Results - Highland Papayas

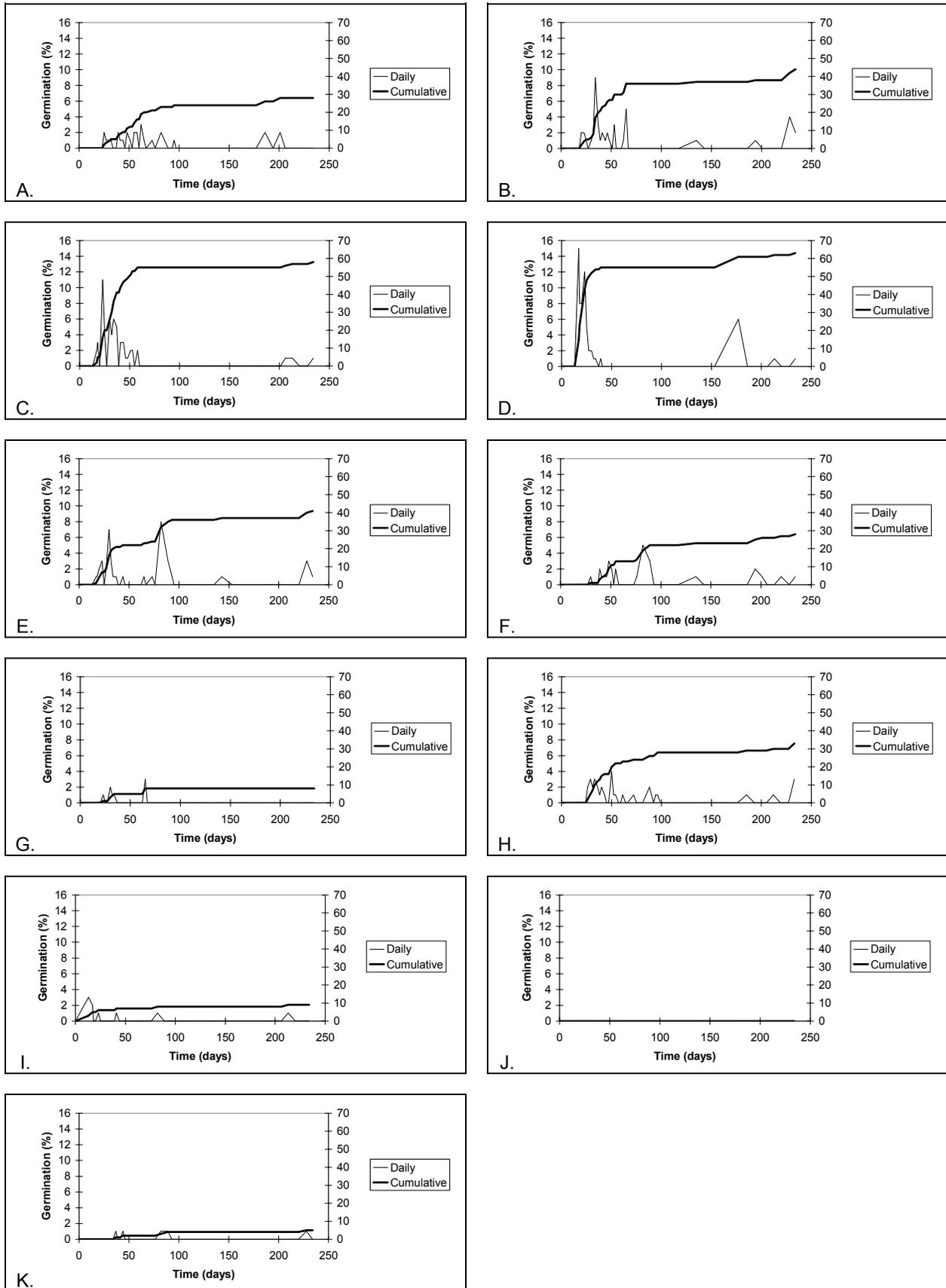


Figure 5.46. Germination of *Vasconcellea* seeds under different pre-applied treatments: A. soaking 24 h in 10 ppm GA₃; B. soaking 24 h in 100 ppm GA₃; C. soaking 24 h in 1,000 ppm GA₃; D. soaking 24 h in 10,000 ppm GA₃; E. soaking 24 h in 0.2 % KNO₃; F. soaking 24 h in H₂O; G. pre-chill 3 days 5 °C; H. pre-chill 3 days 5 °C + soaking 24 h in H₂O; I. chemical scarification (30 min. H₂SO₄); J. partly removal sclerotesta; K. control

Table 5.11 shows germination percentages and their significant differences. Due to irregular germination and often considerable differences between replications within one treatment, only a few significantly different pre-application treatments could be evidenced.

It is clear that pre-application of gibberellic acid (GA₃) has a positive effect on *Vasconcellea* germination, especially at higher concentrations. Germination is fast and attains high total germination percentages. No significant differences were found between 1,000 ppm and 10,000 ppm. Considering economical arguments, a pre-application of 1,000 ppm must be considered as an optimal treatment to fasten and maximalise germination. Based on the obtained results, a pre-application of 1,000 ppm gibberellic acid was used to improve germination of *Vasoncellea* species at the 'Universidad Nacional de Loja' without noting any adverse effects on seedling growth. Potassium nitrate, another typical dormancy-breaking chemical, also has a positive effect on germination. Taking into account that local farmers are not able to obtain gibberellic acid nor potassium nitrate, soaking in water for 24 hours, if necessary combined with a pre-chill, can be used. The germination curves show better germination for this pre-application than the control. However, statistical analysis does not show any significant effect.

The curves show that most germination occurs within the first 100 days. During this period however, germination can be very irregular, except at high GA₃ concentrations. Striking is the very poor germination in the control were no pre-application treatment was used. This illustrates the sometimes difficult germination in *Vasconcellea*. Sulphuric acid clearly is too strong for the corky seeds.

As the results of *V. cundinamarcensis* germination presented in Table 5.10 were obtained on a seeds originating from one accession, and the results presented in Table 5.11 are based on a mixture of *V. cundinamarcensis* accessions, both tables can not be compared, due to the big differences in germination within the species. The low germination obtained in the control in Table 5.11 contrasts with the relatively high germination percentage (Table 5.10) obtained in the first experiment, without application of gibberellic acid and can only illustrate the huge differences in germination within the species.

Germination of Different Species and Accessions

Different accessions of *V. cundinamarcensis*, *V. stipulata* and *V. × heilbornii* were submitted to a germination test. Seeds of *Carica papaya* were included as reference. Seeds were collected from female and monoecious plants in case of *V. cundinamarcensis* and female and hermaphrodite in case of *C. papaya*. Other seeds which had been conserved during 2 years at 3 °C, were also included to study the effect of storage.

Figure 5.47 shows the germination curves of the different accessions with and without application of gibberellic acid.

Results - Highland Papayas

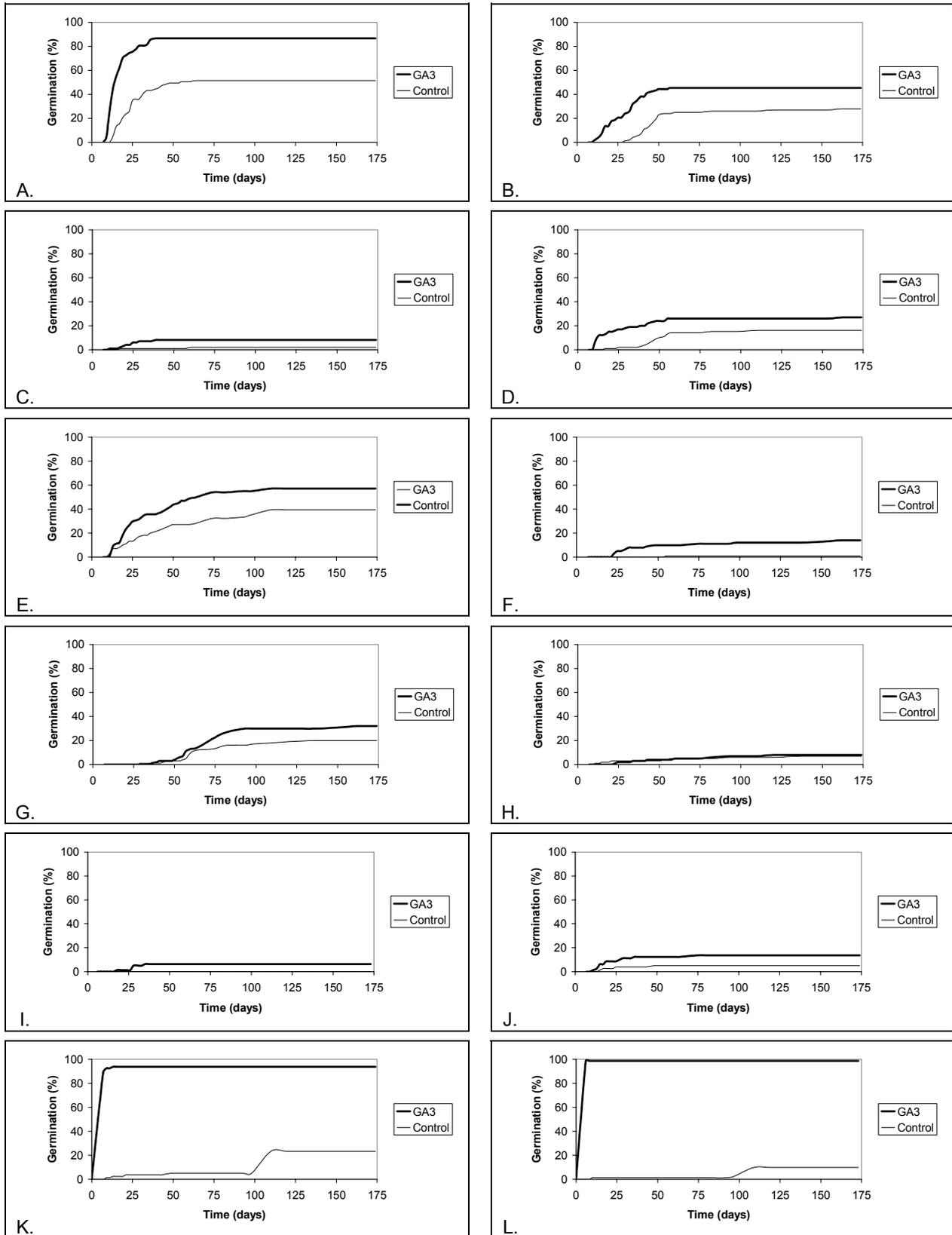


Figure 5.47. Germination of seeds of different *Vasconcellea* accessions and species under control conditions and after pre-application of GA₃: A. fresh *V. cundinamarcensis* seeds, female plant (fem. 1); B. fresh *V. cundinamarcensis* seeds, female plant (fem. 2); C. fresh *V. cundinamarcensis* seeds, monoecious plant (mon. 1); D. fresh *V. cundinamarcensis* seeds, monoecious plant (mon.2); E. two-years old *V. cundinamarcensis* seeds, female plant (fem. 3); F. two-years old *V. cundinamarcensis* seeds, monoecious plant (mon. 3); G. fresh *V. stipulata* seeds (acc. 1); H. two-years old *V. stipulata* seeds (acc. 4); I. fresh *V. × heilbornii* seeds (acc. 1); J. two-years old *V. × heilbornii* seeds (acc. 4); K. fresh *C. papaya* seeds, female plant (fem. 1); L. fresh *C. papaya* seeds, hermaphrodite plant (her. 1)

Application of 1,000 ppm GA₃ has a positive effect on germination of all accessions. Especially the effect on *C. papaya* is striking. In the control, little germination occurs during the first 100 days whereas application of gibberellic acid results in an almost complete germination within the first 10 days. This germination pattern found in *C. papaya* is different from that of *Vasconcellea* accessions. In *Vasconcellea* germination is slow, rather erratic and sometimes very low.

V. cundinamarcensis shows the best germination. Statistical analysis (Table 5.12) shows significant differences between the two female fresh seeds accessions whereas no significant difference could be found between a fresh and a conserved accession. This indicates the large variability that exists in germination of *Vasconcellea* species. This is probably an indication of the genetic variability. Seed conservation at low temperatures during 2 years does not seem to pose a problem. Monoecious *V. cundinamarcensis* accessions show significantly lower germination, much more pronounced than in hermaphrodite *C. papaya*. Once more, no differences between fresh seeds and conserved seeds could be found for the monoecious accessions.

Table 5.12. Germination of different *Vasconcellea cundinamarcensis* accessions using 1,000 ppm gibberellic acid

| Accession | Total germination percentage* | |
|-----------------------------------|-------------------------------|------------------|
| | After 50 days | After 174 days |
| Female 1, fresh seeds | 87 ^a | 87 ^a |
| Female 2, fresh seeds | 44 ^b | 45 ^b |
| Female 3, two-years old seeds | 27 ^{bc} | 39 ^b |
| Monoecious 2, fresh seeds | 24 ^{bc} | 27 ^{bc} |
| Monoecious 3, two-years old seeds | 10 ^c | 14 ^c |
| Monoecious 1, fresh seeds | 8 ^c | 8 ^c |

* significant differences (95 % probability) according to Tukey test

V. stipulata shows very poor germination (Table 5.13) with a maximum of 32 % germination for one accession. Accession 2, where seeds were extracted from fresh fruits, did not germinate. After 174 days, significant differences can be found between the accessions, while once more no differences between fresh and conserved seeds can be evidenced.

Table 5.13. Germination of different female *Vasconcellea stipulata* accessions using 1,000 ppm gibberellic acid

| Accession | Total germination percentage* | |
|-------------------------------|-------------------------------|-----------------|
| | After 50 days | After 174 days |
| Accession 1, fresh seeds | 4 ^a | 32 ^a |
| Accession 4, 2 year old seeds | 4 ^a | 8 ^b |
| Accession 3, fresh seeds | 2 ^a | 2 ^c |
| Accession 2, fresh seeds | 0 ^a | 0 ^c |

* significant differences (95 % probability) according to Tukey test

V. × heilbornii (Table 5.14) shows even lower germination than *V. stipulata* with a maximum germination of 14 %. Once more differences between accessions are more important than the conservation status of the seeds. *V. × heilbornii* is the supposed

result of a natural hybridisation and the often seedless fruits are generally formed by parthenocarpy. The fact that the few remaining seeds still possess some germinative power was surprising and offers some interesting possibilities for plant breeding purposes.

Table 5.14. Germination of different female *Vasconcellea* × *heilbornii* accessions using 1,000 ppm gibberellic acid

| Accession | Total germination percentage* | |
|----------------------------------|-------------------------------|-----------------|
| | After 50 days | After 174 days |
| Accession 4, two-years old seeds | 12 ^a | 14 ^a |
| Accession 1, fresh seeds | 6 ^{ab} | 6 ^{ab} |
| Accession 2, fresh seeds | 1 ^b | 1 ^b |
| Accession 3, fresh seeds | 0 ^b | 3 ^b |

* significant differences (95 % probability) according to Tukey test

5.2.4.3 CONCLUSIONS

Differences between *Carica papaya* and the recently re-established genus *Vasconcellea* can also be found at the level of seed structure and germination. At seed level, the protuberances show a more regular pattern in *Carica*. Germination is much higher and regular in *Carica* when gibberellic acid is pre-applied. Nevertheless, Bertocci *et al.* (1997) mention a germination of only 15 % in papaya when applying 300 ppm GA₃. Our experience showed that a higher dose of 1,000 ppm GA₃ leads to a germination of 99 % within 10 days.

The gelatinous sarcotesta, present in both *Carica* and *Vasconcellea*, forms a hindrance in seed conservation and germination experiments. This sarcotesta can be removed by fermentation or application of Na₂CO₃ without influencing the germination potential. Application of Na₂CO₃ is less time-consuming and is considered as the preferred methodology. CAF (1992) mentions a fermentation of 48 h in water as a method to remove the sarcotesta. Our experiences however, did find fermentation for 48 hours not sufficient to remove the sarcotesta and suggest a fermentation of 21 days in order to remove the fermented sarcotesta.

Germination of seed of *Vasconcellea* species, often irregular, slow and erratic, can be improved considerably by using gibberellic acid. In some cases, this can lead to germination rates up to 25 times higher than under normal conditions. Taking into account the financial aspect, pre-application of 1,000 ppm GA₃ during 24 h is considered as optimal. For low resource farmers, an application of H₂O, if possible combined with a pre-chill at 5 °C, can improve germination, although the differences with the control were not significant. Ellis *et al.* (1985b) mention applications of 10 – 1,000 ppm GA₃ as partly successful dormancy breaking treatments in *Carica papaya*. Our experience showed a concentration of 10 GA₃ as not significant for improving *Vasconcellea* germination. Alarcon *et al.* (1997) mention a germination for *V. cundinamarcensis* that rose from 36 % in recently extracted seeds to 87 % in seeds conserved for 4 months at room temperature.

Interspecific variability of germination was very high. This probably indicates a considerable genetic diversity. *V. stipulata* and *V. × heilbornii* showed very low germination, seldom higher than 10 %. This is confirmed by Jiménez *et al.* (1998) who found a germination of 0 and 5 % in *V. stipulata* and 5 % in *V. × heilbornii*. In *V. cundinamarcensis* an important difference was found between seed origins. Seeds originating from female plants tend to have a much better germination, up to 87 %, than the ones from monoecious accessions which had a germination of maximum 27 %. Jiménez *et al.* (1998) found similar results, 68 % germination in female plants and only 16 % in monoecious plants after 48 days. An explanation for this phenomenon could not be found. No significant differences between fresh seeds and seed conserved for 2 years at 4 °C could be found, giving an indication about conservation possibilities for *Vasconcellea* seeds. Alarcon *et al.* (1997) however, report a decrease in germination to only 2 % after conservation for 12 months at room temperature for *V. cundinamarcensis*, illustrating the importance of a good conservation strategy based on temperature (and humidity).

The fact that some seeds of *V. × heilbornii* do germinate, although erratic and only significant with application of gibberellic acid, is surprising. *V. × heilbornii* fruits are supposed to show a high degree of parthenocarpy (Badillo, 1993) resulting in seedless fruits. However, seeds may be formed by pollination with pollen from other *Vasconcellea* species. Horovitz & Jiménez (1967) already described the natural presence of low-seeded accessions beside parthenocarpic seedless *V. × heilbornii* accessions. They emphasise the importance of the pollen source, excluding apomixis, and confirm the interesting perspectives of these backcrosses. Our research found that applying gibberellic acid to these *V. × heilbornii* seeds considerably improves germination and must be considered as an important tool in *Vasconcellea* breeding.

5.2.5 ALTERNATIVE USES

Besides its potential as a fruit crop, *Carica papaya*, is also an important source of papain, which is extracted from the latex of green fruits. The term papain is commonly used to describe the extracted product, although in reality it consists of a group of proteolytic enzymes. As *Vasconcellea* species are closely related with *Carica papaya*, proteolytic activity of *Vasconcellea* latex was submitted to a biochemical analysis.

Table 5.15 shows the proteolytic activity of dried latex of different *Vasconcellea* species with papaya as reference. *Vasconcellea stipulata* has a proteolytic activity of approximately ten times higher. The unclassified varieties of *V. × heilbornii* show similar values with a maximum of 177 mU BAPNA/mg dried latex, which is 17 times higher than the papaya reference. *V. × heilbornii* 'Babacó' and *V. cundinamarcensis* both show activities of on average 50 mU BAPNA/mg dried latex. These are lower values than those obtained for *V. stipulata* and the unclassified varieties of *V. × heilbornii*, but still a fivefold of the activity of papaya latex. No differences were found

between female and monoecious *V. cundinamarcensis* accessions.

Table 5.15. Proteolytic activity of dried latex of different *Vasconcellea* species and the reference *Carica papaya*

| Species | Accession | Proteolytic activity (mU BAPNA/ mg dried latex) |
|---|--------------------|--|
| <i>Carica papaya</i> | Accession 1 | 10.1 |
| | Accession 2 | 10.4 |
| <i>V. cundinamarcensis</i> | Accession 1 (fem.) | 52.2 |
| | Accession 2 (fem.) | 48.0 |
| | Accession 3 (fem.) | 53.9 |
| | Accession 4 (fem.) | 57.0 |
| | Accession 5 (mon.) | 51.0 |
| | Accession 6 (mon.) | 56.7 |
| <i>V. stipulata</i> | Accession 1 | 127.6 |
| | Accession 2 | 121.0 |
| | Accession 3 | 129.4 |
| | Accession 4 | 93.0 |
| | Accession 5 | 102.6 |
| | Accession 6 | 102.8 |
| | Accession 7 | 93.8 |
| | Accession 8 | 106.9 |
| <i>V. × heilbornii</i> | | |
| <i>V. × heilbornii</i> 'Babacó' | Accession 1 | 73.1 |
| | Accession 2 | 38.1 |
| | Accession 3 | 43.4 |
| <i>V. × heilbornii</i> var. <i>chrysopetala</i> | Accession 1 | 127.6 |
| <i>V. × heilbornii</i> unclassified variety | Accession 1 | 112.5 |
| | Accession 2 | 105.7 |
| | Accession 3 | 109.4 |
| | Accession 4 | 97.8 |
| | Accession 5 | 177.7 |
| | Accession 6 | 113.9 |

These results prove that highland papayas also offer promising possibilities for the extraction of papain. Nevertheless these results must only be used as a first indication and further research and confirmation of the trends observed are necessary. Due to the lack of proper *Vasconcellea* cultivation, no data on latex yield per hectare could be made. A look at yield data (Table 5.16) show that one fruit of *Carica papaya* holds more latex but this in a considerably bigger fruit volume.

Table 5.16. Average proteolytic activity, latex yields per fruit and fruit volume for *Vasconcellea* species and the reference *Carica papaya*

| Species | Proteol. act. (mU BAPNA/mg dried latex) | Fresh latex/fruit (g) | Dry latex/fruit (g) | Fruit volume (cm ³) |
|---------------------------------|---|-----------------------------|------------------------|------------------------------------|
| <i>Carica papaya</i> | 10.2 | 14.15 | 3.81 | 22,607 |
| <i>V. cundinamarcensis</i> | 53.1 | 0.95 | 0.30 | 1,103 |
| <i>V. stipulata</i> | 109.7 | 1.75 | 0.55 | 1,089 |
| <i>V. × heilbornii</i> 'Babacó' | 51.5 | 2.01 | 0.60 | 6,162 |
| <i>V. × heilbornii</i> * | 120.6 | 1.63 | 0.47 | 1,029 |

* with exception of *V. × heilbornii* 'Babacó'

Taking into account an average *C. papaya* fruit is approximately 20 times bigger than an average *Vasconcellea* fruit, dry latex yield is only 6 to 7 times higher than that of *V. stipulata* or the unclassified varieties of *V. × heilbornii*. *Vasconcellea* fruits are thus an important source of dry latex with considerable proteolytic activity. The few data on fruit yields available for *Vasconcellea* fruits indicate similar yields as for *Carica papaya*. It can be presumed that, taking into account the clearly higher proteolytic activity, *Vasconcellea* species can form a good alternative for papain extraction. Further research is also needed on the process of latex extraction, which is easier on the bigger papaya fruits, and the exact enzymatic composition of the *Vasconcellea* latex.

Results on proteolytic activity of *V. cundinamarcensis* confirm earlier findings by Baeza *et al.* (1990) who found a five- to eight-fold higher proteolytic activity in *V. cundinamarcensis* latex than in *C. papaya* latex. Dhuique Mayer *et al.* (2001) however, report proteolytic activity of babaco latex to be equivalent or slightly higher than that of papayas. Our results however, show that babaco latex shows a four to seven times higher activity.

5.2.6 OVERALL DISCUSSION AND CONCLUSIONS

Southern Ecuador is an important centre of diversification of highland papayas. Besides the well-known and cultivated *Vasconcellea × heilbornii* 'Babacó', other species and varieties are only known locally, leading to considerable differences on knowledge and cultivation within Loja Province. Concerning local nomenclature, people do not make any distinction between the different species and generally consider all highland papayas, with exception of the larger babaco, as 'toronche'. Striking is the notable preference for *V. stipulata*, which is preferred over babaco especially in areas where the species is clearly present. Despite this preference, cultivation practices and commercialisation of *V. stipulata*, and of *V. cundinamarcensis*, are almost non-existent. Locally, most highland papayas are used in processed products such as juices and jams. This study also showed that within a rather small area as Loja Province, knowledge about highland papayas is not only very locally determined, but is also decreasing with decreasing age of respondents. As the loss of knowledge leads to the loss of use and hence crops are forgotten, this decrease in knowledge makes *Vasconcellea* species very vulnerable to genetic erosion.

Loja Province shows areas that are evaluated as very suited for growing *Vasconcellea* species, making it an ideal centre for cultivation of and research on these fruits. This study assessed the climate and soil preferences of the different *Vasconcellea* species and preliminary determined the best cultivation zones for Loja Province.

Morphologically, *Vasconcellea* species, especially *V. stipulata* and *V. × heilbornii*, tend to be very variable and often difficult to classify. This variability offers however, a wide range of possibilities for future selection programmes.

Application of sodium carbonate was found to be the best method to remove the gelatinous sarcotesta, which is essential for seed conservation and management. Germination of *Vasconcellea* species improves considerably when using gibberellic acid through a pre-application of 1,000 ppm during 24 h. Nevertheless, germination still can remain very low and erratic, especially in *V. stipulata* and *V. × heilbornii*. Despite this low germination, generative propagation may be an interesting tool in breeding programmes as, due to the large variability combined with natural hybridisation and backcrossing, progeny can show interesting properties.

The high papain content of the latex of highland papayas, which is much higher than that of *C. papaya*, also offers a potential for future cultivation. Especially the latex of *V. stipulata* and of some as yet unclassified *V. × heilbornii* varieties show very high proteolytic activities. The preliminary results are very promising but further research, especially on exact enzymatic composition and yields, is required.

Soil and climate preferences of *V. × heilbornii* are intermediate between *V. cundinamarcensis* and *V. stipulata*, its supposed progenitors. Looking at results of other research however, *V. × heilbornii* seems to show more resemblance with *V. stipulata*. This is especially the case in the morphological characterisation where some accessions could not be differentiated, but also at the level of germination and papain content. Studies at DNA level, using AFLP analysis, on the same material (Van Droogenbroek *et al.*, 2002) confirm this close affiliation of some *V. × heilbornii* accessions with *V. stipulata*. Further study on genetic and taxonomical levels should clarify the exact position of *V. × heilbornii* and provide exact identification keys.

It is the task of local research institutes and organisations to continue the research on these promising fruits based on the preliminary results obtained by this study. Future activities also should include conservation actions and campaigns to increase interest and cultivation by local farmers. Especially *V. stipulata* and the large variability of unclassified, mostly low-seeded *V. × heilbornii* varieties must be targeted. These species are endemic and little studied, but at the same time show the biggest variability, which can even be widened using seed propagation. They also offer the best possibilities as fruit or as a source of papain and are widely accepted by the local population that is familiar with them. At this moment however, they are not or only locally commercialised.

6. GENERAL CONCLUSIONS

Southern Ecuador offers an unequalled variety of ecological zones resulting in a rich biodiversity. Nevertheless, the economic situation, especially that of resource poor farmers in the Andes, is very problematic. It has even worsened during the last years due to natural disasters as the 'El Niño' phenomenon and economic mismanagement. In spite of the very narrow agricultural portfolio of Ecuador, dominated mainly by banana, a survey of the vast and unique floral diversity of this country has revealed the presence of 334 wild edible species, mainly fruits, in southern Ecuador (Van den Eynden *et al.*, 1999).

The present research work studied the potential of one species and one genus out of these 334 species, i.e. cherimoya (*Annona cherimola* Mill.) and highland papayas (*Vasconcellea* spp.). These species are considered to be promising at local level while internationally the selected species are considered as under-utilised fruit species that need and warrant further investigation (National Research Council, 1989; FAO, 1992; Castillo, 1995; Campbell, 1996; Libreros & Lastra, 1999).

The study showed that both cherimoya and highland papayas indeed demonstrate a lot of unexploited potential but also evidenced a high risk of genetic erosion. During a visit in 2001 some rare *Vasconcellea* × *heilbornii* accessions that had been accessed during earlier visits had already been cut by local farmers. An ethnobotanical study described the local knowledge and actual use patterns, and revealed a striking lack of adapted cultivation practices and on-farm selection, and decreasing farmer knowledge. By collecting and using data from wild or semi-wild plants, a study on crop ecology indicated the species' preferences on climate and soil conditions. Using a GIS, these edaphoclimatic preferences lead to the delimitation of potential cultivation zones in Loja Province. Germplasm collection, characterisation and selection illustrated the large morphological variability and, in the case of highland papayas the need for a detailed taxonomic study. Preliminary selection of some promising accessions showed that indeed local fruits have a lot of cultivation potential that is not exploited for the moment. Some cherimoya accessions are superior or at least comparable to commercial cultivars currently grown in cherimoya exporting countries. A study on generative propagation, a technique that would promote future seed conservation and management programmes, evidenced erratic germination and the positive effect of gibberellic acid (GA_3) on both cherimoya and highland papayas. In the latter case, these germination enhancement methods could form a useful tool in future selection programmes. *Vasconcellea* species show an additional potential as an important source of proteolytic enzymes, whose activity was shown to be up to 17 times higher than that of *Carica papaya*, which is currently used worldwide.

This was the first long-term extensive study on these species in their centre of origin. It aimed at widening the knowledge on and determining the potential of these species. The results obtained should raise interest with local scientists, NGOs and international researchers. As the work on under-utilised species ultimately aims at poverty alleviation through income generation, the efforts to promote a specific crop should have the desired effects with respect to the interests and needs of poor

farmers, maintenance of diversity and strengthening sustainability of production (Padulosi *et al.*, 2001). On international level, special care must be taken to focus not exclusively on species and crop development, as happened with kiwi, but also to pay attention to the local socio-economic situation and production. On a national level a good cooperation between scientists and extensionists, governmental or non-governmental, must be built in order to combine further research with diffusion of results and if possible and/or needed plant material. Finally, conservation of genetic diversity must be ensured through *ex situ*, or preferably *in situ*, conservation activities. Once outstanding accessions are selected, the challenge will be to combine the cultivation of this selected material and the conservation of the germplasm. Complementary conservation strategies (combining *in situ* and *ex situ* conservation) and conservation-through use (achieving conservation through use) are considered strategies that strengthen the farmers' available options in their crop selection and cultivation decisions for income generation and may be used in the case of cherimoya and highland papayas. The Ecuadorian National Institute for Agricultural and Livestock Research (INIAP) would be the appropriate organism to take up the responsibility to oversee and conserve the diversity of these species (both *in situ* and *ex situ*).

Results presented in this study are only preliminary and in a number of cases indicative. Research should be continued to confirm and deepen the results obtained. Special attention should go to further characterisation and evaluation of local germplasm. More accessions should be collected although the priority should be to submit selected accessions to a more detailed evaluation. Particular interest must hereby go to aspects such as yield and resistance to local pests and diseases, which are characteristics that need long-term follow-up. In order to obtain reliable results this characterisation and evaluation should be executed in multi-location collection gardens where different climate and soil characteristics prevail. This should lead to a definitive selection of outstanding local accessions that could be distributed among the local farmers. This introduction of selected fruit plants with local farmers should be accompanied by a thorough follow-up, supervision and instruction on cropping practices. This should and must lead to a raised interest with local farmers. Without this interest, which at this moment is relatively low, all cultivation and introduction attempts are doomed to fail. Once outstanding accessions are selected, limited pre-breeding activities could consist of mass screening of seed progeny, making use of the allogamous character of cherimoya and highland papayas. It is clear that all these activities will require a considerable amount of time and space, and thus a substantial financial input. Vegetative propagation should be another focal point, and should especially concentrate on grafting in cherimoya and propagation by cuttings in highland papayas. Clonal propagation of outstanding accessions will be necessary in order to maintain the obtained qualities and avoid a regression to heterogeneous properties.

Besides the widening of the obtained results in order to fully exploit the existing potential in southern Ecuador other disciplines need to be improved to reach a successful introduction of the crop. The existing local commercialisation channels

form an important constraint for the local farmers and need to be adapted, through an equal sharing of benefits. The post-harvest properties of both cherimoya and highland papayas do not favour long transport and form an important obstacle for an eventual export. Research on optimal conservation conditions (longer shelf life) should be addressed together with the optimisation of the techniques, taking into account the adaptability for local farmers, to obtain processed products as juices and jams.

Recently, participatory approaches for the identification or breeding of improved crop cultivars have gained interest at international level. Participatory varietal selection (PVS) is a rapid and cost-effective way of identifying farmer-preferred cultivars while participatory plant breeding (PPB) includes the farmers in the evaluation of segregating material (Witcombe *et al.*, 1996). Whereas participatory varietal selection was successfully applied, e.g. sorghum in Ethiopia (Mulatu & Belete, 2001), rice in Nepal (Joshi *et al.*, 1997) and pearl millet in Niger (Baidon Forson, 1997), the potentials of participatory plant breeding are still to be explored (Witcombe & Joshi Arun, 1996). These participatory approaches all start from established annual crops and no cases of this methodology were reported using perennial crops. Nevertheless, participatory varietal selection methods could be used in cherimoya and highland papayas to simultaneously raise awareness of the existing potential and select locally preferred varieties.

At this moment and based on the presented results, further research is going on, both nationally and internationally and on a governmental and non-governmental level. In Ecuador, the non-governmental organisation 'Fundación Científica San Francisco' continues research on *Vasconcellea*, with both *in situ* conservation and diffusion of results to local farmers. The Universidad Nacional de Loja, started a programme on cherimoya, financed by the International Plant Genetic Resources Institute (IPGRI) in collaboration with the Ecuadorian National Institute for Agricultural and Livestock Research (INIAP) and researchers from the Research Station 'La Mayora' in Malaga, Spain. INIAP is conducting research on resistance against *Fusarium oxysporum* and *Meloidogyne incognita* on different highland papayas to find a resistant rootstock for the more commercially advanced babaco. Research on taxonomy of *Vasconcellea*, both at morphological and genetic level, started in Ghent University, while the Brussels 'Université Libre' continues biochemical research on the proteolytic enzymes present in *Vasconcellea* latex.

Due to the basic local research circumstances, typical for remote areas in developing countries, the methodologies used in these investigations are mostly simple, needing little outside inputs and may easily be applied in other developing countries or on other crops. Currently the NGO 'Fundación Científica San Francisco' is realising similar research on *Rubus* species in southern Ecuador. *Rubus* species in Southern Ecuador form another interesting taxa, showing a lot of potential though under an increasing risk of genetic erosion and must also be further investigated.

The presented study, which highlights only a few of the 334 native edible plant species present in southern Ecuador has already lead to numerous Ecuadorian and international spin-off projects. However, the challenge still remains to improve the economic situation of the local farmers in southern Ecuador.

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ANNEX

The enclosed CD contains all data files as well as survey forms, graphs, figures and additional pictures of cherimoya and different *Vasconcellea* species that were the result of the research realised by the project '*Conocimientos y Prácticas Culturales sobre los Recursos Fitogenéticos Nativos en el Austro Ecuatoriano*'. The arrangement of the files is similar to the chapters of the Ph.D. text.

- **Subdirectory Study Area**

This subdirectory contains data on the study area as presented in Chapter 2.1 (Literature Review – Study area). It contains mainly maps of Loja Province and climatic data.

| File Type | File extension | File content |
|--------------------------|--|---|
| IDRISI | *.rdc / *.rst *.vdc / *.vtc *.map *.smp | Raster layers (file name indicates content) Vector layers (file name indicates content) Map composition files (file name indicates content) Palette files (file name indicates corresponding raster layer) |
| MS Excel | *.xls | Climate data of some stations in Loja Province with climatograms |
| CorelDraw | *.cdr | Map with county and municipality borders |
| General Graphic files | *.bmp *.wmf | Graphic file of maps (file name indicates content) Graphic file of maps (file name indicates content) |

- **Subdirectory Ethnobotanical Survey**

This subdirectory contains data used in the ethnobotanical survey as presented in Chapters 4.1 (Materials and Methods), 5.1.1 (Cherimoya) and 5.2.1 (Highland Papayas). It contains the questionnaire and the obtained data.

| File Type | File extension | File content |
|--------------------------|----------------|--|
| MS Excel | *.xls | Results of ethnobotanical survey (file name indicates content) |
| MS Word | *.doc *.rtf | Questionnaires of ethnobotanical survey (file name indicates content) Questionnaires of ethnobotanical survey (file name indicates content) |
| SPSS | *.sav | Data of ethnobotanical survey (file name indicates content) |
| Adobe | *.pdf | Questionnaires of ethnobotanical survey (file name indicates content) |
| Coreldraw | *.cdr | Maps (file name indicates content) |
| General Graphic files | *.wmf | Graphic file of maps (file name indicates content) |

- **Subdirectory Crop Ecology**

This subdirectory contains data used in the study on crop ecology as presented in Chapters 4.2 (Materials and Methods), 5.1.2 (Cherimoya) and 5.2.2 (Highland Papayas). It contains all climate and soil data, frequency maps (raw and reclassified) and histograms.

| File Type | File extension | File content |
|--------------------------|---|---|
| IDRISI | *.rdc / *.rst *.vdc / *.vtc *.map *.smp, *.smo | Raster layers (file name indicates content) Vector layers (file name indicates content) Map composition files (file name indicates content) Palette files (file name indicates corresponding raster layer) |
| MS Excel | *.xls | Soil data (derived from soil analysis) and site data (derived from thematic maps) (file name indicates content) |
| SPSS | *.sav *.spo | Soil data (derived from soil analysis) and site data (derived from thematic maps) (file name indicates content) Output (histograms) (file name indicates content) |
| General Graphic files | *.bmp *.jpg | Graphic file of maps (file name indicates content) Graphic file of histograms (file name indicates content) |

- **Subdirectory Germplasm Collection**

This subdirectory contains data used in the germplasm collection, characterisation and selection as presented in Chapters 4.3 (Materials and Methods), 5.1.3 (Cherimoya) and 5.2.3 (Highland Papayas). It contains the descriptors, characterisation data, histograms, boxplots and dendrogram.

| File Type | File extension | File content |
|-----------------------|----------------|--|
| MS Excel | *.xls | Data of germplasm characterisation (file name indicates content) |
| MS Access | *.mdb | Data of germplasm characterisation (file name indicates content) |
| MS Word | *.doc *.rtf | Descriptors of germplasm characterisation (file name indicates content) Descriptors of germplasm characterisation (file name indicates content) |
| SPSS | *.sav *.spo | Characterisation data used in PCA and cluster analysis (file name indicates content) Output (histograms) (file name indicates content) |
| Adobe | *.pdf | Descriptors of germplasm characterisation (file name indicates content) |
| General Graphic files | *.jpg | Graphic file of histograms, boxplots and dendrogram (file name indicates content) |

- **Subdirectory Generative Propagation**

This subdirectory contains data used in the study on generative propagation as presented in Chapters 4.4 (Materials and Methods), 5.1.4 (Cherimoya) and 5.2.4 (Highland Papayas). It contains the figures on seed structure and data of germination tests.

| File Type | File extension | File content |
|-----------------------|----------------|---|
| MS Excel | *.xls | Data of germination tests (file name indicates content) |
| SPSS | *.sav | Data of germination tests (file name indicates content) |
| General Graphic files | *.jpg | Graphic file of figures on seed structure (file name indicates content) |

- **Subdirectory Alternative Uses - Papain**

This subdirectory contains data used in the study on papain content of highland papayas as presented in Chapters 4.5 (Materials and Methods) and 5.2.5. It contains the figures on seed structure and data of germination tests.

| File Type | File extension | File content |
|-----------|----------------|--|
| MS Excel | *.xls | Data of proteolytic activity |
| MS Word | *.doc *.rtf | Data on collection sites Data on collection sites |

- **Subdirectory Photographs**

This subdirectory contains 177 photographs obtained during the study on distribution and potential of cherimoya and highland papayas. It contains images of cherimoya and all collected *Vasconcellea* species.

| File Type | File extension | File content |
|-----------------------|----------------|---|
| General Graphic files | *.jpg | Photographs (file name indicates content) |

- **Subdirectory Manuscript**

This subdirectory contains the complete Ph.D. text in digital format.

| File Type | File extension | File content |
|-----------|----------------|---------------------|
| MS Word | *.doc | Complete Ph.D. text |
| Adobe | *.pdf | Complete Ph.D. text |

CURRICULUM VITAE

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UNIVERSITY STUDIES

2000-2002 : Ph.D in Applied Biological Sciences (Agronomy)
Ph.D. prepared at the Faculty of Agricultural and Applied Biological Sciences, Ghent University

Ph.D. Thesis :

"Study of the cultivation potential of native fruit species in Loja province, Ecuador: cherimoya (*Annona cherimola* Mill.) and the complex of highland papayas (*Vasconcellea* spp.)"

Ph.D. Training Programme :

Applied Molecular Biology and Genetics, Plant Breeding, Applied Plant Taxonomy, Taxonomy of Seed Plants

1989-1994 : Agronomist (M.Sc)
Obtained at Faculty of Agricultural and Applied Biological Sciences, Ghent University

Degrees :

1989-1990 : *Summa Cum Laude* (Highest Distinction)
1990-1991 : *Summa Cum Laude* (Highest Distinction)
1991-1992 : *Magna Cum Laude* (High Distinction)
1992-1993 : *Magna Cum Laude* (High Distinction)
1993-1994 : *Magna Cum Laude* (High Distinction)

M. Sc. Thesis :

"Modelling the Potential Production of de maize (*Zea mays* L.) In the North-West province of Cameroon using a Geographical Information System (GIS)"

| |
|----------------------------------|
| PROFICIENCY AND KNOWLEDGE |
|----------------------------------|

LANGUAGE PROFICIENCY :

Dutch : Excellent (Technical)
English : Excellent (Technical)
French : Very good
Spanish : Excellent (Technical)
German : Fair

SOFTWARE PROFICIENCY :

Geographical Information System (GIS) :
IDRISI32, ARC/INFO 3.4D, ARCVIEW 3.2
Database Management :
MS ACCESS 2000
Statistical Software :
SPSS 10.0, SIGMASTAT 2.0, NTSYSpc 2.1
Webdesign :
MS FRONTPAGE 2000
Non-Specific Software :
WINDOWS 98, MS OFFICE 2000, CORRELDRAW 9.0

MEMBERSHIPS :

“International Society for Horticultural Science” (ISHS)
“Belgian Soil Science Society”

| |
|-------------|
| EXPERIENCES |
|-------------|

WORK EXPERIENCE :

February 2000 – present : Ghent, Belgium

Scientific Assistant at the Department Plant Production, Faculty of Agricultural and Applied Biological Sciences, Ghent University. Preparation Ph.D : “Study of the cultivation potential of native fruit species in Loja province, Ecuador: cherimoya (*Annona cherimola* Mill.) and the complex of highland papayas (*Vasconcellea* spp.)”. Supervision of Belgian and foreign thesis students. Participation in Lectures (Crop Development).

October 1997 – January 2000 : Loja, Ecuador

Scientific Assistant at the Department Plant Production, Faculty of Agricultural and Applied Biological Sciences, Ghent University. Project Manager of the research and development project “Conocimientos y Prácticas Culturales sobre los Recursos Fitogenéticos Nativos en el Austro Ecuatoriano”, financed by the Directorate General of International Cooperation (DGIC, Belgium) and carried out at the “Centro Andino de Tecnología Rural” (CATER) of the National University of Loja, Ecuador. The project focussed on broadening the knowledge (diversity, propagation, crop ecology, cropping systems, ...) of some promising native fruit species (Annonaceae, Caricaceae, Passifloraceae), to use the obtained information to promote the cultivation of the selected fruit species and in this way improve the economic situation of local farmers. The project worked also on formation of local and Belgian scientists and students.

Main activities were :

- collection, characterisation, evaluation and selection of germplasm
- establishment of experimental plots and *in vivo* conservation
- research on vegetative and generative propagation
- research on edaphoclimatic preferences and zoning (using GIS)
- study of possibilities of processing and commercialisation
- introduction with local farmers of obtained knowledge
- organization of « First International Symposium on Cherimoya » (ISHS, Acta Horticulturae 497)
- management of the project « Conocimientos y Prácticas Culturales sobre los Recursos Fitogenéticos Nativos en el Austro Ecuatoriano » (budget 320.000 euro)

September 1995 - September 1997 : Loja, Ecuador

VVOB Expert (Flemish Association for Development Cooperation and Technical Assistance) in the "Centro Andino de Tecnología Rural" (CATER), of the National University of Loja, Loja, Ecuador. Responsible for horticultural research in the project "Conocimientos y Prácticas Culturales sobre los Recursos Fitogenéticos en el Austro Ecuatoriano". Research on the potential and introduction of some native fruit species (Annonaceae, Caricaceae, Passifloraceae).

APPRENTICESHIP :

October 1994 – March 1995 : Peradeniya, Sri Lanka

Apprenticeship realized in the "Weed Science Project" (Ghent University) carried out at the Peradeniya University, Sri Lanka under supervision of Prof. P. Van Damme. The work consisted of a survey on upland and paddy weeds.

SYMPOSIUMS - CONGRESSES - COURSES :

- May 8, 1995 : "47th International Symposium on Crop Protection", Ghent, Belgium.
Poster: "Weed distribution in relation to soil texture and pH in the Dry and Low Intermediate Zone of Sri Lanka"
- July 1–5, 1996 : Course "Manejo de SPSS como instrumento de procesamiento estadístico de datos recolectados a traves de encuestas", Loja, Ecuador.
- July 29–31, 1996 : Congress "La Gestión de los Recursos Naturales y el Desarrollo Rural", Loja, Ecuador.
Presentation: "Colección, Caracterización y Selección de Chirimoya (*Annona cherimola* Mill.) en la Provincia de Loja"
- November 12, 1998 : Participation on exposition organized by the Municipality of Loja and the NGO San Francisco, Loja, Ecuador.
Presentation: "Variabilidad de Caricaceae de clima templado en la provincia de Loja"
- January 13–19, 1999: Exposition "Ecuador Alternativo", Quito, Ecuador.
Presentation project "Conocimientos y Prácticas Culturales sobre los Recursos Fitogenéticos en el Austro Ecuatoriano"
- March 16–19, 1999 : "Primer Simposio Internacional sobre Chirimoya - First International Symposium on Cherimoya", Loja, Ecuador.
Co-organisator
Presentations: "Edaphoclimatological Study of Cherimoya (*Annona cherimola* Mill.) in Loja Province, South Ecuador" / "Collection and Characterization of Cherimoya (*Annona cherimola* Mill.) in Loja Province, South Ecuador" / "Promising Cherimoya (*Annona cherimola* Mill.) Accessions in Loja Province, South Ecuador" / "Seed Structure and Germination of Cherimoya (*Annona cherimola* Mill.)"
- April 17–18, 1999 : Congress "Primeras Jornadas de Cultivo de Babaco, Tomate Rifón y Pimiento", Quito, Ecuador.
Presentation : "Investigaciones en Caricaceae de Clima Templado en el Austro Ecuatoriano"
- October 26–28, 1999 : "II Congreso Internacional de Anonáceas", Tuxtla Gutiérrez, Mexico.
Presentation: "Potencial del Cultivo de Chirimoya (*Annona cherimola* Mill.) en la Provincia de Loja, Ecuador"
- February 3-4/8-9, 2000 : Course "Application of Project Cycle Management in University Development Co-operation (South Research / IDPM-RUCA)", V.I.I.R., Brussels, Belgium.
- May 9, 2000 : "52nd International Symposium on Crop Protection", Ghent, Belgium.
Participation.
- June 12–16, 2000 : "International Conference on Science and Technology for Managing Plant Genetic Diversity in the 21st Century" (IPGRI), Kuala Lumpur, Malaysia.
Posters: "Potential of and Threat to Cherimoya (*Annona cherimola* Mill.) Conservation in its Centre of Origin" / "Collection and Selection of Cherimoya (*Annona cherimola* Mill.) in Loja Province, Southern Ecuador" / "Potential of Local Highland Papayas in Southern Ecuador" / "Edaphoclimatological Evaluation and its Possibilities: A Case Study of Local Highland Papayas in Loja Province, Ecuador"
- December 13-15, 2000 : "Symposium on Biodiversity, from Genes to Landscapes", Louvain-la-Neuve, Belgium.

- Posters: "Potential of Cherimoya (*Annona cherimola* Mill.) diversity from Southern Ecuador for new varieties development" / "Potential use of *Carica* diversity in Southern Ecuador for new varieties development and alternative uses (phytopathology, papain)"
- May 8, 2001 : "53rd International Symposium on Crop Protection", Ghent, Belgium. Participation.
- May 16-20, 2001 : "EUCARPIA XVI Genetic Resources Section Symposium", Poznan, Poland. Poster: "Collection and Selection of Cherimoya (*Annona cherimola* Mill.) in Loja Province, Southern Ecuador"
- May 27-30, 2001 : "9th Meeting on Nordic Botanical Research in the Neotropics", Utrecht, Netherlands. Presentation: "Vasconcella (Caricaceae) species in Southern Ecuador : Agronomical Potential and Need for Taxonomical Research"
- September 24-28, 2001: "Conservación de la Biodiversidad en los Andes y la Amazonia", Cuzco, Perú. Poster: "Potential of highland papayas (*Vasconcella* spp.) in southern Ecuador" / "Potential of cherimoya (*Annona cherimola* Mill.) in southern Ecuador"
- October 19-20, 2001: "Botanical Biodiversity and the Belgian Expertise", Meise, Belgium. Poster: "Potential of highland papayas (*Vasconcella* spp.) in southern Ecuador" / "Potential of cherimoya (*Annona cherimola* Mill.) in southern Ecuador"
- November 6-9, 2001: "International Symposium on Sustainable Use of Plant Biodiversity to Promote New Opportunities for Horticultural Production Development", Antalya, Turkey. Presentation : "Horticultural Potential of Andean Fruit Crops Exploring their Centre of Origin"
- December 3, 2001 : United Nations University Biodiversity, Ghent, Belgium Seminar "New Crop development and Horticultural Potential of Andean Fruits"
- April 9, 2002 : International Plant Genetic Resources Institute (IPGRI), Aleppo, Syria Lecture "Ditribution and Potential of Cherimoya (*Annona cherimola* Mill.) and Highland Papayas (*Vasconcellea* spp.) in Ecuador"

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| PUBLICATIONS |
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A. Publications in International Journals with Referee System

- VAN RANST, E., **SCHELDEMAN, X.**, VANMECHELEN L., VAN MEIRVENNE, M. & KIPS, PH. (1996). Modelling the land production potential for maize. In: North-West Cameroon using GIS. In: Surveillance des sols dans l'environnement par télédétection et systèmes d'information géographiques (Monitoring soils in the environment with remote sensing and GIS), R. Escadafal, M. Mulders, L. Thiombiano (editors). ORSTOM Editions, Collection COLLOQUES et SEMINAIRES, Paris, 1996, 489-502.
- VAN MELE, P., VAN DAMME, V., **SCHELDEMAN, X.**, MEYLEMANS, B. & VAN DAMME, P. (1996). Herbicide use and occurrence of *Echinochloa* sp. in rice fields in the Dry and Intermediate Zones of Sri Lanka. *International Rice Research Notes*, 22(1): 47.
- VAN DAMME, P. & **SCHELDEMAN, X.** (1999). Promoting cultivation of cherimoya in Latin America. *Unasyva*, 50(1999/3, 198): 43-47.
- VAN DAMME, P., VAN DAMME, V. & **SCHELDEMAN, X.** (2000). Ecology and cropping of cherimoya (*Annona cherimola* Mill.) in Latin America. New data from Ecuador. *Fruits*, 55(3): 195-206.

B. Publications in International Journals without Referee System

- SCHELDEMAN, X.** & VAN DAMME, P. (1998). Promising cherimoya (*Annona cherimola* Mill.) accessions in southern Ecuador. *California Cherimoya Association Newsletter*, Volume 11, No. 2.
- JIMÉNEZ, Y., ROMERO, J. & **SCHELDEMAN, X.** (1999). Colección, caracterización y descripción de *Carica x heilbornii* nm. pentagona B.; *Carica pubescens* (A.DC) Solms – Laub y *Carica stipulata* B., en la provincia de Loja. *Revista de Difusión Técnica y Científica de la Facultad de Ciencias Agrícolas de la Universidad Nacional de Loja*, 29, 1-2, 1998: 43-54.

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C. Publications in Proceedings of Congresses and Symposiums

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