Session II

Genetic resources, conservation and utilization

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Plants, people and a portfolio approach to the conservation of biodiversity: the case of potatoes in Peru

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Abstract

Biodiversity is of growing concern in many developing countries. It is of particular interest in Peru where the potato is one of the country's most spectacular examples of biodiversity. Given the number of ecologies, varieties, and production centers, no single law, program, organization, business or project is capable by itself of sustaining the potato's biodiversity. Rather, different activities, actors and alliances may well be not only necessary but desirable in an overall effort to sustain biodiversity of the crop. This paper outlines a portfolio approach to sustaining biodiversity in which diverse actors with diverse goals each play an important and complementary role. The Peruvian experience may well be of interest to other developing countries seeking to sustain the biodiversity of their tropical roots and tubers, but lack a comprehensive framework to address the problems and promise such biodiversity represents.

Keywords: *Ex situ, In situ,* marketing, environmental services.

Introduction

Environmental conservation encompasses the entire world and touches a constellation of issues. Among the most complicated, if not controversial, is biodiversity. The issue is of particular interest in Peru where the potato is perhaps the most spectacular example of Peru's mega biodiversity. According to Alberto Salas, taxonomist at the International Potato Center (CIP), Peru is home to between 2,000 and 2,500 native varieties. Given the number of ecologies, varieties, production centers and pressures on the potato sector, no single law, program, organization, business or project is capable by itself of sustaining the potato's biodiversity. Rather, different activities, actors and alliances may well be not only necessary but desirable in an overall effort to sustain biodiversity of the potato.

This paper outlines a portfolio approach to sustaining biodiversity in which diverse goals and diverse organizations each play an important and complementary role. The Peruvian experience may well be of interest to other developing countries seeking to sustain the biodiversity of their tropical roots and tubers, but lack a comprehensive framework to address the problem and promise such biodiversity represents. In so doing, the paper also seeks to promote greater synergies among actors and secondary objectives with the intent of making the principal goal of sustaining biodiversity easier and more rewarding for all involved.

Why think of the collective efforts to sustain biodiversity from a portfolio perspective? One reason is that an investment portfolio typically encompasses multiple objectives like total return on investment, preservation of capital and, increasingly, concern over social responsibility. In analogous fashion, sustaining biodiversity also entails multiple objectives such as food security, food production/economic growth and poverty alleviation/ corporate social responsibility. Like different investments in a portfolio, each aiming to give priority to a particular goal, each being based on a different time horizon (from long to short-term), and each having a different risk/ reward ratio, recent years has seen a variety of mechanisms utilized to sustain the biodiversity of the potato. Prior to optimizing the composition of an investment portfolio, one first needs to understand the different investment instruments represented.

Conservation in situ and ex situ

For centuries, small farmers have maintained the biodiversity of the potato in their own fields, or *in situ*, utilizing an array of strategies as crop rotation, seed rotation, or exchange of potato varieties with other farmers in their own or nearby communities (Brush et al., 1992). The principal objective of these farmers was preserving their annual source of food as well as the cultural traditions and beliefs that potato cultivation represents for them. Maintaining a diverse collection of varieties--some more resistant to certain biotic or abiotic constraints than others-- was their way of pursuing those objectives.

Beginning in the second half of the last century, various organizations in Peru—public, private, national, international—have employed different techniques or strategies to help maintain the biodiversity of native potato varieties both on and off (i.e., *ex situ)* the farm. Conservation *ex situ* can be done either *in vivo, in vitro* or both. *In vivo* conservation involves planting the same native varieties year after year in experimental plots managed by trained professionals. *In vitro* conservation entails maintaining the native potato varieties in the form of plant cuttings growing in test tubes in a controlled laboratory environment. Said cuttings are done in such fashion so as to ensure they are sufficient to reproduce each and every variety.

These efforts have focused largely on identifying, then ensuring secure and continuous access to the genes of these varieties in an accelerated process to develop new and improved varieties. In order to guarantee the continuity of this work and in the process simultaneously avoid the possibility that one or other native potato variety might disappear forever due to a severe frost, drought or landslide in a particular farmer's field, collections of native potato varieties were established to conserve these potatoes *ex situ*. At the macro level, these potato breeding efforts have sought to respond to the growing demand for food resulting from continued population growth and accelerating rural-to-urban migration in the developing world.

In Peru, several different public organizations maintain collections of native potatoes and in different forms. The National Institute for Agrarian Innovation (Instituto Nacional de Innovación Agraria (INIA) has collections at its Experiment Stations in Ayacucho (Canaan), Cajamarca (Baños del Inca), Cusco (Andenes), Huancayo (Santa Ana) and Puno (Ilpo). In addition, several universities and NGOs have collections. For example, the NGO IDEAS in Cajamarca is working with over 130 native varieties. Many of these organizations—INIA, Universities, NGOs work with local growers "*conservacionistas*", i.e., producers dedicated to plant and in so conserve their own personal/family collections of native potato varieties. INIA is in the process of creating a unified national data base containing information about the native potato varieties that are in the collections of its Experiment Stations, the different Universities and the NGOs, among other reasons, to reduce the number of duplicate accessions where possible and the cost of maintaining the various collections. However, it should be pointed out that this is not a quick and easy undertaking.

The International Potato Center (CIP) maintains the world potato collection that includes not only the native potato varieties of Peru, but also those of other countries, e.g. Bolivia, as well. CIP`s system of maintenance of the world germplasm collection for potatoes includes four different components. In Peru, CIP maintains the collection both in the field (*in vivo*) and in the laboratory (*in vitro*). In addition, CIP coordinates the maintenance of the same collection at the new "doomsday" germplasm bank located in a cave in the ice on the island of Svalbard, Norway near the Arctic Circle (See http://www.scientificamerican.com/blog/60-second-science/post.cfm?id=doomsday-vault-aims-to-save-the-wor-2009-02-27. A second complete collection sits in a so-called "black box"—the entire collection is held under lock and key, not be opened except under emergency conditions, with the National Institute for Agricultural Technology (Instituto Nacional de Tecnologia Agropecuaria, INTA) in Argentina.

Conservation via the market

Concern over maintaining biodiversity in Peru began to receive greater attention in recent years partly due to the growing recognition of the link between the cultivation of native potato varieties and the incidence of severe poverty. A review of government statistics on the incidence of poverty showed that while nationwide the percentage of people living in poverty was declining, poverty-- in particular the poorest of the poor, or severe poverty, was still prevalent in the countryside as opposed to the cities. Furthermore, severe poverty was particularly acute in the rural highlands. A mapping exercise intended to explore the relation, if any, between severe poverty and potato production, in fact, found a high correlation between those districts with a high incidence of severe poverty and production of potatoes. Follow-up, ground-truthing research found that many

of these areas were isolated communities with cropping areas above 3,500 m where little else but native potato varieties could be grown.

For decades, potato producers in Peru—even those commercial growers that cultivate almost exclusively improved varieties given their higher yields—have maintained production of some native potato varieties for their own consumption given their superior culinary and nutritional traits. In recent years, however, many growers--even small growers that heretofore produced potatoes almost entirely for their own use, have expressed an interest in marketing native potatoes. Simultaneously, growing interest on the part of chefs in the New Andean cuisine combined with a resurgent demand on the part of the general public for traditional food commodities produced in the highlands has generated greater effective demand for native potatoes in major urban markets. This coincidence of wants led to a growing number of activities, organizations and enterprises offering monetary incentives to producers to continue planting native potato varieties as a form of conservation *in situ* through marketing.

One of the oldest forms of conservation through the market has been the wholesale marketing of native yellow potatoes in Lima. That activity has gone on for over half a century (Scott, 1985). Nevertheless, the traditional sale of yellow potatoes started to change in 1996 when a small, Lima-based produce business launched a new form of packaging yellow potatoes for retail sale: washed, graded and bagged in small, plastic mesh bags (Alarcon & Ordinola, 2002). Improved packaging for yellow native potatoes fit well with the concept of retail innovation in the form of superior quality and service–even for a product as basic as potatoes--being put forward by the growing number of supermarkets that up to that timer no one had thought of doing. These firms were looking to lure tradition-bound Lima consumers away from established food-purchasing practices tied to frequenting municipal stall markets to do their shopping based largely on price. It is noteworthy that this innovative form of packaging is now standard practice for all types of potatoes as well for other vegetables.

Innovation in the packaging of yellow potatoes led to a wave of other new and/or improved potato products based on native varieties in which the link to sustaining biodiversity of the potato was much more explicit. In many instances, the launching of these products was the result of strategic alliances between private entrepreneurs, community-based producer associations, and research and development organizations. Most, if not all, of these varieties had not been sold before in any appreciable quantities in major urban markets. These products include: a variety of potato chips; skin creams and lotions; improved white *chuño* (or *tunta*), a traditional processed product; and, small, plastic bags containing a mix of native varieties and a mini recipe booklet sold in supermarkets (Anonymous, 2008; Ordinola *et al.*, 2007a, 2007b). Other products in various stages of development include instant mashed potatoes from native, yellow flesh potatoes (Cortez, 2008) and community-based restaurants and cooking festivals as part of promoting gastronomic or experiential tourism. Selling points for nearly all these products are that the native varieties are organically produced; they have superior culinary and nutritional traits including anti-oxidants, and they are grown by poor, small farmers in threatened ecologies.

Conservation via payment for environmental services

Not all producers in the vast, rugged highlands of Peru can participate in the types of programs outlined above. Some growers whether for geographic isolation or other reasons will fall outside established safety nets for conserving biodiversity. Under these circumstances, and for the purpose of both alleviating severe poverty and sustaining biodiversity, a third option comes up for consideration. It consists of an evaluation of the economic and social feasibility of paying for environmental services as a mechanism to sustain cultivation of native potato varieties.

The idea of offering payment for environmental services is something entirely new in Peru. Hence, it raises a whole series of questions about who (or whom) would pay for such services? How, where, in want form, to how many producers, and for how long could one envision such payments form part, albeit limited and experimental, of an overall portfolio of activities intended to sustain the biodiversity of native potatoes in Peru?

In summary, a whole series of activities, actors and alliances are engaged in sustaining the biodiversity of native potato varieties in Peru. The array of initiatives in their totality constitutes, *de facto*, a portfolio of investments—public, private, national, international. Among the unfinished tasks at hand to sustain the overall effort are: 1) legally registering of Peru's native potato varieties before the recognized, official authority in that domain; said registration is independent of that done with National Service of Agrarian Health (SENASA) in order to become

eligible for multiplication as certified seed; 2) continuing to evaluate—not only the technical, but also the economic feasibility of new and/or improved products using native potato varieties; 3) publicize more extensively the results achieved to date in order to, among other things, facilitate greater synergies among both actual and potential participants in the aforementioned activities; and, 4) in the context of these developments and the series of existing and about-to-be-signed trade agreements, aggressively seek out ways to institutionalize the process as part of an overall strategy aimed developing the potato sector in the years ahead— and as have already done the neighboring countries (See, for example, IICA-MADR, 1998).

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Model genebank concept: CIP Genebank as an example

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Abstract

There are more than 1300 genebanks in the world holding about 6.1 million accessions. Most of the smaller genebanks especially in developing countries require strengthening both in terms of genebank management system and competency of genebank staff. The Model Genebank Concept as the name indicated will establish a series of genebanks in the world where other genebanks can model upon. This paper described the concept and used the genebank of the International Potato Center as an example to illustrate the idea.

Keywords: Model genebank, genebank management, best practices, dynamic conservation, conservation strategy.

Introduction

There are some 1300 genebanks holding about 6.1 million accessions in the world (FAO 1996). Many of the smaller genebanks especially in developing countries require strengthening both in terms of genebank management system and competency of technicians. This is to certain extent related to sufficient funding in the CGIAR (Consultative Group on International Agricultural Research) genebanks where the World Bank has to intervene and funded a multi- millions two-phase Global Public Goods Project known as GPG1 (2004 to 2006) and GPG2 (2007-2009) specifically to upgrade the infrastructure and equipment, and to process the backlog of germplasm accessions accumulated. These two projects also focus on the standardization of genebanking management and processes in order to establish a series of best practices for genebanks in the CGIAR system and for general application in other genebanks. Some of these results have already been posted in CGIAR System-wide Genetic Resources Programme at http://cropgenebank.sgrp.cgiar.org/. The successful outcome of the projects at CIP is that the CIP Genebank became the first genebank in the world to obtain the high standard ISO 17025 accreditation. The Model Genebank Concept as proposed in this paper and as the name indicated will establish a series of genebanks in the world where other genebanks can model upon. This paper described the concept and used the genebank of the International Potato Center as an example to illustrate the idea.

History on the development of plant genetic resources conservation

The development of human civilization is connected to plants. The domestication of plants into crops about 10,000 years ago simultaneously in the old and new world allowed people to settle and establish concentrated populations and building ancient cities. Through generations of cultivation and selection, crop diversity and crop genetic resources were created. For example, Pliny (AD 23-79), a Roman naturalist was able to record fact about plants in his 37-volume *Historis Naturalis* (Natural History). However, the field of plant genetic resources was made significant through the work of N.I. Vavilov on the centers of origin of crops in the world (Vavilov 1926). Genebanks were built to store the collected germplasm. A master degree course on the Conservation and Utilization of Plant Genetic Resources was first offered in 1969 by the University of Birmingham, United Kingdom and this continues to date. This was followed by the publication of two landmarked books on plant genetic resources which served both as a standard and reference textbook at the early stage of the development in this field (Frankel and Bennett 1970; Frankel and Hawkes 1975). The IBPGR (the International Board for Plant Genetic Resources) was founded in 1974 by CGIAR in response to the rapid loss of crop genetic resources and the threat to agricultural development and food security. It was housed at the Food and Agriculture Organization of the United Nations (FAO) to coordinate and fund germplasm collection and conservation activities, and to set up standards and procedures relating to these. One of the earlier standard crop descriptors published was for cultivated potato by the International Potato Center in 1977, and to date descriptors of 94 crops have been published and the newest one is also of cultivated potato on 'Key access and utilization descriptors for cultivated potato genetic resources' in 2009 (http://www.bioversityinternational.org/publications/publications/latest.html).

A practical seed handling and storage book documenting the experience – 'Principles and practices of seed storage' relating to seed genebanking was published (Justice and Bass 1978). This was followed by the publication of a series of genebanking handbooks by IBPGR where the procedures and standards established then are still the norm today. As the result, several key genebank manuals were published (http://www.bioversityinternational.org/publications/publications/latest.html).

This was followed by a period of increased construction of modern genebanks both in CGIAR centers and national genebanks in many countries which number about 1300 (FAO 1996). The IBPGR was evolved into the International Plant Genetic Resources Institute (IPGRI) in 1991 where its activities became more focus on research than the coordinating roles of the IBPGR. In 2006, IPGRI transformed itself into a research institution and renamed as Bioversity International.

Following the increasing utilization of the germplasm and the extensive use of plant breeders rights and patents to protect the derived new cultivars and sometimes extend to the original germplasm genetics used, the debate on the intellectual property rights of the original farmers and their fore-fathers in the development of the genetic resources became an agenda in the 1980s mainly at FAO under the intergovernmental Commission on Genetic Resources for Food and Agriculture and the International Undertaking on Plant Genetic Resources. It was at the 1992 United Nations Conference on Environment and Development in Rio de Janeiro that the full force of the resulting deliberation was tabled where 150 countries signed the Convention on Biological Diversity (CBD) which became effective in November 1993. The status of genetic resources as the common shared heritage and property of humankind became the nature resources of individual sovereign nations. The rules of engagement in germplasm collection, conservation and use change where prior consent on the use, equitable sharing of benefits resulting from the use of the genetics and associate knowledge, and the farmer rights became the matter of bilateral negotiation and agreements between users of germplasm and provider country. The exchange of genetic resources was notably reduced as the result. FAO through its Global Plan of Action for the conservation and use of plant genetic resources was finding a way to revert this and an International Treaty on Plant Genetic Resources for Food and Agriculture was signed and rectified, and went into force in June 2004 (http://www.planttreaty.org/index_en.htm) to create a multi-lateral germplasm exchange and use system with a pass-on standard material transfer agreement (SMTA). To-date, 120 parties have sign-on and the world is in a transition to learn and live with these two systems of germplasm use.

Dynamic conservation strategy as a discipline

Crop genetic resources conservation is, currently, separated into two main strategies: (1) *ex situ* conservation strategy where germplasm samples are collected or acquired and then conserved outside their natural state, and (2) *in situ* conservation strategy when germplasm variation are maintained in their natural environment by the farmers and farming communities, i.e. the genepool is evolving according to the environment pressure offered. The *ex situ* conservation strategy has been the main focus to these days. Well established genebanks have been built like the CGIAR genebanks. In the past fifty years, an interdisciplinary field involving knowledge from more than 25 scientific disciplines has been developed as shown in Fig. 1. However, the knowledge and experience has been concentrated on the *ex situ* conservation strategy in seed and clonal genebanks.

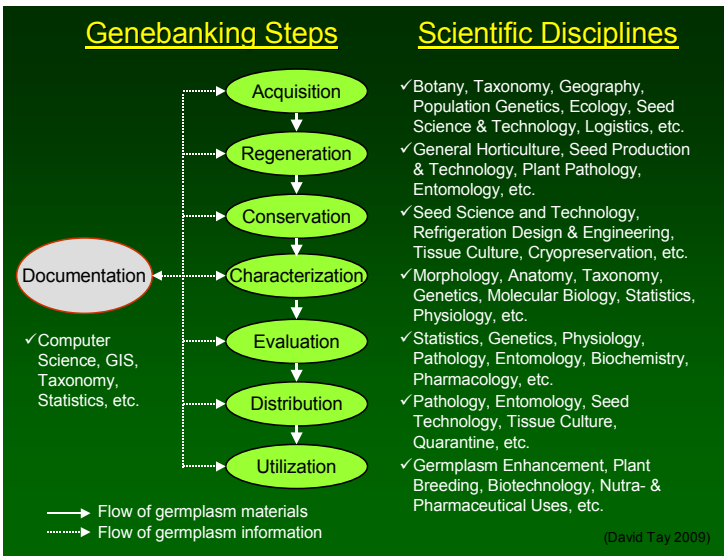

Figure1. The processes and the knowledge relating to the scientific disciplines required in managing and operating a standard *ex situ* genebank for both seed and clonal crops

On the other hand, the *in situ* conservation strategy by nature of its social sciences component has been exploratory and *ad hoc*, and endeavors have been mainly on natural areas and buffer zones relating to the conservation of landraces and wild related species. The interaction of the *in situ* and *ex situ* conservation strategy and their complimentary function were limitedly explored and tested.

At CIP in the last ten years, the interaction of *in situ* and *ex situ* conservation was put to test at San Jose de Aymara, Huancavelica, Peru and the 'Potato Park' in Pisac, Cusco, Peru. Communal genebanks of native potato were established with their own native cultivars collected in their own locations when the project was established and in addition, CIP repatriated all the cleaned virus-indexed native potato from its *ex situ* genebank collected about 30-40 years ago to the respective communities. Technologies and know-how on clean seed maintenance and selection, seed storage, scientific characterization of the cultivars and register and database were provided as a package. Plastic greenhouses were built in support of clean seed production and distribution to neighboring communities. After about ten years of interaction at San Jose de Aymara, the communal genebank is, currently, independently managed by the community and some special cultivars were produced for seed trade and niche markets and thus a self-sustainable *in situ* conservation system is in operation. The community, in addition, takes on the scientific task to produce clean seed for CIP *ex situ* collection for its repatriation program in Peru.

With the success in San Jose de Aymara, an agreement between the Association of the Potato Park Communities, Asociacion ANDES (a non-profit organization in Cuzco) and CIP was signed in December 2004 for five years to duplicate the experience. The results of this project have been widely reported in popular press and news media.

These successes coupled with the experience at CIP in its repatriation program which from 1998-2008 distributed 3608 samples of 1250 accessions of native potato to 41 communities in Peru have led CIP to formulate a regional project to cover the whole range of the Andes from Venezuela in the north to Argentina in the south to establish communal genebanks in their local communities at the micro-centers of agro-biodiversity along the Inca ancient highways. This is 'La Ruta Condor' project – 'the flight path of condor' (Fig. 2). The strategy is to establish site by site based on communal interest and funding availability. At the moment, three more sites are under negotiation in collaboration with the Andean Community and the Mountain Institute.

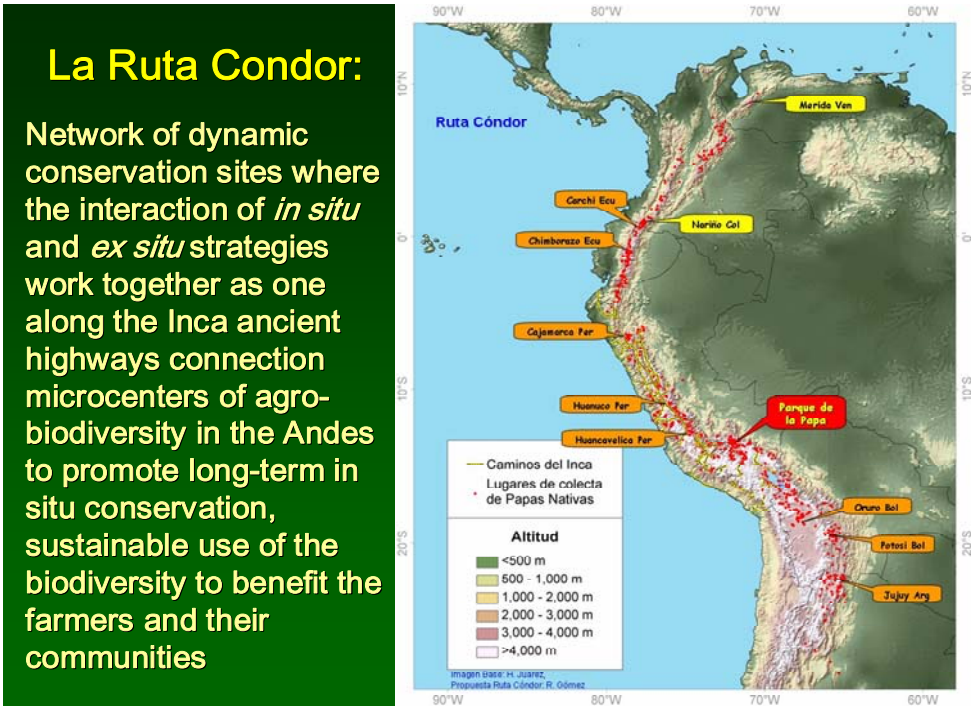

Figure 2. The 'La Ruta Condor' project – a dynamic conservation strategy where the *in situ* and *ex situ* conservation work together in a dynamic way to complimenting the comparative advantage of each

The crop genetic resources conservation in the fifty years has developed and grown into a discipline of study in its own right. There are three scientific journals that specifically publish works in this discipline. We thus recommend the use of dynamic conservation strategy, a discipline of the complimentary use of both *in situ* and *ex situ* methods, in genetic resources conservation program.

What is a model genebank system?

This concept is proposed for the first time to create a network of genebanks of excellence to act as models for other genebanks to follow and to draw expertise from. The primary goal of a model genebank should be to conserve germplasm and related data and information for our future generations. It could be a seed and clonal genebank or a combination of both. A practicing dynamic conservation program where *ex situ* and *in situ* conservation methods interact to compliment each other is a plus as described in the above section. Thus, a model genebank should have the following features:

- A working genebank with comprehensive competency and authority in genebanking of at least one crop
- Implementing international conventions, treaties, standards such as CBD, IT, etc.
- Hands-on in generating genebanking processes and standards in the world or a region
- Proactive in genebanking research and capacity building and training of other genebank staff

What makes a model genebank? It should have a set of qualities as follows:

- Committed staff, i.e. competence & discipline
- Sufficient infrastructure and equipment
- Best practices and discipline
- Distributing clean germplasm
- Full backup of germplasm and information
- Efficient and cost effective
- Sufficient funding

Committed staff. The staff should individually believe in the primary goal of the genebank to curate the genetic resources for our future generations. They should thus be motivated and passionate on their curatorial responsibilities, knowledgeable and skillful in what they do and eager to teach and learn, transparent in handling of germplasm and information (not withholding germplasm and information), take on research only on issues relating to genebanking activities, etc.

Sufficient infrastructure and equipment. The infrastructure requirement is more of functional accuracy and reliability rather than complexity and a well monitoring and maintenance program should be in place and functioning. Seed and *in vitro* cold storage rooms should have a reliable alarm system and a standby generator when main grid electric fell.

Best practices and independent accreditation. The development of genebank management system has evolved since the 1970s where management was based on ones own experience and that of others and then developed to these days a best practice system as illustrated in Fig. 3. Following the creation of IBPGR, common standards were established and genebank management handbooks were published. Common standards were followed such as the -18°C was set for seed long-term storage, and the 15°C and 15% relative humidity for slow seed drying to 3-7% seed moisture content. In the mid 1980s based on these standards FAO initiated a genebank assessment program and each participating genebanks was given a report on its status (personal experience at the AVRDC genebank). However, this program was not continued. This was followed by the use of

genebank own operation manual which was a norm in the 1990s to date. The GPG1 and GPG2 have developed best practices for the different genebanking processes. CIP's genebank took the efforts one step further to become the first genebank in the world to obtain the ISO 17025 accreditation where all the best practices are documented, and infrastructure and equipment are calibrated and monitored at set interval. All nonconforming occurrences are documented and corrective actions are formulated and updated on the ISO operation procedures so that the same nonconforming activities will be prevented. It is thus a self-improving best practice system.

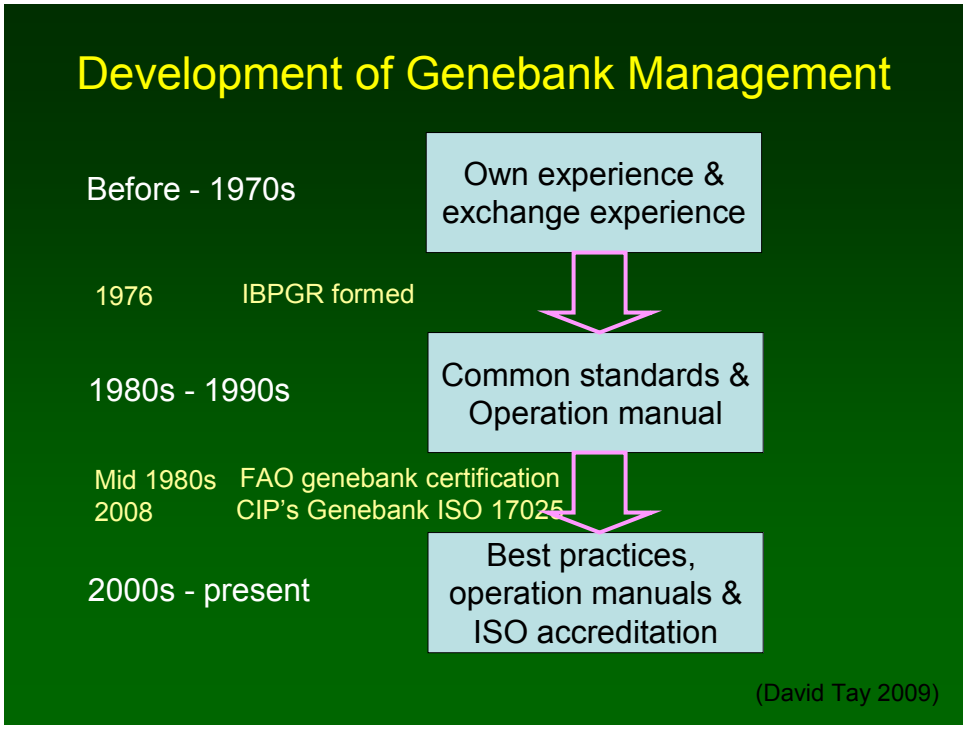

Figure 3: The development and evolution of genebank management system from the 1970s to present

Distributing clean germplasm. This is a must in clonal genebanks where clean vegetative material should be only distributed to prevent the spread of diseases around the world. FAO specific crop guidelines should be the minimum standards to be followed and in addition the recipient country quarantine requirement should be followed. Similar, seed genebank should adopt the best practice in seed production to harvest clean seed and to follow national and international quarantine requirement.

Duplications of germplasm and information. Germplasm collections should be 100% backup at another site of different risk factors. Similarly, hard copy of all germplasm collection, characterization, evaluation and associate information should be kept in an 'information blackbox' and also scanned for digital backup. Similarly, digital images should be backup together with germplasm databases.

Sufficient funding and efficiency and cost effectiveness. Quality work can only be achieved with sufficient fund. However, a genebank should be efficient and cost effective in its operation. Constant supervision and evaluation of the operation processes and outcomes by experience genebank manager is the mean. Periodic benchmarking with similar genebanks will provide the guidance to achieve state of the art.

Why CIP's Genebank is a model genebank?

The CIP's Genebank has 37 years of experience in both seed and clonal which included field-, seed, *in vitro*, cryo-) collections and a supporting DNA bank, a herbarium and a 10-year dynamic conservation program where *in situ* and *ex situ* methods compliment each other in farming communities as described in the above section. In fact, the seed collection at CIP is larger than its clonal collection in term of number of accessions. Some of its qualifications include the following:

- Home of the largest *in vitro* genebank.
- World first genebank with ISO 17025 accreditation – a self-improving continuous best-practice approach.
- Leader in barcode technology, pocket PCs and wireless system where pens and pencils are sparingly use CIP's *in vitro* genebank.
- Quality than quantity collection – where duplicates in the clonal native potato collection have been identified with the most appropriate science of the time and converted to seed and a system to confirm the individual accession identity is carried out .
- Committed to the distribution of only cleaned clonal germplasm where in potato 9 viruses and 1 viriod, and visible bacteria are eliminated and in sweetpotato 11 viruses and other viruses as can be detected by indicator plants and visible bacteria.
- Full implementation of IT and the use of SMTA (Standard Material Transfer Agreement) and CBD requirement in its germplasm distribution.
- Cryo-bank with more than 750 accessions of potato in storage.
- Ten-year experience in dynamic conservation strategy – the complementary role of *in situ* and *ex situ* conservation.
- Recognitions and values the contribution of farmers in the development of the original genetic resources (landraces) and applies this believe in its dynamic conservation projects.
- Genebank operation is fully cost.

CIP Genebank is a leader globally in these key qualifications. It leads the GPG2 task force in these areas in the CGIAR genebanks. CIP genebank is thus a model genebank.

CIP is committed to take on the role as a model genebank and this is in its Cooperate Plan 2009-2018 for implementation (under final revision). The goal is CIP, community conservationists and other stakeholders will collaborate on dynamic *in situ* and *ex situ* conservation of potato, sweetpotato and underutilized root and tuber genetic resources to enhance their use by present and future generations and respond to climate change. The four strategic objectives to fulfill this role as a model genebank are:

1. The holdings of the genebank will represent the entire genepool of potato, sweetpotato and their wild relatives.
2. The holdings of the CIP genebank will be completely characterized for priority traits and the information about it will be freely available and accessible to all users.
3. ISO-accredited quality management systems will cover all relevant aspects of genebank activities to ensure secure conservation and a safe, responsive, decentralized maintenance and distribution service for plant genetic resources.
4. A strategy, methods and tools for CIP, community conservationists and other stakeholders implemented to collaborate on dynamic *in situ* and *ex situ* conservation.

Conclusion

We are at the stage where both the technical aspect of genebanking and the international operational framework in the fair and equitable transfer and use of genetic resources are in place. The model genebank concept with its model genebanks will enhance the development of more high quality genebanks in the world where a genebank can benchmark itself against a model genebank to identify areas that require further improvement. Currently, such a system is not available. CIP genebank as one of the CGIAR genebanks is in an appropriate position to take on this challenge as the first model genebank.

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Gaining and maintaining the world's first ISO Accreditation for a genebank

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CIP is committed to provide quality germplasm to its clients around the world. In 2006 the decision was made to formalize current practices under an international standard as an additional safety net for consistent quality. Implementing an ISO standard means effectively applying the principles of scientific peer review to product delivery and is thus very compatible within the social culture of a research institute. CIP is the first genebank in the world that opted to implement the ISO 17025 standard for testing laboratories which includes technical review of procedures and protocols on top of management review. The implementation of the formal framework further streamlined processes and protocols. As a side effect documentation management took advantage of modern online tools to auditing document changes and facilitating remote access by external auditors reducing the need for international travels. The implementation process up to the initial accreditation took a year; this is relatively fast and due to the availability of formal pre-existing documentation of main processes as well as the support by advanced laboratory information systems capable of providing complete audit trails on accession transactions. Last but not least the high staff motivation was crucial for the implementation. After another year the new system proved its viability since several former key staff left but thanks to the existing documentation and procedures new staff could quickly be incorporated. The ISO accreditation thus proved its worth and its internal scope is being extended.

Keywords: genebank, management, quality, accreditation, bar code, laboratory information management systems.

Introduction

One of the primary functions of a genebank is the distribution of plant materials; these materials are products and the results of technical processes. As for any product it is important to ensure that the product is 'fit for use': this includes in the case of plants vigor and health. In 2008the International Potato Center adopted a formal 'quality management system' based on ISO 17025 for the distribution of its in-vitro genebank materials.

The *International Standards Organization* (ISO, www.iso.org) is a non-governmental organization and has among its members individual countries and collaborates with other organizations like the United Nations. The quality standards produced by ISO includes the general standard for the implementation of a quality management systems – ISO 9001 and the standard that covers the requirements for the competence of laboratories - ISO 17025. Both these are potentially of interest to research organizations and some some governments have already started to insist that organisations in the field of plant production and distribution comply with these standards. For Examples of this include the the Dutch genebank that has implemented ISO 9000 certification for all its operations (T. van Hintum, pers. comm.) and the CIMMYT genebank has implemented ISO 17025 for itsits seed health testing unit (T. Payne, pers. comm.) at the request of the respective governments. It is important to note that ISO 9000 refers to the overall management of the quality system and ISO 17025 expands this to cover the technical implementation of the processes in terms of the competence to carry these out and comply with current technical best practices. ISO 9000 is assessed through *certification*, whereas ISO 17025 is assessed through *accreditation*.

The principles of an ISO compliant *quality management system* are related to good scientific practice: the whole process should be (a) transparent and fully document the process of how e.g. the final product is derived from the raw materials, (b) include internal controls to check on adherence to procedures, protocols and standards, and (c) include regular external review by experts in the field. ISO quality management aims to provide for continuous self-improvement through critical review.

Materials and methods

The principal processes (in-vitro laboratory; virology laboratory) included for the ISO accreditation process had counted with extensive process and protocol level documentation before the project start. In addition, an information technology infrastructure allowing real-time data logging was available using advance bar-code based tracking applications for laboratory information management.

A crucial element was the support of upper management that allowed the hiring of a consultant (DG) dedicated to project. The project depended largely also on the activation of in-house expertise through motivation and concurrent improvements in infrastructure and process management.

Results

ISO 17025 was accredited by UKAS (United Kingdom Accreditation Service) according to the planned schedule in February, 2008, for "the acquisition, maintenance and distribution of in-vitro plant material following pathogen screening techniques using symptom detection on grown on plants and on host range plants after inoculation with sap; the detection of pathogens using DAS-ELISA and NASH diagnostic techniques. This is for both potatoes and sweetpotatoes…". This gives a visible additional assurance of quality to all users of CIP genebank materials. The overall time-line of the project was about one year from the hiring of the consultant to accreditation. This was extremely fast and built on the prior established workflows and associated documentation.

During the one year period of the implementation several amendments were made including : (a) the use of internet based wiki-pages using Confluence (www.atlassian.org) for managing all documents with version control in a central repository; (b) the standardization of all documents and addition of missing ones; (c) the training of internal auditors; (d) the appointment of an ISO manager to coordinate audits; (d) completion of a validation of the processes to clearly demonstrate their effectiveness (e) the concurrent further improvement of protocols, procedures, and supporting IT infrastructure to further minimise risks.

Wiki technology is used to maintain a complete trail of changes to all documents related to management of processes under ISO quality management. This traditionally requires a lot of additional effort for updating documents. Using a web based system also allowed to easily centralize documentation while maintaining access for 'process and protocol owners' and simultaneously obviate the majority of the administrative red-tape. It also allowed transparent access for the external UK based evaluators early on in the process and thus saved on international travel and assessment costs. . The centralization of documentation served also as an opportunity to standardize documents according to the ISO requirements and helped identify gaps. One important addition was the establishment of a formal internal audit system. The intensive internal review both by the quality management system specialist and the technical teams involved served further to improve on processes, protocols and information technology to enhance specifically transparency and ease of use by users and auditors. This included for example real-time photo documentation of virus infection on indicator plants with wireless enabled digital cameras.

The ISO quality management system has proved to be sustainable for more than a year, without need for the input from external consultants as documented by the successful continuation of the accreditation. The improved documentation and procedures has helped to manage the departure of several key staff during 2008 by ensuring that there is a very well organised knowledge base that smoothed the transfer of responsibilities for technical procedures to the new staff.

Discussion

The successful implementation of the project to acquire ISO 17025 accreditation was largely due to (a) senior management support, (b) existing advanced and well documented procedures and protocols along with efficient and effective information systems, (c) early and continued involvement of all staff to contribute and improve documentation; (d) validation of processes helping to improve the efficiency of germplasm pathogens; (e) sustainability through staff commitment and community building.

The accreditation gives credit to the innovative genebank and senior management since CIP was the first genebank world wide to do so and gives the genebank the the highest level of recognition possible (Jorge, 2008).

CIPs improved in-house capability in formal quality management systems is now being used to extend the scope of ISO accreditation in a step-wise manner to other areas of genebank management and beyond.

CIPs move to adopt ISO 17025 served as a use case in a recent study from CG genebank community under the GPG2 (Global public goods – phase 2) project funded by the World Bank to assess the applicability of formal quality management systems for genebanks (Jorge, 2008). Jorge and Galsworthy summarize that recent developments relevant to the genebank community suggest that quality management should be based on the ISO approach and preferably use ISO 17025. Thus, the introduction of ISO 17025 at CIPs genebank already proved its value beyond and underlines its potential as a model genebank.

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Status and impact of *in vitro* conservation of root and tubers at the International Potato Center (CIP)

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Abstract

Potato *in vitro* slow growth storage and pathogen elimination techniques were established at CIP in the 1973-79 period. These developments pioneered the *in vitro* conservation and worldwide dissemination of the most important Andean root and tuber crops. The CIP's *in vitro* genebank, existed now for 36 years, and currently stores potato (5,551 accessions), sweetpotato (5,454), oca (491), ulluco (418), mashua (47), yacon (29), achira (11) and arracacha (2) germplasm; additionally, breeding and research accessions of potato (3,906), sweetpotato (45) and other crops (158) are maintained. Since 2000, the use of bar-coding-assisted databases has increased the conservation efficiency and pathogen-tested materials production. Currently, 48% of the whole collection is available for international distribution under the International Treaty on Plant Genetic Resources for Food and Agriculture (IT). The establishment of safety back up copies and cryopreservation use has enhanced the long-term conservation of collections. In February 2008, CIP's *in vitro* genebank was awarded with the Accreditation Certificate for applying the highest quality practices, by the UK Accreditation Service in accordance with the International Standard ISO/IEC 17025:2005. The most relevant milestones achieved are: 1) 16,843 accessions of root and tubers are safely conserved *in vitro* ; 2) 44,858 pathogen tested samples were distributed to 121 countries, contributing to research, conservation and productivity; 3) since 1998, CIP has repatriated more than 3,600 samples of over 1,200 pathogen-tested native potatoes varieties to 41 Andean farmer communities and 6 national institutions to restore diversity affected by crop diseases/pests, migration and poverty, and to increase productivity; 4) cryopreservation has increased efficiency through reducing labor and loss risks. Currently, the potato cryo-collection contains more than 700 accessions with 95% confidence of recovering samples after long-term preservation.

Introduction

The international genebanks maintained by the Consultative Group on International Agricultural Research (CGIAR) centers have the aim of securing the in-trust genetic resources collections to perpetuity and promoting their use through the development and application of efficient practices and conservation methods. At CIP, considerable progress has been made in the development and application of *in vitro* tissue culture conservation techniques for conserving Andean tuber and root crops clonally. The CIP's *in vitro* genebank activities started in 1973 with the development of potato *in vitro* conservation and pathogen elimination methods (Roca, *et al.*, 1978). Achievements on potato paved the way for the development of methods for sweetpotato, oca, ulluco, mashua, yacon, achira and arracacha. Technologies for *in vitro* conservation, production of pathogen tested materials, cryopreservation, and germplasm distribution developed by CIP (Table 1) have been disseminated to most genebanks of root and tuber crops worldwide, and transferred to more than 500 scientists involved in germplasm conservation and utilization that were trained in CIP's genebank. This report describes the status of the main *in vitro* genebank activities that made possible the efficient conservation of 16,112 accessions, as well as, shows the achievements and impact reached by the use of high quality procedures.

Material and methods

The *in vitro* introduction, maintenance and pathogen elimination methods used were those developed by CIP and collaborators, for potato (*Solanum* spp.), sweetpotato (*Ipomoea batatas*), oca (*Oxalis tuberosum*), ulluco (*Ullucus tuberosus*), mashua (*Tropaeolum tuberosus*), yacon (*Smalanthus sonchifolia*), arracacha (*Arracacia xanthorriza*), and achira (*Canna indica*) (Roca, *et al.*, 1978; Schilde-Rentschler and Roca, 1987; Espinoza, *et al.*, 1992; Lizarraga, *et al.*, 1992; Golmirzaie and Panta, 1997; Golmirzaie, *et al.*, 1999; CIP, 2009). Protocols and media used for *in vitro* conservation are shown in Tables 2 and 3. *In vitro* stocks viability was evaluated using specific indicators (Table 4). Multiplication and distribution of pathogen tested materials was done following CIP

standard procedures (Golmirzaie and Panta, 1997; CIP, 2009). In 2000, barcode technology and mobile informatics tools were incorporated to the *in vitro* genebank monitoring system. This system uses the following resources: mobile computers (PDAs); bar-coding comprising accession identification, heath status and culture medium data; specific barcoding labels and ribbons; thermal printers; hand barcode readers; and wireless networking (Rojas, *et al.,* 2005). Safety back-up of potato collection was placed in INTA-Argentina in 2004, and a complementary copy collection was placed in CIP's-Huancayo station, in 2007. Sweetpotato back-ups were placed in CIAT-Colombia in 2006; and CIP's-Huancayo and -San Ramon stations, in 2008. Copies of the other Andean crops were placed in CIP's-Huancayo, in 2007. Monitoring by viability evaluation of *in vitro* plantlets was done every 4 months, and collections were re-placed every 2 years, every year and twice per year for potato, sweetpotato and the other Andean root and tuber crops (ARTC), respectively. Cryopreservation methods used were those developed jointly by CIP and the Catholic University of Leuven (KULeuven), Belgium (Panta, *et al.,* 2006). In 2007, all protocols and procedures applied in the *in vitro* genebank were updated (Table 1), and after following internal and external audits CIP applied for the highest quality practices Accreditation Certificate, to the UK Accreditation Service (UKAS) in accordance with the International Standard ISO/IEC 17025:2005.

Results and discussion

In vitro conservation

The CIP's *in vitro* collection currently comprises 16,112 accessions (Table 5); out of these 12,003 are in the long-term collection and 4,109 are transitory holdings; and 50% (8,068 acc) of the collection is pathogen-tested. The collections are conserved under by long-term or transitory status, the first group mainly comprises accessions designated under the International Treaty (IT), and the transitory conservation is for research or breeding material upon specific scientist request. Arrangements for establishing safety copies of *in vitro* potato and sweetpotato collections (black boxes) at INTA and CIAT, respectively, were successfully done. From 2004, potato black box have been replaced three times; currently a new shipment comprising 3,770 accessions in long-term conservation will be placed in INTA, and conserved in a chamber at 8°C, photoperiod of 16 h, 2000 lux and 70% of relative humidity. Each sample consists of 2 tubes (18x150mm) per accession containing 2 plantlets per tube in slow grow medium [MS salts (Murashige & Skoog, 1962) supplemented with 2 mg/l glycine, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.4 mg/l thiamine, 2% sucrose, 4% sorbitol, 0.7% agar]. Because of Argentina quarantine regulations potato accessions positive to APMV and PMTV can not enter into the country; for this reason 1,781 accessions under long-term conservation are being placed in CIP's Huancayo station in a chamber at 18°C, photoperiod of 16 h, 2000 lux. There is a strategic plan for cleaning these accessions from APMV and PMTV. Regarding safety back up of sweetpotato about 4,500 accessions are placed at CIAT-Colombia; the materials are sent in three groups per year for conserving at 19-21°C, photoperiod 16h light and 2000 lux luminance. Each sample consists of 3 tubes 18x150mm per accession containing 2 plantlets per tube in sweetpotato conservation medium (MS salts supplemented with 2 mg/l calcium panthotenate, 100 mg/l calcium nitrate, 100 mg/l L-arginine, 200 mg/l ascorbic acid, 20 mg/l putrescine HCl, 3% sucrose, 0.3% phytagel). Currently, a group of about 1,000 accessions is being prepared to sent to CIAT at the end of the year. Sweetpotato accessions infected with systemic bacteria contaminants are not able to storage in CIAT, for this reason safety copy of these materials (482 acc), are being conserved in two places CIP-Huancayo and –SanRamon stations, 1 tube and 2 tubes, respectively. In Huancayo they are stored at 18°C and in San Ramon at 23°C. Response of cultures in these conditions is being evaluated; up to date there are indications that higher survival is found at 18°C. Safety back-up of oca, ulluco and mashua collections are maintained at CIP's Huancayo station in a chamber at 18°C, photoperiod of 16 h, and 2000 lux. Experiments for improving *in vitro* conservation of oca, ulluco, and mashua are in progress. These collections are subjected to low temperature (6-8°C) in comparison with incubation at 20°C. Using low temperature the conservation period of ulluco and mashua has extended from 8 to 14 months, for oca from 8 months to 12 months. The method is being validated by a third assay.

Production of pathogen tested materials

In the last 10 years, 4,129 accessions were virus-tested for all known viruses (HS2): 2,211 of potato, 1,538 of sweet potato and 380 of ARTC. The method comprises an initial health testing by serological, molecular and host range tests; then, therapy by combining incubation at high temperature with meristem culture; and a final diagnosis for verifying the virus elimination. The efficiency of this process was 83% overall. The result showed that PVS and SPFMV were the most common in potato (72%) and sweetpotato (17%), respectively. In 2005, bacteria contaminants were found in more than 900 sweetpotato accessions. Procedures for bacteria cleaning were developed and to date 600 accessions have been cleaned. This method combines 2-4 weeks of *in vitro* antibiotic

treatment using Cefotaxime 200 mg/l, Ceftriaxone 200 mg/l, and Rifampicin 300 mg/l; growing in the greenhouse for 2-3 months and re-introducing to *in vitro*. In 2009, 150 accessions are under processing with this therapy.

Cryopreservation

Since cryopreservation is the best alternative for long term safe conservation of clonal crops, CIP has worked to adapt different cryo- procedures for the potato. During 1994-1999, more than 400 genotypes were done with the ethylene glycol-based vitrification Steponkus' technique (Gonzales-Arnao, et al., 2008). Not all genotypes withstood cryopreservation and the overall viability decreased as more genotypes were tested. Based on these results, in 2004, research for developing an improved potato cryopreservation method started. The PVS2 droplet vitrification method (Panis, *et al.,* 2005), currently utilized for the long-term conservation of *Musa spp* germplasm, was adapted to potato landraces developing a workable protocol (Panta, *et al.,* 2006). Currently, CIP is applying this droplet-vitrification technique and has successfully cryopreserved more than 700 potato landraces. In recent research, the effect of culturing shoot-tip-donor-plantlets at low temperature (6°C) was compared with normal culture (22°C). Using 434 accessions, it was demonstrated that 209 (48%) accessions showed no significant difference in recovery rate in both culture treatments (6°C and 22°C), 132 (30%) accessions had higher recovery rate in the 6°C treatment, and 58 (13%) accessions had higher recovery rate with 22°C treatment (Table 6). The results suggested use of 6°C shoot-tip-donor-plantlets culture favor the response to cryopreservation; currently the routine potato cryopreservation is done following the 6°C treatment, and storing 120 samples per accession. All recovered accessions are currently cryo-stored in liquid nitrogen and viability is tested by thawing 20 samples. Shoot recovery rate equal or higher than 20% assures that from cryopreserved accessions, after long-term, at least one plant will be recovered with 95% confidence. This was estimated using the statistical formula developed by Dussert and collaborators (Dussert, *et al.*, 2003). The accessions with lower recovery rate will be processed again until fulfill the Dussert's formula requirements. The cryopreservation success on potato has paved the way for the development of sweetpotato, oca and ulluco cryopreservation. Currently, research for adapting the potato cryo-technique to these crops is going on.

Germplasm distribution and repatriation

The CIP's *in vitro* genebank existed more than 30 years; during this period 10,660 accessions (44,858 samples) were distributed to 121 countries (4,299 acc.) and to CIP scientists (6,361 acc.). The most requested material was improved varieties and breeding materials (advanced cultivars and breeding lines) and the main purpose was breeding research. This activity has played a basic and important role in the dissemination of potato crop and increasing the productivity around the world.

Using *in vitro* pathogen tested clones; CIP's scientists produce clean tuber seed. From this material type, since 1998, CIP has repatriated more than 3,600 samples of more than 1,200 varieties of native potatoes of high sanitary quality to 41 Andean farmer communities and six institutions to restore and improve diversity affected by crop diseases/pests, migrations and poverty, and to increase productivity through the use of clean seed (Table 7).

Informatics technology for in vitro genebank monitoring

Significantly improvement has been achieved by installing the bar code system for *in vitro* storage, using mobile computers, wireless connectivity and scanning technologies; and setting up dynamic reports on *in vitro* stocks through CIP-intranet (http://sol/appdb/research/RIU/REPORTSD/). These tools have increase efficiency on tracking materials, inventorying, preparing material lists, labeling, monitoring of materials following health testing and pathogen elimination treatments, checking material availability for distribution, and monitoring germplasm acquisition and distribution.

Best practices supporting Quality Accreditation (ISO 17025)

The CIP *in-vitro* genebank succeed in the process of implementing an overarching quality system to cover all the activities of *in vitro* germplasm acquisition, management and distribution. The system structure is based around the world-wide accepted quality system standard for the competence of laboratories – ISO/IEC 17025. The *in vitro* genebank followed a technical assessment against this Standard in January 2008 by a recognized world expert in genebank management in order to gain accreditation from the United Kingdom Accreditation Services (UKAS). Previously, all activities protocols and workflows were updated, staff was trained, monitoring of environment and equipment was implemented, and internal audits were performed. After clearance of certain issues raised at the expert visit, the *In vitro* Genebank was awarded accreditation on February 2008. This

accreditation was renewed in 2009 and currently the Genebank is following the third UKAS audit. The accreditation renewal is annual and requires a continuous improvement of all activities.

Conclusions

One of the major CIP's *in vitro* genebank milestones is the constant improvement of practices for conserving the most valuable genetic resources of the Andean root and tuber crops. The expertise of this genebank is worldwide recognized and is making possible the efficient conservation of 16,112 accessions of eight crops. The conservation and therapies applied to this germplasm allowed the potato dissemination to 108 countries. As well as have played an impact role on the socio-economic benefit of Andean communities, since high sanitary quality samples of native varieties are repatriated restoring diversity and increasing productivity by the use of cleaned seed. The high quality practices reached in the CIP's *in vitro* genebank assure the safe and long-term conservation of the genetic resources in trust and the safe movement of the germplasm worldwide distributed. As well as the Accreditation ISO 17025 is defining the CIP's genebank as a leader in the application of Quality Assured (QA) practices across CGIAR's and worldwide genebanks.

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Table 1. Techniques and procedures utilized for germplasm *in vitro* conservation and distribution in CIP's genebank

Techniques	Procedures	Activities
Introduction to *in vitro* culture *In vitro* multiplication *In vitro* conservation Meristem culture Virus testing Virus elimination Bacteria elimination Cryopreservation	Best practices for *in vitro* culture Best practices in greenhouse Data monitoring Preparation of medium, solutions and materials for *in vitro* culture Equipment monitoring Environment monitoring Safety measures: alarms	Germplasm acquisition *In vitro* conservation: short and medium term Long term conservation Production of pathogen tested materials Distribution of *in vitro* materials Safety back up of collections Identity verification

Table 2. Storage protocols and procedures applied for *in vitro* conservation of eight Andean crops

Crop	Temp-erature (°C)	Light intensity	Type of vessels	Number of replicates/ accessions stored	Storage duration (months) Aver./Min./Max.		
Potato	6 - 8	1000 lux	25x150 mm tubes	3 (4 explants each)	24	8	36
Sweetpotato	19 - 21	2000 lux	18x150 mm tubes	6 (2 explants each)	10	6	12
Oca	6 - 8	1000 lux	25x150 mm tubes	3 (3 explants each)	18	12	20
Ulluco	6 – 8	1000 lux	25x150 mm tubes	3 (3 explants each)	18	12	20
Mashua	6 - 8	1000 lux	25x150 mm tubes	3 (4 explants each)	18	12	20
Achira	18 - 22	2000 lux	25x150 mm tubes	10 (1 explant each)	3	3	4
Yacon	18 - 22	2000 lux	25x150 mm tubes	7 (1 explant each)	3	3	4
Arracacha	18 - 22	2000 lux	25x150 mm tubes	7 (1 explant each)	3	3	4

Table 3. Media composition for *in vitro* conservation of potato, sweetpotato and other root and tuber crops

	Potato (*)			Sweet potato	Oca	Ulluco	Mashua	Achira	Yacon	Arracacha
	No. 22	No. 32	No. 42							
MS salts	1	1	1	1	1	1	1	1	1	1
Ascorbic acid (g/L)	-	-	-	0.2	-	-	-	-	-	-
ANA (mg/L)	-	-	-	-	-	-	-	-	0.05	
BAP (mg/L)	-	-	-	-	-	-	-	4	-	4
Calcium nitrate (g/L)	-	-	-	0.1	-	-	-	-	-	-
Calcium panthotenate (mg/L)	-	-	-	2	2	2	2	-	2	-
Giberellic acid									2	
Glycine-HCl (mg/L)	2	2	2	-	2	2	2	2	2	2
L-Arginine (g/L)	-	-		0.1	-	-	-	-	-	-
myo-inositol (mg/L)	100	100	100	-	100	100	100	100	100	100
Nicotinic acid (mg/L)	0.5	0.5	0.5	-	0.5	0.5	0.5	0.5	0.5	0.5
Putrescine-HCl (mg/L)	-	-	-	20	-	-	-	-	-	-
Pyridoxine-HCl (mg/L)	0.5	0.5	0.5	-	0.5	0.5	0.5	0.5	0.5	0.5
Thiamine-HCl (mg/L)	0.1	0.1	0.1	-	0.1	0.1	0.1	0.1	0.1	0.1
Sucrose (g/L)	20	20	20	30	20	20	20	25	30	25
Sorbitol (g/L)	20	30	40	-	30	30	30	-	-	-
Agar (g/L)	6.5	6.5	6.5	-	7	7	7	7	7	7
Phytagel (g/L)	-	-		3	-	-	-	-	-	-
pH	5.6	5.6	5.6	5.7	5.6	5.6	5.6	5.6	5.6	5.6

(*) For accessions with good growth under osmotic stress, use 3 tubes with conservation media No. 42; for mean growth use 2 tubes with conservation media No. 42 and one with No. 32; for regular growth use 2 tubes with conservation media No.22 and 1 with No.22

Table 4. Indicators used for viability evaluation of *in vitro* collections maintained by CIP

Crop and Viability categories		Indicators by main factors affecting viability	
POTATO and MASHUA		Stem Necrosis	
	Good	0-10%	
	Medium	10-30%	
	Bad	30-70%	
	Lost (died)	100%	
SWEETPOTATO		**Shoot/ Stem Necrosis**	**Defoliation**
	Good	0-10%	0-20%
	Medium	10-30%	20-50%
	Bad	30-70%	50- 70%
	Lost (died)	100%	100%
YACON, ACHIRA and ARRACACHA		**Stem Necrosis**	**Defoliation**
	Good	0-10%	0-20%
	Medium	10-30%	20-50%
	Bad	30-70%	50- 70%
	Lost (died)	100%	100%
OCA and ULLUCO		**Oxidation (browning)***	**Stem Necrosis**
	Good	No	0-5%
	Medium	Light	5-30%
	Bad	Medium - High	30-70%
	Lost (died)	High	100%

* Brown color observed in the culture medium and plantlets foliage

Table 5. Currently inventory of CIP *in vitro* genebank indicating backlogs fro pathogen elimination (2009)

Collection		Accessions				
		Number *In vitro*	Virus tested			Backlog to clean
			HS1 [2]	HS2 [3]	HS2%	
Potato	Long-term holdings	5551	1326	3087	56	1138
	Under International treaty	4682	748	2963	63	971
	Landraces	4441	747	2744	62	950
	Wild and weedy	24	1	6	25	17
	Improved material	217	0	213	98	4
	No under International treaty [1]	869	578	124	14	167
	Transitory Holdings [1]	3906	443	2181	56	1282
	Total	**9457**	**1769**	**5268**	**56**	**2426**
Sweetpotato	Long-term holdings	5454	15	2396	44	3043
	Under International treaty	3900	14	1864	48	2022
	Landrace	3677	13	1731	47	1933
	Wild	6		2	33	4
	Improved material	217	1	131	60	85
	No under International treaty [1]	1554	1	532	34	1021
	Transitory Holdings [1]	45	1	24	53	20
	Total	**5499**	**16**	**2420**	**44**	**3063**
ARTC	Long-term holdings	998	20	352	35	626
	Under International treaty	998	20	352	35	626
	Arracacia xanthorriza	2	0	0	0	2
	Canna indica	11	7	0	0	4
	Oxalis spp	3	0	1	33	2
	Oxalis tuberosum	488	2	258	53	228
	Smalanthus sonchifolia	29	0	27	93	2
	Tropaeolum tuberosus	47	0	5	11	42
	Ullucus spp	2	0	2	100	0
	Ullucus tuberosus	416	11	59	14	346
	Transitory Holdings [1]	158	5	28	18	125
	Total	**1156**	**25**	**380**	**33**	**751**
	Total of Long-term holdings	12003	1361	5835	49	4807
	Total of Transitory Holdings	4109	449	2233	54	1427
Total		**16112**	**1810**	**8068**	**50**	**6234**

1) Collections comprise research and breeding material

2) HS1 : Plant material tested negative to viruses of economic importance and pathogens of quarantine significance; PLRV, APLV, PVY, PVX, PVS, APMoV, PSTVd and PVT, for potato; and SPCSV for sweetpotato.

3) HS2 : Plant material tested negative to all known pathogens

Table 6. Potato landraces subjected to two shoot tip-donor-plantlet culture temperature treatments and showing at least 20% recovery from cryopreservation

Species	Number of cryo-tested accessions	Accessions recovered • 20%, by culture treatment								Difference between 22° and 6°C (T-test, *P*>0.05)
		22° & 6°C[1]		22°C[2]		6°C[2]		Total		
		Num.	%	Num.	%	Num.	%	Num.	%	
S. tuberosum subsp. *andigenum*	379	123	32	35	9	93	25	251	66	**
S. stenotomum subsp *stenotomum*	26	8	31	7	27	4	15	19	73	ns
S. phureja	18	7	39	1	6	3	17	11	61	ns
S. stenotomum subsp. *goniocalyx*	11	5	45	1	9	2	18	8	73	ns
Total	434	148	33	44	**10**	102	**24**	289	67	**

[1] Accessions responding to both 22°C and 6°C treatments with recovery • to 20%.
[2] Accessions with recovery •20% only in one treatment.

Table 7. Repatriation of native potato varieties from CIP genebank to farmer communities of Peru (1998-2009)

Communities	No. Samples	Purpose	Communities	No. Samples	Purpose
Dpt. Apurimac			Dpt. Huanuco		
Tintay (Aymaraes)	55	Diseases	Iscopampa	51	Diseases
			Huamally	73	Diseases
			PRAA-Huánuco	53	Diseases
Dpt. Arequipa					
Chuquibamba Inst. Agrop (Condesuyos)	25	Prize	Dpt. Junin		
Chuquibamba (Condesuyos)	25	Prize	Racracalla (Concepción)	88	Restoration
			Mamac (Concepción)	88	Restoration
Dpt. Ayacucho			Andas (Concepción)	88	Restoration
Llamanniyoc (Huanta)*	86	Terrorism	Pahualtupo (Concepción)	95	Restoration
Chiara, INIEA-Canaan	80	Diseases	Cayash (San Pedro de Cajas)	68	Diseases
			Cascas (Tarma)	68	Diseases
Dpt. Cajamarca			La Libertad (Concepción)	50	Diseases
Cajamarca-ONG	28	Diseases	Muqui (Jaula)	100	Restoration
INIA-Baños del Inca	112	Diseases	Tarmatambo (Tarma)	20	Restoration
Dpt. Cusco			Dpt. Lima		
Urinsaya Anansaya Ccollana	172	Diseases	Cochas-Paca (Cajatambo)*	109	Restoration
Chisicata (Espinar)			Laraos (Yauyos)	22	Restoration
Chahuaytire, Pampallacta, Paru-Paru,	410	Restoration	Miraflores (Yauyos)	47	Prize

Communities	No. Samples	Purpose	Communities	No. Samples	Purpose
Amaru, Cuyo Grande, Sacaca			Huancayo (Yauyos)	47	Prize
Instituto Técnico Agropecuario	60	Restoration	Laraos (Yauyos)	47	Prize
Bilingüe Patacancha			Curquish (Cajatambo)*	55	Prize
Conservacionistas de papas nativas	156	Restoration			
			Dpt. Pasco		
Dpt. Huancavelica			UNDAC-Pasco	33	Diseases
San José de Aymará (Tayacaja)*	344	Restoration	Chinchan (Huariaca) Dpt.	22	Restoration
Collpatambo (Tayacaja)	244	Restoration			
Castrovirreyna	35	Diseases	Dpt. Piura		
Ticrapo	35	Diseases	Sondorillo (Huancabamba)	15	Restoration
Paucará	35	Diseases			
Chopkas, ONG RuruInka	50	Diseases	Dpt. Puno		
Chopkas, ONG Yanapai	172	Restoration	INIA-Puno	45	Diseases
Chonta, MP Churcampa	150	Restoration			
Churcampa (Castrovirreyna)	50	Diseases			
Total = 41 communities and 6 institutions 3608 (= 1250 cvs)					

Identification of duplicate accessions within a sweetpotato germplasm collection using morphological characterization and AFLP markers

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Abstract

The International Potato Center (CIP) sweetpotato germplasm collection consists of 8,018 accessions. In 1988 CIP Annual Report, it was reported about 66% of the Peruvian collection represent duplicates. This high percentage of duplicates is also anticipated in the Latin American collection. The cost of maintenance of duplicates is high because they are maintained both in greenhouse and in vitro collections. For this reason the rationalization is necessary through identification and elimination of duplicates.

Three hundred and sixty accessions collected from Perú grouped into 119 synonym groups were studied. The morphological and molecular characterization were done using 33 morphological descriptors according to descriptors for sweetpotato (CIP, AVRDC and IBPGR, 1991) and seven AFLP primer combinations following procedures of Vos et al. (1995) with IRDye in LICOR 4300 System. Cluster analysis for morphological and molecular data was based on Average taxonomic distance and Jaccard coefficients, respectively and the unweighted pair-group method using an arithmetic average (UPGMA) algorithm with NTSYS-pc version 2.1.

The result showed that the 360 accessions studied can be reduced to 119 unique genotypes and the remaining 241 accessions consist of 197 duplicates and 44 require further evaluation.

Keywords: *Ipomoea batatas*, morphological characterization, AFLP, duplicate accessions.

Introduction

The sweetpotato germplasm collection consists of 8,017 accessions. In the 1988 CIP Annual Report, it was reported about 66% of the Peruvian collection represent duplicates. This high percentage of duplicates is also anticipated in the Latin American collection. The maintenance cost of duplicates is high because they are maintained both in greenhouse and in vitro collections. For this reason the rationalization in the identification of duplicates is very important. We are using firstly morphological characterization in the field using the international standard descriptors and then to complement with the molecular characterization using AFLP markers.

Materials and methods

Germplasm studied

Three hundred and sixty accessions collected from Perú grouped into 119 synonym groups were used in this study. The plant material was obtained from screenhouses at San Ramon and La Molina and CIP in vitro Genebank in Lima, Peru. All expected duplicates were grouped by their similarities of morphological descriptors by cluster analysis, (Huamán, 1997).

Morphological characterization

All accessions were planted with 2 replications and characterized with 30 morphological descriptors according to descriptor for sweetpotato (CIP,AVRDC and IBPGR) in May- June in la Molina field with a Pocket PC. The list of morphological descriptors were:

1	Plant type	16	Petiole pigmentation
2	Ground cover	17	Petiole length
3	Vine internode diameter	18	Storage root shape
4	Vine internode length	19	Storage root surface defects
5	Predominant vine color	20	Storage root cortex thickness
6	Secondary vine color	21	Predominant skin color
7	Vine pubescence	22	Intensity of predominant skin color
8	General outline of the leaf	23	Secondary skin color
9	Leaf lobes type	24	Predominant flesh color
10	Leaf lobe number	25	Secondary flesh color
11	Shape of central leaf lobe	26	Distribution of secondary flesh color
12	Leaf size	27	Storage root formation
13	Leaf vine	28	Latex production storage roots
14	Mature leaf color	29	Oxidation in storage roots
15	Inmature leaf color	30	Twining

Molecular characterization

Leaf samples. At four months of sowing, five to seven (100 mg.) healthy young leaves of each accession were collected from plants in the field. These leaves were placed in a tube containing CTAB 2X buffer and transferred into icebox.

Extraction of DNA. Genomic DNA was isolated from young fresh leaf tissue using a modification of the CTAB method (Doyle & Doyle, 1987 as modified by NCSU, 1990). Quantification and quality of the DNA extracted were checked in 1% agarose gel using lambda DNA standard as control. DNA samples were diluted to 100 ng/ul for AFLP analysis.

AFLP procedure. AFLP markers were obtained using 7 primers combinations (Table 1) marked with IRDyes (Infrared dyes) and visualized in a LI-COR 4300 Hightroughput System. The AFLP protocol used follow the procedure described by Vos et al. (1995) with the modifications listed below. The DNA was digested using two restriction enzymes EcoRI / MseI. AFLP adapters for both restriction enzymes were then ligated to the restriction fragments. Subsequently, template DNA was pre-amplified using primer combinations based on the sequence of the adapters but 3' – extended without selective nucleotide (EcoRI + 00/MseI + 00).

The second amplification reaction used primers marked with IRDye* infrared dyes.

PCR was completed in a PTC-100 thermocycler programmed for 1 cycle of 30sec at 94ºC; 30sec at 65ºC; 2min at 72ºC foll1owed by 12 cycles in which the annealing temperature decrease 1.0ºC per cycle, followed by 30 cycles of 30sec at 94ºC; 30sec at 56ºC; 2min at 72ºC.

Data analysis

Morphological characters. The morphological data were recorded using a 0-9 scale for all 30 descriptors. Cluster analysis of the morphological data was performed with NTSYS-pc version 2.1 (Rohlf,1993) based on Average taxonomic distance coefficient (DIST) and the unweighted pair-group method using an arithmetic averages (UPGMA)

DNA markers

The AFLP profiles generated by the 7 primer combinations were scored for the presence (1) or absence (0) of each fragment and missing data (9). Only those fragments with high intensity were counted. Scores were recorded using SAGA MX GT Generation 2 Software. The NTSYS –PC software package, version 2.1 (Rohlf 1993) was used to compute a matrix using Jaccard coefficients, based on this matrix we perform cluster analysis with the option UPGMA (Unweighted pair-group method) producing a cluster dendrogram.

Table 1. AFLP primer combinations for identification of duplicates in sweetpotato

Primer Combinations	Primer Name	Sequence (5'-3')	Annealing temperature (°C)
E32-M48	E32-AAC	GACTGCGTACCAATTCAAC	56
	M48-CAC	GATGAGTCCTGAGTAACAC	56
E35-M36	E35-ACA	GACTGCGTACCAATTCACA	56
	M36-ACC	GATGAGTCCTGAGTAAACC	56
E35-M48	E35-ACA	GACTGCGTACCAATTCACA	56
	M48-CAC	GATGAGTCCTGAGTAACAC	56
E36-M50	E36-ACC	GACTGCGTACCAATTCACC	56
	M50-CAT	GATGAGTCCTGAGTAACAT	56
E38-M61	E38-ACT	GACTGCGTACCAATTCACT	56
	M61-CTG	GATGAGTCCTGAGTAACTG	56
E39-M49	E39-AGA	GACTGCGTACCAATTCAGA	56
	M49-CAG	GATGAGTCCTGAGTAACAG	56
E42-M35	E42-AGT	GACTGCGTACCAATTCAGT	56
	M35-ACA	GATGAGTCCTGAGTAAACA	56

Results and discussion

Morphological markers. A dendrogram showing the relationships between accessions is presented in Figure 1.

At 100% of similarity, from 226 accessions we identify 83 unique genotypes, in other group of 90 accessions we identify 36 unique genotypes and the remaining 44 accessions need further evaluation. No differences were found between the two replications.

DNA markers. The 7 primer combinations generated 270 clearly scorable polymorphic fragments for the 360 accessions. (Fig 1). At 100% of similarity, from 229 accessions we identify 80 unique genotypes, in other group of 87 accessions we identify 37 unique genotypes and the remaining 48 accessions need further evaluation.

Morphological and Molecular markers. The result showed that the 360 accessions studied can be reduced to 119 unique genotypes and the remaining 241 accessions consist of 197 duplicates and 44 require further evaluation.).

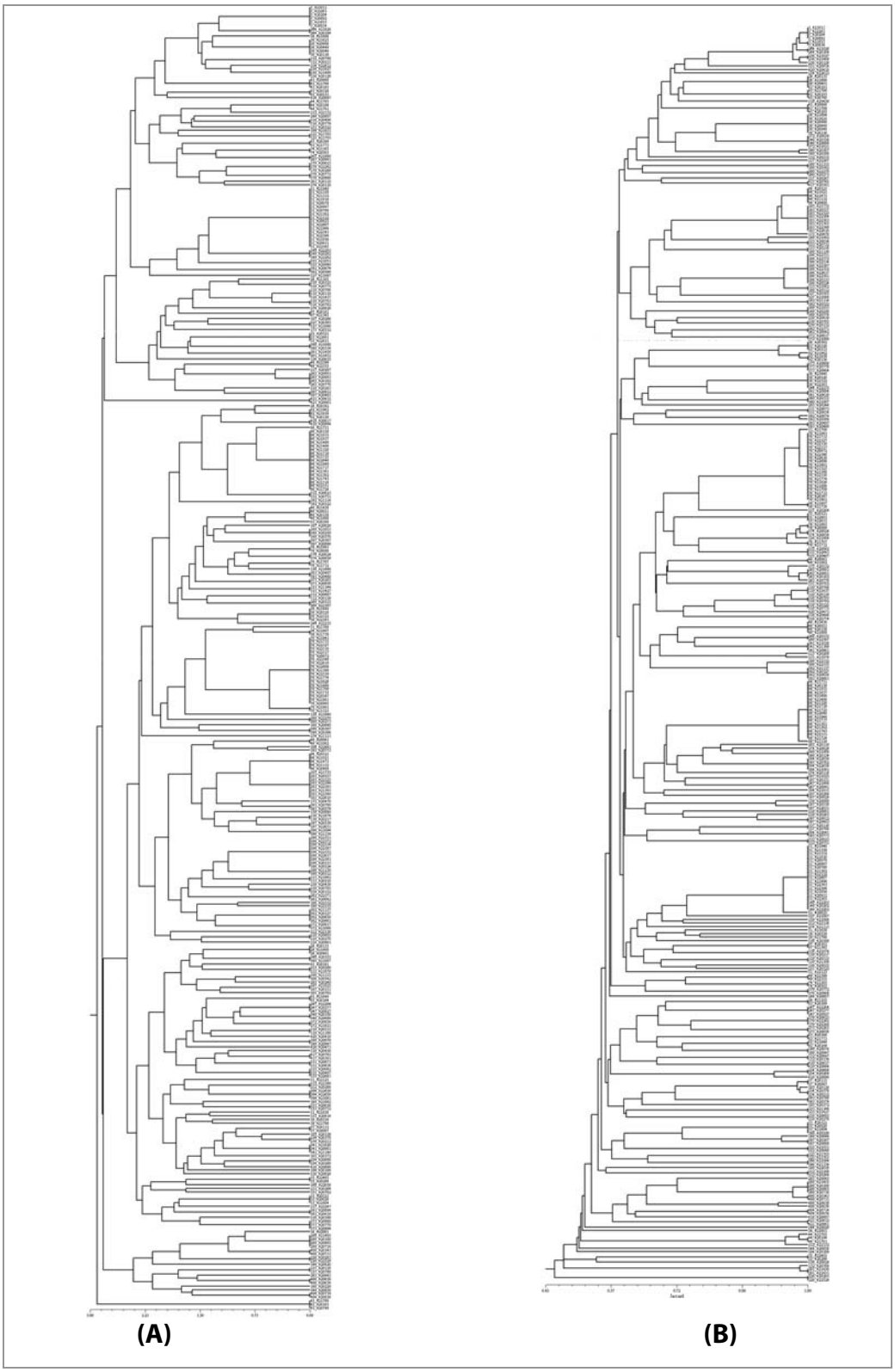

Figure 1. Cluster analysis for morphological (A) and molecular (B) characterization for the 119 synonym groups

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Ex situ conservation of underutilized Andean roots and tubers

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General survey

Astute Andean farmers domesticated at least nine roots and tubers thousands of years before the arrival of European explorers. According to their economic importance, they include oca, ulluco, mashua, and maca from the highlands; and arracacha, yacón, achira, ahipa and mauka from the warm Andean valleys (Table 1). These crops are grown from southern Venezuela to northwestern Argentina with the highest cultivar diversity and uses from central Peru to central Bolivia.

Although the first attempt to maintain *ex situ* oca, ulluco, and mashua collections was carried out by the Bolivian agronomist Walter Cevallos as early as 1910 by collecting and conserving 9 accessions of oca, 5 of ulluco, and 2 of mashua in Oruro, Bolivia to study their morphological variations (Alandia, 1994), systematic ex situ conservation of ART however can be seen in four stages. In the first one, comprising from 1926 to 1933, the team leaded by N.I. Vavilov collected, maintained and studied morphologically, physiologically, and bromatologically oca, ulluco, and mashua in Leningrad. One of the main conclusions of Vavilov was that oca, ulluco, and mashua were of short day reaction, and variation was high in Peru, and Bolivia (Bukasov, 1930).

The second stage lasted from 1958 to 1965 under the auspices of the Interamerican Institute for Cooperation in Agriculture (IICA) of the American State Organization-Andean Zone with funding from the Rokefeller Foundation. Thus, under the leadership of Martín Cárdenas of Universidad Mayor de San Simón, Bolivia, it was collected and studied morphological variation in 148 accessions of oca, 91 of ulluco, and 60 of mashua of Peru, Bolivia, Ecuador, Colombia, Argentina, Venezuela, Chile, and México (Cárdenas, 1985). The collections were maintained until the project came to and end in 1965, in which the material was distributed to the Universities of Cusco, Huancayo, Ayacucho, and Cajamarca, Peru (Rea, pers. comm.; Valladolid, pers. comm.). Evaluation of this material for more than 10 years in Huancayo resulted in the release of two new varieties of oca namely *Huanca,* and *florencio* (López, pers. comm.).

The third stage took place from 1979 to 1989 under the auspices of the International Board for Plant Genetic Resources of FAO (IBPGR, later IPGRI, now Bioversity International) supporting collections of Andean roots and tubers in Peru, Bolivia, and Ecuador). The International Development Research Center (IDRC), Canadá, and IICA also cooperated to the IBPGR funded project of oca, ulluco, mashua, arracacha, achira, yacón, maca, and mauka (Arbizu, 1981; Arbizu and Robles 1986). The main purpose of IBPGR was to safeguard diversity of roots and tubers in the Andes, and to carry out morphological characterization and preliminary evaluation.

The forth stage started in 1990 when the International Potato Center, with funding from the German Ministry for Economic Cooperation (BMZ) and the Agency for Technical Cooperation (GTZ), supported key exploration and/or collections of oca, ulluco, mashua, arracacha, achira, yacón, and maca and their wild allies in Peru, Bolivia, Argentina, Ecuador, Colombia, and Chile to be maintained, characterized and evaluated in Quito, Ecuador under the leadership of Michael Hermann. More intensive work on ex situ conservation of ART was followed in 1993 with funding of Swiss cooperation (COSUDE) to safeguard and utilize the biodiversity of nine Andean roots and tubers mainly in Peru, Bolivia and Ecuador. The main contribution of the forth stage has been safe conservation of nine ART in INIAP, Ecuador; PROINPA, Bolivia; University of Cajamarca, Peru, University of Cusco, Peru, and CIP.

It has been reported the conservation of 122 collections of ART (24 of oca, 22 of ulluco, 22 of mashua, 19 of arracacha, 13 of yacon, 11 of achira, 7 of maca, 3 of mauka, and 1 of *Pachyrhizus*) carried our by some 20 institutions from 1958 to 2001. Most of them maintained important ART collections provided that there was external funding available, but collections were dramatically reduced, or lost when funded projects came to an end. Thus, the number of accessions maintained by Andean gene banks have shown ups and downs from 1958 to 2001 (Table 2).

Table 1. Main features of Andean roots and tubers

Crop	Botanical name	Family	Altitude (m)	Edible part	Fresh	Traditional processing
Oca	*Oxalis tuberosa*	Oxalidaceae	3000-4000	Tuber	Boiled, baked	*Kaya*
Ulluco	*Ullucus tuberosus*	Basellaceae	3000-4000	Tuber	Soups, stews, salads	*Lingle/chullqi*
Mashua	*Tropaeolum tuberosum*	Tropaeolaceae	3000-4000	Tuber	Boiled, baked	*Tayacha*
Arracacha	*Arracacia xanthorrhiza*	Apiaceae	1000-3300	Root	Boiled, baked, soups, stews, fried, puddings, instant soup, baby food	Kawi
Achira	Canna indica	*Cannaceae*	2000-2900	Rhizome	Baked, boiled, industrial starch	starch
Yacon	*Smallantus sonchifolius*	Asteraceae	1300-3300	Root	Snacks, syrup	Sugar
Maca	*Lepidium meyenii*	Brassicaceae	3900-4500	Hypocotyl	Baked, salad	Juices, bakery, biscuits, drink
Mauka	*Mirabilis expansa*	Nyctaginaceae	2300-3200	Root	Boiled, stews, soups, fried, puddings	Grated
Ahipa	*Pachyrhizus ahipa*	Fabaceae	1500-3000	Root	Snacks, salads	

Table 2. Accessions of ART maintained by Andean gene banks from 1958 to 2001

Crop	1958	1968	1978	1988	1992	1993	1996	2001*
Oca	148		400	950	585	3282	4396	3486
Ulluco	91			746	874	2034	2517	2156
Mashua	60			470	180	725	1082	810
Arracacha		37		123	29	921	687	807
Yacón				39	22	105	479	537
Achira					23	108	417	478
Maca				3	33	48	53	35
Mauka						2	117	93
Pachyrhizus						2	72	10
TOTAL	**299**	**37**	**400**	**2331**	**1746**	**7227**	**9820**	**8412**

* Adapted from Talledo et al., 2001

a. Blank spaces: No information available
b. Reporting institutions (an archive is kept by the author).
1958: IICA=Instituto Interamericano de Cooperación para la Agricultura
1968: UNC=Universidad Nacional de Cajamarca, Perú.
1978: UNSAAC=Universidad Nacional San Antonio Abad del Cusco, Perú
1988: INIA-Cajamarca, -Huancayo, -Huaraz=Instituto Nacional de Investigación Agropecuaria, Perú; UNMSM=Universidad Nacional Mayor de San Marcos, Perú.

1992: CERRGETYR=Centro Regional de Recursos Genéticos de Tubérculos y Raices, CICA= Centro de Investigación de Cultivos Andinos, UNSAAC; CIP=Centro Internacional de la Papa; IBTA=Instituto Boliviano de Tecnología Agropecuaria, Bolivia; UNMSM.

1993:CERRGETYR; CNPH-EMBRAPA=Centro Nacional de Pesquisa de Hortalizas-Empresa Brasileña de Pesquisa Agropecuaria, Brasil; CICA; CIP; IBTA; INIA-Lima; INIAP=Instituto Nacional de Investigaciones Agropecuarias, Ecuador; PROINPA=Programa de Investigación en Papa, Bolivia; UNC; UNCP=Universidad Nacional del Centro del Perú; UNDAC= Universidad Nacional Daniel Alcides Carrión, Perú; UNSCH=Universidad Nacional de San Cristóbal de Huamanga, Perú; UNMSM.

1996: CICA; CIP; CRIBA=Centro Regional de Investigacón en Biodiversidad Andina, UNSAAC; INIA, Perú; INIAP; PROINPA; UNC; UNDAC; UNMSM; UNSCH.
2001: CICA; CIP; CORPOICA=Corporación Colombiana de Investigación Agropecuaria; Colombia; CRIBA; INIA, Cusco, Perú; INIAP; PROINPA; UNC.

Conservation strategies

Once the genetic resources of ART are collected, they need to be maintained for current and future use. Conservation is carried out by means of three main strategies: field collection, in vitro, and seed. Additionally, DNA and herbarium have been considered lately. Furthermore, a bar code system has been developed since 2002 by CIP to optimize management of ART throughout conservation strategies (Rojas et al., 2008).

Field conservation

This strategy is the ancients one and relatively easy to perform. Most Andean gene banks have maintained field collections since they were set up with their holdings going up and down according to availability of external funding.

a. Tuber crops. Field conservation of oca, ulluco, and mashua implies clonal conservation of each accession and should take place at 3500- 3800 m above sea level in well drainage soils with high levels of organic matter, and frost free. If the collections were planted at lower altitudes, virus, bacterial, and fungal diseases would put at risk the collections of oca, ulluco and mashua due to the fact that they are planted out of their ecological niches. Thus for instance, the fungus *Verticillium dahliae* can threaten seriously ulluco field collections planted at about 3200 m by axilary bud proliferation in stems and thin sprout proliferation of tubers (Otazú et al., 1998). Also, our data suggest that virus diseases can put at risk collections of oca, and ulluco planted at low altitudes (3200 m.).

Oca weevil (*Adioristidius tuberculatus,*Curculionidae), and ulluco weevil (A*mathynetoides nitidiventris,*Curculionidae) (www.mcknight.org), which are serious pests for oca and ulluco field collections respectively, can be prevented by planting them after fallowing complemented with plastic fences. Also, it is recommended that the field collection of oca, ulluco, and mashua be allocated as far away as possible of potato fields, as three potato virus diseases are shared with ulluco (PLRV, APLV, PVT), two with ocas (PBRSV, and PVT), and one with mashua (PVT) (Fuentes and Chuquillanqui, 2004; Lizárraga et al., 1997). Mashua does not show neither serious pests nor diseases to threaten its field conservation explained by isothiocyanates and other repellent factors.

Planting time usually takes place at the onset of the rainy season, in this case each tuber seed used for planting usually weights 20-40 g and the recommended density for oca and ulluco is 0.85-1.20 m between rows and 0.30-

0.40 m within rows. Whereas, planting density for mashua can be 1.10-1.70 m between rows, and 0.30-0.50 m between plants. A total of 5-13 plants per accession are planted. Harvest of oca, ulluco, and mashua takes place 7-8 months after planting once the rainy season comes to an end, and the dry one starts in the Andes. At this time tubers are enough expanded and mature. Some 20-60 tubers (20-40 g each) per accession of oca, ulluco, and mashua are put in paper bag, or mesh plastic bag, or wooden box to store the collection during the dry season for conservation during 4-5 months at ordinary room temperature ranging from 8-16 °C, until the onset of the new growing season. To prevent any risk of losing valuable genetic material in the field, some tubers should be kept extra time (5-6 months) to guaranty safe continuity of the field conservation program (Talledo et al., 2001).

There are sometimes accessions of oca, ulluco, or mashua with tuber seed scarcity for planting, or tuber seeds are too small to plant in the field, suggesting that the accession is under risk of losing in the field. To guaranty safe conservation of the accession, rapid multiplication technique can be used to increase tuber seed as fast as possible by means of tuber sprouts, or juvenile cuttings or lateral shoot cuttings (Bryan at al., 1981; López, 2004). Successful rooting of juvenile cuttings or lateral shoot cuttings can easily be achieved by sinking 30% of the basal part just in tap water for about one an a half weeks, after which the plantlets are transferred to jiffy seven for 3-4 weeks, and later on to pots or to the field to complete their growing period with tuber production at the end.

b. Roots crops. The main criterion is again to maintain clonally each accession of arracacha, yacon, achira or mauka. That is, they must be planted and harvested once a year. Planting usually takes place at the onset of the rainy season. Crop husbandry however, to a certain extent, are rather different for the crops involved (Table 3). Once the growing period comes to an end, propagules are stored in wooden boxes or mesh plastic bags and left at room temperature ranging from 11 to 14 °C (Talledo et al., 2001).

Exception to the above field conservation occurs in achira as most of the clonal accessions of both the cultivated and its wild allies can be grown in the field for more than 10 years provided that the pants are pruned, fertilized, and watered. Some clones of yacon also have shown this valuable feature allowing reduction on the field conservation costs of the collections.

Table 3. Specifications for maintaining field collection of Andean root crops

Plant	Planting propagule	Weight of each propagule (g)	Distance between plants (m)	Distance between rows (m)	No. of plants per accession	Growing period (months)	Storage time (days)
Arracacha	Cormels ("colinos")	20	0.3-0.6	0.8-1.1	5-15	8-12	0-60
Yacón	Offsets	20	0.5-1.0	0.8-1.2	5-10	1012	0-30
Achira	Rhizome tips	50-60	0.5-1.0	0.8-1.1	5-10	9-12	0-45
Mauka	Stem cutting	15	0.5-1.0	0.7-1.1	10-15	9-12	0-60

2. *In vitro* conservation. There has been considerable progress on *in vitro* active collection of oca, ulluco, and mashua since the works pioneered by Estrada and coworkers in the eighties. The most appropriate protocols have been developed for slow growth of oca and ulluco by using low temperature (6-8°C) combined with sorbitol osmotic stress complemented with light intensity of 1000 lux for 16 hours, giving as a result the storage of oca and ulluco for at least one year before transferring to fresh medium (Panta and Roca, 2008; Inouye, pers. comm.; Panta, pers. comm.). Arracacha, yacon, and achira conservation on the other hand, have been performed by in vitro subculture every 3-4 months since protocols development by the middle of the nineties (Panta and Roca, 2008; Panta et al., 1994: Toledo et al., 1994).

As far as in vitro base collection is concerned, although this is still in its infancy, preliminary tests using droplet-vetrification protocols have shown promising results as recovery after cryopreservation or freeze preservation

ranged from 13 to 35% in ulluco, and 7 to 15% in oca, and depends on genotypes (Panta, 2007; Sánchez et al., 2008).

In vitro strategy has facilitated pathogen elimination by thermotherapy followed by meristem culture to distribute pathogen free material to users. Pathogen free material has played a role in restoring diversity and productivity of oca, ulluco, mashua, and yacon in traditional farming communities of the Andes.

Seed conservation

Pachyrhyzus (*P. ahipa*, *P. tuberosus*, and *P. erosus*) and maca are seed propagated crops of orthodox behavior. They are being maintained in a long term conservation program (-20°C) only by CIP. A thousand seeds of these species weight about 325, 598, and 170 g respectively. The same amount of maca on the other hand, weights about 0.8 g.

Other crops routinely maintained as seeds of orthodox behavior in a long term program are achira, mauka and oca to complement their clonal conservation of both the cultivated and wild material. A thousand seeds of these crops weight about 280, 7, 0.5 g. Seeds of the wild *Canna* on the other hand are smaller and a thousand seeds weight about 130 g. Although mashua set considerable amount of seeds, it is necessary however to determine their reaction to conservation under cold room.

DNA conservation.

DNA of each clonal accession can be maintained at –70 °C as an active, and a base collection. This strategy is easy, simple and efficient. It will help to verify the identity of accessions maintained clonally either in vitro or in the field. So far, 585 accessions of oca, 107 of mashua, 46 of arracacha, 36 of yacón, and 13 of ulluco are being maintained by CIP (Zorrilla et al., 2007; Rossel, pers. comm..).

Herbarium

The University of Cajamarca, Perú and CIP house more than 1500 specimens of oca, ulluco, mashua, arracacha, achira, yacon, and mauka (Seminario, pers. comm.). They are being used to check identity of the material, if necessary.

Characterization and preliminary evaluation

Morphological characterization and preliminary evaluation have been the concern of most Andean curator since the first works of Cárdenas by the end of the fifties. Some 11,527 accessions have been morphologically characterized or have undergone preliminary evaluation since 1958 (Arbizu et al., 1997; Talledo et al., 2001). Morphological characterization of oca, and ulluco has been dynamized by using standard descriptor lists during the current decade. Also, descriptor lists of mashua, arracacha, yacon, achira, and mauka have been tested by Andean gene bank curators. Qualitative morphological characters have shown excellent resolution of the morphotypes for each crop. Most crops maintained by Andean gene banks are highly redundant (20 - >50%) in terms of morphological features. So, molecular characterization is needed to reduce the size of the collections. But, before eliminating the duplicates, they will the transformed into seeds to be maintained in a long term conservation program.

Preliminary evaluation of ART has resulted in the identification of promising material, followed by its multiplication, and distribution to users. Thus, the new cultivars of oca namely *pachatuzan*, and *kayra* were released by CICA, Cusco, Peru in 1976, and 1977 respectively (Cortez, pers. comm.). Also, the oca cvs *huanca*, and *florencio* were released by Salazar at the Universidad Nacional del Centro del Peru in the eighties (López, pers. comm.). In a similar manner, new cvs of ulluco namely INIAP- *puka*, INIAP- *qillo*, and INIAP-*rumi* were released by the Instituto Nacional de Investigación Agropecuaria, Ecuador in 1994. By the end of the nineties, INIAP also released another new cv of ulluco called INIAP-*caramelo* (Holle, pers. comm.).

Conclusions

Conservation of the genetic resources of ART by gene banks has been successful while external funding was available. Promising material has been identified, multiplied, and distributed to farmers to restore diversity and productivity of ART in rural communities that had lost their planting material due to biotic, abiotic, and social factors. Thus, *ex situ* and *in situ* conservation has been dynamized. Close cooperation among Andean gene banks has been established to optimize collection, conservation, and utilization of ART.

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www.mcknight.org. Collaborative Crop Research Program.

Genetic diversity of yacon (*Smallanthus sonchifolius)* in Peru

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Abstract

Yacon -an ancient Andean root crop- is considered a truly functional food because it is an important source of health related compounds, including fructooligosaccharides and antioxidants. The plant is grown from Ecuador to northwestern Argentina, with the highest concentration of diversity between Peru and Bolivia. Genetic diversity of yacon in Peru has been studied only by means of morphological features, employing non-standardized descriptors. In this work a total of 309 polymorphic AFLP markers –obtained from 6 primer combinations- were used to study the diversity of 359 accessions of yacon maintained by six Peruvian genebanks.

Only seven accessions were identified as duplicates and 352 were unique genotypes. However, the genetic diversity index (Nei and Shannon-Weaver) and the analysis of molecular variances (AMOVA) showed low levels of diversity between genebanks, and within them. The cluster and factorial analyses identified three major groups of yacon in the collection: the first and second groups included accessions from northern and southern Peru, respectively; a third group consisting of accessions from northern, central and southern regions. These results could be useful to establish strategies for future collecting missions and germplasm conservation.

Keywords: Genetic diversity, yacón, *Smallanthus sonchinfolius,* AFLP.

Introduction

The yacon, is an Andean root crop domesticated before the pre-inca times. Its reservant roots are rich sources of fenolic compounds and fructooligosacarides (3.8% and 60% of dry weight, respectively) (Seminario et al., 2003). There are evidences that these compounds generate benefic effects in the health and play important functions in the prevention of several chronic diseases, such as diabetes, obesity, dyslipidemia, atherosclerosis and colon cancer (Valentová et al., 2004: Genta et al., 2009).

The yacon is the only species domesticate and eatable of the genus *Smallanthus*. Its natural habitat is the Andean region from the south of Colombia to the northwest of Argentina, and the most probable center of origin is a narrow slope between south of Peru and north of Bolivia (Grau and Rea, 1997). Six institutions of Peru, with a total of 359 accessions, maintain the biggest collections of yacon. These collections have been characterized morphologically in the past under different criteria and doing the evaluations in different environments and conditions. Therefore, there is no reliable information to estimate the total diversity of Peruvian yacon neither the number of morphotypes or genotypes.

At the molecular level, there are few studies reported in yacon. Milella et al. (2005) after testing five yacon clones by RAPD markers suggested that the technique could be useful for identification and differentiation of varieties. Mansilla et al. (2006) using RAPD markers in the collection of CIP reported more diversity in central Peru compared to the north and south. However, the contribution of both publications to assess the total genetic diversity of yacon was low because the numbers of samples were small and not representative. In comparison with the RAPD markers, the AFLP markers can be more useful for studies of diversity because they reveal a larger

number of polymorphisms and can differentiate duplicates genotypes. In addition, AFLP technique does not require DNA sequence information and the PCR technique is rapid and reproducible (Ferreira y Grattapaglia, 1998).

The aim of this study was to assess the genetic diversity of the six more representative *ex situ* collections of yacon of Peru, using AFLP markers.

Materials y methods

Plant material and DNA extraction

A total of 359 accessions of yacon of six institutions of Peru were tested: 48 accessions from CIP, 123 from INIA, 103 from UNC, 62 from UNSAAC, 13 from UNSCH and 10 from UNALM. DNA extraction was made from young leaves using CTAB technique (Doyle and Doyle, 1990) medium scale protocol standardized and reported by CIP (2000). The DNA concentration was calculated in comparison with known concentrations of Phage Lambda DNA digested with *Pst I* restriction enzyme and visualized in agarose gel 1%.

AFLP analysis

AFLP protocols are based on Vos et al. (1995), adapted for use with silver staining of 6% polyacrylamide gels. The protocols of DNA restriction and ligation, PCR amplifications and electrophoresis were used as reported in the manual by CIP (2000).

Data analysis

The AFLP bands pattern for each accession was recorded manually on a binary matrix. The present bands were recorded as 1 and those absent as 0. The identification of unique genotypes and redundancy was made using a cluster analysis based on Jaccard similarity index (1908) and the UPGMA algorithm (Sokal and Sneath, 1963) using the software DARwin5 (Perrier, et al., 2006). Genetic heterogeneity was estimated using the Nei's (1973) and Shannon-Weaver diversity index (1949), the genetic structure was conducted using the Principal Coordinates Factorial analysis and the changes of genetic diversity between collections was evaluated by analysis of molecular variance - AMOVA (Excoffier et al., 1992) using the software Arlequin ver. 3.11 (Excoffier et al., 2005).

Results

Selection of AFLP primers combinations (E+3/M+3)

Six primer combinations were selected from a test of 120 combinations of primers with three selective nucleotides. These primers were selected due to its high polymorphisms index and good resolution of the bands (Table 1).

Table 1. Combination of primers (E+3 / M+3) used in the molecular characterization of yacon

Cod. Lab.	Combination	Primer	Sequence (5'- 3')
E40-M36	EAGC-MACC	EAGC	GACTGCGTACCAATTC-**AGC**
		MACC	GATGAGTCCTGAGTAA-**ACC**
E38-M33	EACT-MAAG	EACT	GACTGCGTACCAATTC-**ACT**
		MAAG	GATGAGTCCTGACTAA-**AAG**
E39-M36	EAGA-MACC	EAGA	GACTGCGTACCAATTC-**AGA**
		MACC	GATGAGTCCTGAGTAA-**ACC**
E42-M35	EAGT-MACA	EAGT	GACTGCGTACCAATTC-**AGT**
		MACA	GATGAGTCCTGACTAA-**ACA**
E36-M55	EACC-MCGA	EACC	GACTGCGTACCAATTC-**ACC**
		MCGA	GATGAGTCCTGAGTAA-**CGA**
E37-M60	EACG-MCTC	EACG	GACTGCGTACCAATTC-**ACG**
		MCTC	GATGAGTCCTGAGTAA-**CTC**

Genotypes duplicates

From 359 accessions evaluated by 309 polymorphic markers generated by six AFLP primer combinations, 355 unique genotypes were found, although most of them share a close genetic similarity. In addition, three genotypes were represented by more than one accession; they were named duplicate accessions (100% of genetic similarity): two genotypes belonging to UNSAAC collection with 3 (ZS-074, ZS-024 and ZS-050) and 2 (ZS-072 and ZS-067) accessions respectively. The third genotype belongs to the CIP collection with 2 accessions (205037 and 205031).

Cluster analysis and geographical distributions pattern

Using a genetic similarity coefficient of 87% into cluster analysis, it was possible to identify 2 large molecular groups (I and II), which include 329 accessions. The 30 accessions remaining do not present a defined cluster because of their low genetic relatedness between them and the groups identified. In addition, the group I contains 4 subgroups: the subgroups A and C are formed by accession of the north, the subgroup B are formed by accessions of the south and the last subgroup and also the group II are formed by accessions from the three regions of Peru (Figure 1).

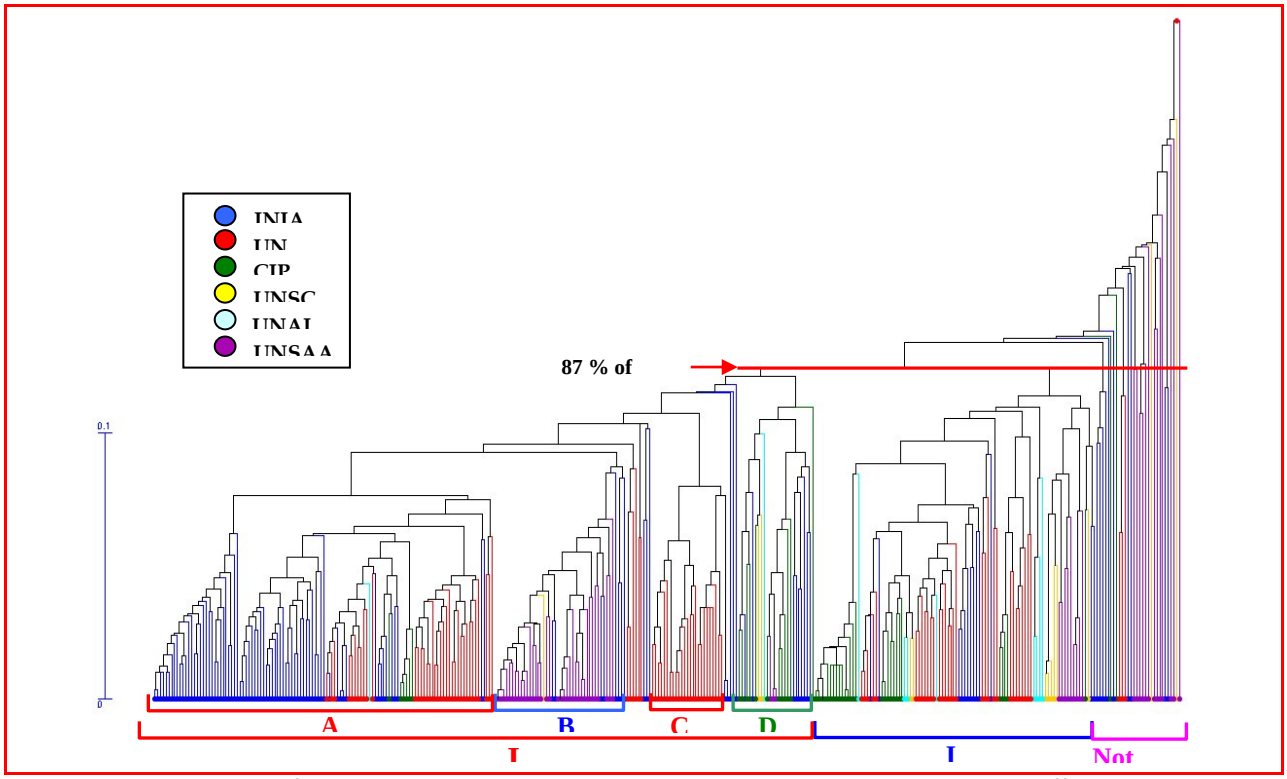

Figure 1. Dendrogram of yacon collections based on 309 AFLP markers using the Jaccard coefficient and UPGMA algorithm. subgroups A and C are formed by accessions from northern, subgroup B is formed by accessions from southern and subgroup D and Group II are formed by accessions from the three regions

The factorial analysis shows the presence of only three groups: 2 groups consisting of accessions from the north and south respectively, and a group of accessions from the three regions. Although these three groups are distinguishable in the analysis, they present a slight overlapping, indicating that the differences are not very distant (Figure 2).

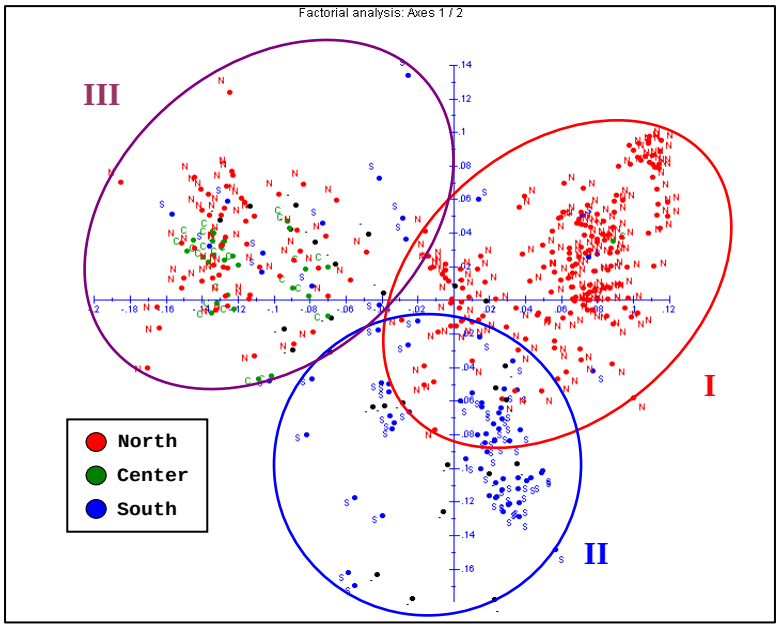

Figure 2. Factorial Analysis of yacon collections. Group I is formed by accessions from northern Peru. Group II is formed by accessions from the south and Group III is formed by accessions from the three regions

Genetic diversity

Table 2. Nei and Shannon-Weaver index

Collections	Number of accessions	Nei index	Shannon-Weaver index
CIP	48	0.14	4.51
INIA	123	0.13	4.51
UNC	103	0.13	4.27
UNSAAC	62	0.15	4.74
UNSCH	13	**0.17**	**5.17**
UNALM	10	0.14	3.92
Total	359	**0.13**	**5.90**

The genetic diversity of the entire collection of yacon is 0.13 (Nei's index) or 5.90 (Shannon-Weaver index). The diversity among collections is very similar and the highest value is presented in the UNSCH collection (Table 2).

The entire collection of yacon presents 37 exclusive bands (bands that are only present in one collection) and the UNSAAC collection has the highest number of exclusive bands. In addition, there are 192 bands (62%) that are shared among all collections (Table 3).

Table 3. Number of present, exclusive and share bands

Collections	Total number of present bands	Number of exclusive bands	Number of share bands
CIP	220	3	192
INIA	249	7	
UNC	232	5	
UNSAAC	**253**	**15**	
UNSCH	220	3	
UNALM	207	4	

Analysis of molecular variance

To perform the AMOVA analysis, we identified 3 geographical regions (northern, central and south) and also the departments that are include in each region. There are significant differences in the genetic heterogeneity of the three regions but with a low value of variation (7.28%), also the fixation index shows a moderate differentiation (Fst = 0.0728). The same situation appears between departments (Fst = 0.1248) but it is higher than regions (Table 4). Additionally, the AMOVA was performed to show the differences between the six collections. There were significant differences among the six collections with a high differentiation (Fst = 0.18 – data not shown).

Table 4. Analysis Molecular Variance - AMOVA from three regions of collection and Fixation index

Source of variation	d.f.	Sum of squares	Percentage of variation	Fixation Index
Among regions	2	525.279	7.28 (+)	0.0728
Among departments within regions	12	589.951	11.57 (+)	0.1248
Within populations	341	5697.916	81.15	0.1885
Total	355	61813.146		
Significance Level = 0.05		Significance (+)	Non significance (-)	

Discussion

The genetic diversity of yacon maintained in the six collections was low (Nei = 0.13, Shannon-Weaver = 5.90). Also, there are narrow ranges of similarity in which groups are formed in the dendrogram (~0.87-1.00 of similarity), which indicates close relation between genotypes and as a result a lack of a better clustering.

All collections represent the total genetic diversity because their diversity indexes were similar to the total index. And the AMOVA analysis shows that these collections have a moderate genetic differentiation (Fst = 0.18 – data not shown). The exclusive bands of each collection and differences in the number of accessions between collections would be the factors that make differences in diversity among collections. The highest diversity value (0.17-0.517) presented in the UNSCH collection could be overestimated due to the presence of low number of accessions.

Almost 99% of the accessions studied were unique genotypes (355), but they have a close genetic relationship (62% of shared bands). According to Grau and Rea (1997), the area from Apurimac in Peru to La Paz in Bolivia, holds the largest area of diversity, which in this work is supported by a large number of unique bands present in the collection of UNSAAC, since in this collection most of its accessions are from southern Peru.

Cluster analysis shows two main groups and subgroups A, B and C show a geographical patterns; although, this analysis also present a high number of accessions with lack of clustering. The factorial analysis shows a better association with geographical regions (north, central and southern Peru). Grau & Rea suggest that the geographical distribution is a very important marker for yacon, due to differences in tuber size that were found between the area of Ecuador, Cajamarca and Cusco in Peru. Also, they found a high variability of tuber flesh color in southern Peru and northern Bolivia compared to Ecuador and Argentina. In this analysis it was identified mainly 3 genetically different groups: the first consisting of accessions from the north, a second formed by accessions from the south and the last consisting of accessions from the 3 regions. Although these three groups can be differentiated in the factorial and AMOVA analysis, they maintained a high genetic relationship. The differences in management and selection of yacon in the north and south may be influence the genetic structure of this crop in the two regions.

The use of morphological data and other important characteristics could improve the results of this research for a better management of the genetic diversity of yacon and develop new strategies for collect and conservation of yacon in the future.

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Farmers' practices and the consequences for the genetic conservation of clonally-propagated RT crops. The case of yam

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Abstract

A growing number of evidences suggest that clonally-propagated RT crops are not maintained in strictly asexual reproduction system. Studies on yam, cassava, potato and taro show or suggest that farmers use the sexual reproduction of the cultivated species and sometime of the wild relative species. The consequences of this mixed reproductive system on the evolution and the adaptive potential of the plants are important. Indeed, the maintenance of sexual reproduction allows the creation of new diversity and thus maintains the adaptation potential while the use of asexual multiplication allows the maintenance of the best genotypes. The consequences for conservation, however, strongly depend on farmers' practices and management.

Here, we review results obtained on yam (*Dioscorea* sp.), a clonally-propagated RT crop mainly cultivated in West Africa. Results showed that farmers introduce the products of hybridization between wild and cultivated yams into the cultivated pool. Moreover, varieties consist in only one genotype and some closely-related genotyped differing by some mutations instead of a mix of different genotypes originated from sexual events. The consequences of these results on the conservation and use of wild and cultivated yams are discussed.

Keywords; Farmers' practices, conservation, diversity, clonally-propagated crops, yam.

Introduction

Among root and tuber crops, the main species (cassava, yam, taro, potatoes) are cultivated in traditional tropical agrosystems by clonal propagation. In such crops, opportunities for sexual recombination are greatly reduced, since propagation by farmers does not require seed production. Indeed, many clonally-propagated crops show disruption of flowering and fruiting mechanisms as well as unbalanced chromosomal arrangements (Zohary 2004). Consequently, it is often believed that the evolution of clonally-propagated crops occurs in the absence of sexuality and is thus slowed down because selection can act only on few new genotypes created by mutations (Hurst and Peck 1996; Barton and Charlesworth 1998).

However, this general belief in the lack of sexuality of clonally-propagated crops lacks evidence. On the contrary, flowers and seeds can frequently be found in the field (potatoes, Johns and Keen 1986; cassava, Elias *et al.* 2000, 2001; yam, Sadik and Okere 1975). Moreover, RT crops have been cultivated for thousand of years and present a high genetic diversity incompatible with purely asexual reproduction (Zhang *et al.* 1998; Birmeta *et al.* 2002; Lakhanpaul *et al.* 2003; Sardos *et al.* 2008). It has recently been shown that traditional farmers' practices actively use this residual sexuality (Elias *et al.* 2000, 2001; Scarcelli *et al.* 2006a, b). Depending of the plants, farmers collect seeds (potatoes, Johns and Keen 1986) or select plants spontaneously growing in the wild (yam, Dumont and Vernier 2000; taro, Sardos *et al.* 2008) or in the field (cassava, Elias *et al.* 2000). The new plant is then introduced in an existing variety or is given a new name. The consequences of this mixed reproductive system on the genetic diversity of clonally-propagated crops are not well known but they will depend on the way sexuality is used by farmers and will then be different for each plant.

Here we review recent results about the role of sexuality in yam (*Dioscorea* sp.) genetic diversity and structure. First we will look at the evidences that farmers use the sexuality of wild and cultivated yams, then we will look at

the role of sexuality and mutation in the genetic diversity observed in yam varieties. Finally we will discuss the consequences for the use and conservation of yam genetic resources.

The use of yam sexuality by farmers

The traditional practice of ennoblement

Yam farmers never collect seeds nor use seeds to grow a new plant. However, several sociological studies have documented a practice named 'ennoblement' (ex. Dumont 1998, Baco 2000, Dumont and Vernier 2000, Okry 2000, Houemassou Bossa 2001, Tostain *et al.* 2003, Vernier *et al.* 2003). Farmers collect tubers of wild yams and plant them in their fields. They select tubers for their likeness to cultivated varieties, e.g. in northern Benin, they look for plants with large green stems, with large tubers and white flesh and without spines. According to farmers, some of these plants develop — after 3-6 years of special cultivation practices — a tuber that is morphologically close to those of cultivated varieties. The tubers are then multiplied and cultivated if farmers are satisfied with their morphology. The biological processes underlying the change in tuber morphology and its maintenance over generations are unknown.

Genetic evidences

Using AFLP and microsatellites, Scarcelli *et al.* (2006a, b) gave the first evidence that farmers actually collect wild and hybrid yams (Figure 1a). Hybrid yams result from spontaneous hybridizations of wild (*D. abyssinica* and *D. praehensilis*) and cultivated yam (*D. rotundata*). These studies also showed that a part of the plants selected by farmers is not wild or hybrid but has a cultivated genotype. Those plants can have two distinct origins: volunteers, i.e. a fragment of tuber forgotten in a field and that managed to survive when the field became a fallow; or progenies of cultivated varieties, i.e. new recombinant genotypes. It was impossible to discriminate between the two origins.

As a result of this selection of new genotypes by farmers, studies found wild and hybrid genotypes within the cultivated varieties (Figure 1b). This means that through the practice of ennoblement, farmers cultivate new genotypes created by the sexual reproduction of wild and cultivated yam.

The genetic struture of cultivated yam

Previous results and limitations

The genetic structure of cultivated yam has never been fully understood, mostly because of technical limitations. Several studies used morphological and genetic markers to analyse the genetic diversity within yam varieties (ex. Hamon *et al.* 1986, Dansi *et al.* 1999, 2000a, 2000b). Results varied among varieties and studies but most of them showed intra-variety diversity. These results, however, were not obtained on varieties, but on 'cultivar groups' – each group corresponding to a mix of varieties with similar morphology. Those cultivar groups were created in order to organize the huge morphological diversity but they cluster together distinct varieties. As a result, an analysis on cultivar group may overestimate the genetic diversity because the analysis was not done on the actual intra-variety diversity. Moreover, the genetic markers available at that time were not appropriate because they were not polymorphic enough (isozymes) or because they were not reproducible enough (RAPDs).

New data on the role of mutation and sexual reproduction

In an attempt to clarify the genetic structure of cultivated yams, Scarcelli *et al.* (submitted) used microsatellite to analyze the genetic diversity of few varieties. This study was done in only one village in Benin and only one ethnic group in order to be sure that the name given to a variety by different farmers corresponds to the same plant. However, because of the limited scale of the study, results may no be extrapolated to different regions.

Results revealed intra-variety diversity. An analysis of the relationship between the genotypes found in each variety showed that the intra-variety diversity is the result of mutations while each variety is a result of sexual reproduction. In this part of Benin, a variety can thus be considered as a product of sexual reproduction that has evolved by mutations. This result suggests that product of sexual reproduction introduced by the ennoblement practice are mostly given a new name rather than introduced in an existing variety. This result also suggests that farmers' management are able to avoid mixing between varieties.

Implications for genetic conservation and improvement

The concept of variety

Varieties are usually considered as an entry point to the genetic diversity and its conservation. In Guyana, cassava seeds spontaneously germinate when a new field is opened. Farmers select plants and introduce them in the morphologically closest variety or create a new variety if the phenotype is distinct enough (Elias *et al.* 2000, 20001). This suggests that within-variety diversity should be high, in term of mutants as well as product of sexual reproduction, and is in accordance with the founding of Jarvis *et al.* (2008). In this system, focusing on varieties only will not be enough as intra-variety diversity may be lost by drift. This conclusion however may be different for other RT crops. Results presented here suggest that focusing on varieties is a good way to preserve yam diversity.

Taking farmers into account

As it was shown or suggested by different studies, several RT crops have a mixed reproductive system (Johns and Keen 1986, Sardos *et al.* 2008, Elias *et al.* 2000 and 2001, Scarcelli *et al.* 2006a, b). This use of sexuality in asexually propagated crops has some evolutionary consequences. Indeed, farmers combine the advantages of both sexuality and asexuality. By testing and selecting new combinations created by sexuality, farmers maintain the potential for future adaptation, and, at the same time, they preserve their best genotypes from recombination by using asexual reproduction. This suggests that traditional practices associated to the use of sexuality should be preserved in order to preserve the adaptive potential of clonally-propagated RT crops.

In the specific case of yam, results suggest that the use of wild yam diversity through the ennoblement practice should be maintained in order to maintain the genetic diversity of cultivated yams. Thus, the *on-farm* conservation of cultivated yams should address both the *in-situ* conservation of wild yam diversity and the conservation of traditional knowledge and farmers' practices.

What can be learnt for genetic improvement?

Yam improvement is not an easy task, particularly because of the difficulty to master flowering and crossing. Until today, few improved varieties have been produced and even fewer have been widely accepted by farmers. Wild yams have been mostly neglected in these programs and their potential has never been tested. The recent studies showed that farmers use this potential by selecting and cultivating F1 hybrids between wild and cultivated yams. They suggest that improvement programs can beneficiate from studying the agronomical properties of spontaneous hybrids, as well as from introducing those hybrids in the selection schemes.

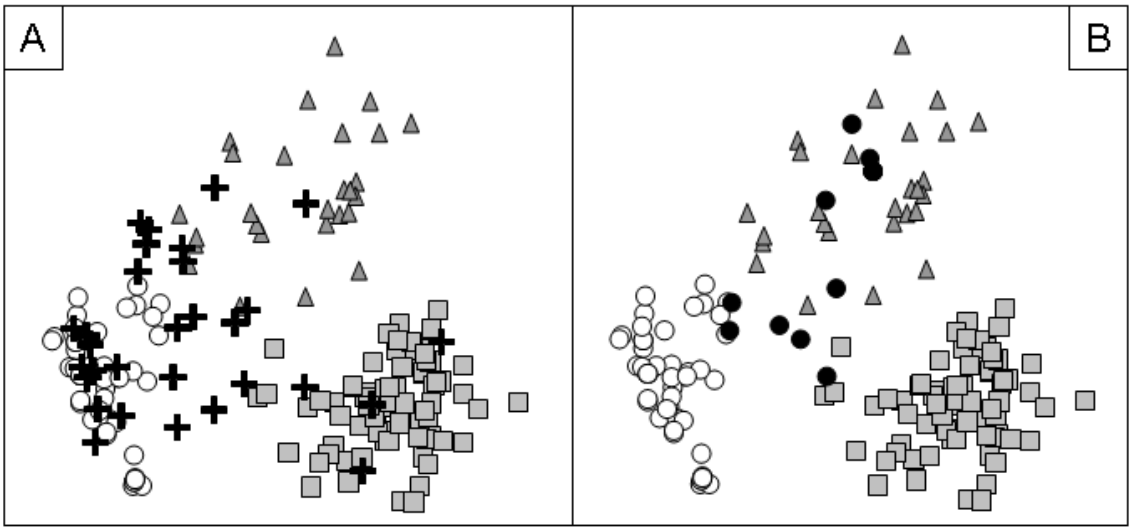

Figure 1. Principal component analysis (PCA) of the genetic diversity of wild and cultivated yams. ■= wild yam *D. abyssinica*; ▲ = wild yam *D. praehensilis*; ○= cultivated yam *D. rotundata*. Genotypes were assessed at 11 microsatellite loci. (A) Plotting of spontaneous yams (✚) selected and tested by farmers through ennoblement. A part of the spontaneous yams clusters with *D. abyssinica*, *D. praehensilis* or *D. rotundata*. The other spontaneous yams are intermediate between the wild and the cultivated species. (B) Plotting of cultivated yams (●) showing a wild or an intermediate genotype. The assignation of each sample to one of the three species has been tested by assignment tests (Scarcelli *et al* 2006b). Moreover, hybrid origins of intermediate genotypes have been tested by assignment tests and the existence of spontaneous hybridizations has been tested by paternity tests (Scarcelli *et al.* 2006b).
Adapted from Scarcelli *et al.* 2008.

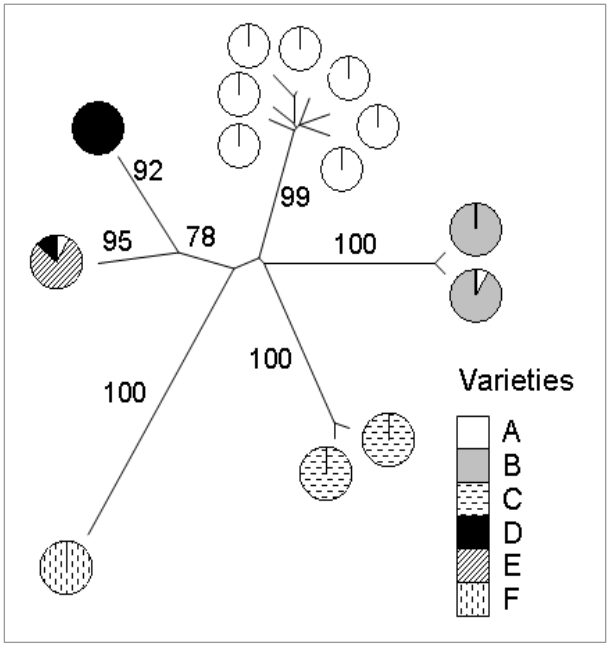

Figure 2. NJ-Tree representing the relationships between genotypes found in different yam varieties. Genotypes were assessed at 13 microsatellite loci. Bootstrap values are given in percentage. Each genotype is represented by a circle. The colour(s) of the circle indicate the presence of the genotype in one or more varieties. Fourteen different genotypes were found. In most of the cases, a genotype is associated with only one of the six varieties. For a given variety, different genotypes could be observed, however these genotypes cluster together in the NJ-tree. According to allele frequencies and mutation rate, the slight differences between genotype inside each cluster were interpreted as the product of few mutations appearing during the clonal propagation phase while the different clusters were interpreted as products of sexual reproduction (Scarcelli *et al.* submitted). As the 6 clusters correspond to the 6 varieties, it can be considered that these yam varieties correspond to different products of sexual reproduction that have evolve by mutation.

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Molecular characterization of the *Oxalis tuberosa* Mol. collection maintained in the CIP's genebank

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Abstract

The Andean ecosystem harbors more than 180 economically important crop plants in a variety of climates and habitats, along its extension. The oca crop (*Oxalis tuberosa* Mol.) is the most important tuber, after potato, in this ecosystem. The genetic diversity and the geographic distribution patterns of oca genebank maintained in the International Potato Center were investigated in the present study using AFLP markers. This collection holds 585 accessions from Argentina, Bolivia, Chile and Peru. Seven primer combinations were tested; obtaining 175 polymorphic markers for genetic diversity analysis. The UPGMA dendrogram showed three main clusters, two including Peruvian ocas and the other formed by ocas from southern Peru, Argentina, Bolivia and Chile. Accessions from southern Peru were found in the three groups, supporting that oca originated in this region. The molecular groups identified showed a relationship with the eco-region where they come from: groups 1 and 3 are found in the Central Andean wet Puna and group 2 in Central Andean Puna. The molecular variance analysis (AMOVA), indicated that Peru is the country with the greatest diversity found in the CIP collection.

Keywords: *Oxalis tuberosa*, oca, AFLP, Genetic diversity.

Introduction

Among the ART´s have oca (Oxalis tuberosa Molina) considered the most important specie between all of the ART´s because its high range of adaptation and its high content of vitamins, micronutrients and starch, also for its grown ability under extremely atmospheric conditions.

The productivity of oca is low because its sow under dry conditions, and support dried and freezing like plague attacks and diseases. In the last years, Oca in Peru, had a harvest ground of 20000 ha, with a production of 116 tm., the centre of major yield is the department of Puno with approximately 8 tm/ha in 2003.

To maintain and protect this species of the genetic erosion we must collect from their origin place and developed genebanks and maintain the major quantity of interest genes of each species. This conservation makes in tidy way maintaining this accessions for undefined time trying to unchange their genetic constitution.

This work find to contribute to knowledge of the genetic diversity, using AFLP markers, determined lines of geographic distribution of the accessions of oca maintained in the genebank of the International potato Center.

Plant material

585 accessions from the genebank maintained at the International Potato Center was studied: 448 from Peru (Piura, Cajamarca, Amazonas, Pasco, La Libertad, Ancash, Junín, Lima, Huancavelica, Ayacucho, Apurimac, Arequipa, Cuzco, Puno y Tacna), 75 from Bolivia (Cochabamba, La Paz, Beni, Oruro, Potosí y Tarija), 52 from Argentina (Salta y Jujuy) y 10 from Chile (Antofagasta y Tarapacá).

Methods

AFLP procedure

AFLP fingerprinting (technique) was carried out using a modified procedure of the one described by Vos et al, 1995, modified in CIP by (Zorrilla, 2006) The digestion of genomic DNA was carried out using enzyme

combination *Eco RI / Mse I*. A pre – amplification with primers complementary to the adapter sequences, having one additional nucleotide on their 3´ end, was done before the final amplification. In this one, primers were used with three additional nucleotides on their 3´ end. The amplification product was loaded on to a 6% (w/v) denaturing polyacrylamide gel and visualized with silver nitrate.

Data analysis

Data recording. The size of the band was calculated by evaluation in a denaturing polyacrylamide gel, was put all the primer combination used in the investigation and also weight markers like plasmid sequencing pUC 18 and ladder 30 pair base. Each produced fragment for each primer combination was taken like an evaluation unit and sequentially numbered. The data was registered in a binary matrix with 1 if is present and 0 is if absent for each AFLP marker band.

Dendrogram construction. The data analysis was made using with Darwin program 5.0.144 (Perrier et al, 2003), the genetic dissimilarity was estimated by the Jaccard index. The genetic distance (1 - genetic similarity) between each accession was calculating using NTSys version 2.01 (Rohlf, 1997), based on the Sokal and Michener (1958) dissimilarity coefficient. Dendrograms were constructed employing the UPGMA (unweighted pair group with mean average) algorithm using the same program. The cophenetic matrices generated from the dendrograms or the similarity matrices used in clustering were compared by performing a Mantel test (Mantel 1967).

AMOVA and population distance (ϕst). Based on the Euclidean distances, the analysis of molecular variance (AMOVA) procedure (Excoffier *et al.,* 1992) was applied to estimate variance components for AFLP genotypes.

Individual variation was partitioned within and between regions (countries).

Nei's genetic diversity. This method was made for genetic diversity analysis (heterozygosis) of a subdivided population, genetic diversity between and among populations.. (Nei, 1973)

Principal Coordinates Analysis (PCoA). Was used for evaluated the distances between population, which is a very big space and very difficult to read, this method find minor dimension spaces where populations are closer for been analyzed.

Result and discussion

AFLP combinations for oca

A total of 292 EcoRI/MseI primer combinations were tested for polymorphic bands, intensity of bands and repeatability. The best 7 combinations were used for the molecular characterization of the 585 accessions of *O. tuberosa* collection. The 82% of the AFLP markers detected were polymorphic, 175 from a total of 213. (*Table1*).

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Table1. AFLP combinations used and percentage of polymorphism

Combination	Fragment Sizes	Total Markers	Polymorphic markers	Percentage of polymorphism
E35ACA/M59CTA	500 -169	31	28	90
E35ACA/M60CTC	515 -118	27	21	77
E39AGA/M40AGC	515 -155	30	20	66
E40AGC/M35ACA	295 -153	29	29	100
E42AGT/M54CCT	515 -130	28	23	82
E42AGT/M60CTC	300 -122	28	24	85
E45ATG/M51CCA	405 -140	40	30	75
Total	**515-118**	**213**	**175**	**82**

The oca shows less polymorphism in comparison to potato; Kim *et al.* (1998) obtained 466 markers with 7 combinations in *Solanum tuberosum* meanwhile we have obtained 213 markers in oca with the same number of

combinations. We believe that it is a consequence of the size of the genome that is smaller for oca. Also, the high number of monomorphic bands would be a consequence of its polyploidy (x=8); thus the absence of a marker can be detected only when it is not present in any of the eight homologous chromosomes.

Clustering analysis

The dendrogram was drawn using the Jaccard's distance and the UPGMA algorithm. Three groups were clearly observed (*Figure 1*). Group I included accessions from departments of the Northern Peru (Cajamarca, Piura, Amazonas y La Libertad), Central Peru (Lima, Ancash, Pasco, Junín, y Huancavelica) and Southern Peru (Ayacucho, Apurimac, Cuzco, Arequipa, Tacna y Puno) and one accession from Bolivia. Group II included mostly accessions from Puno, together with Argentina, Bolivia and Chile. Group III included accessions from the departments of North, Center and South Peru. The Principal Coordinates Analysis indicates shows that the Groups I and III, that include Peruvian accessions, are closely related (*Figure 2*). The South of Peru seems to be the place where it was originally domesticated as it was previously reported (Arbizu y Tapia 1992, Emshwiller 2002, Pissard *et al.* 2006) because accessions from this area are distributed in the three molecular groups.

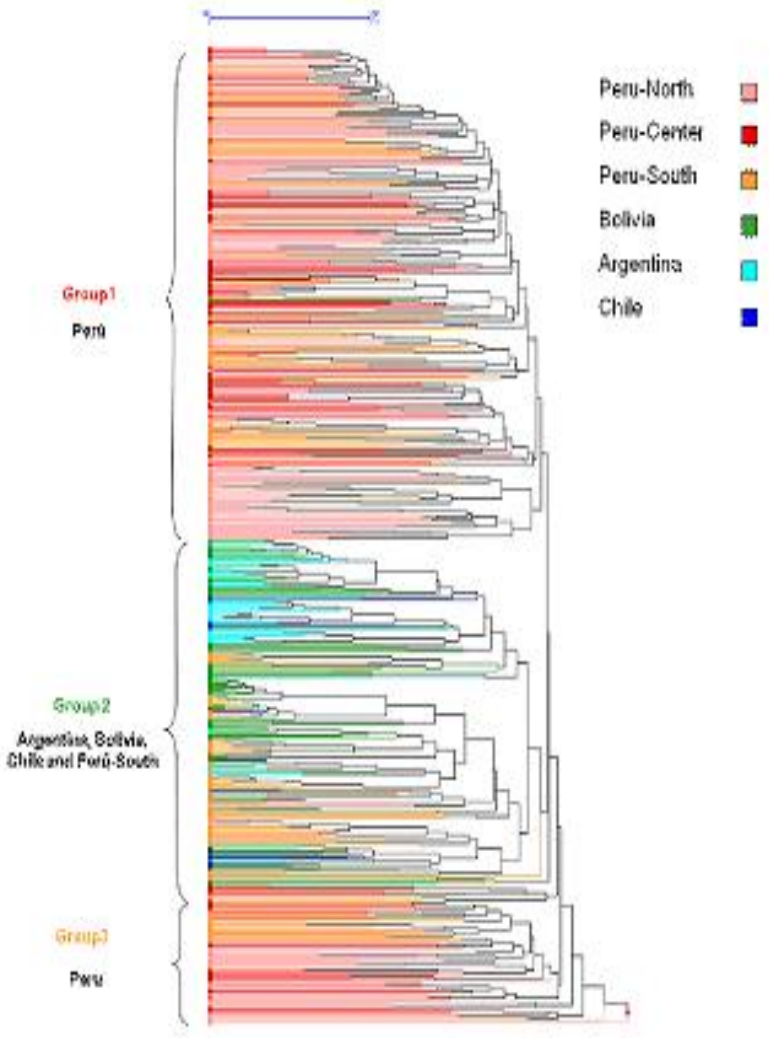

Figure 1.

Clustering analysis of 585 oca accessions using Jaccard's distance and UPGMA algorithm. The dendrogram was drawn with the software Darwin 4.0.

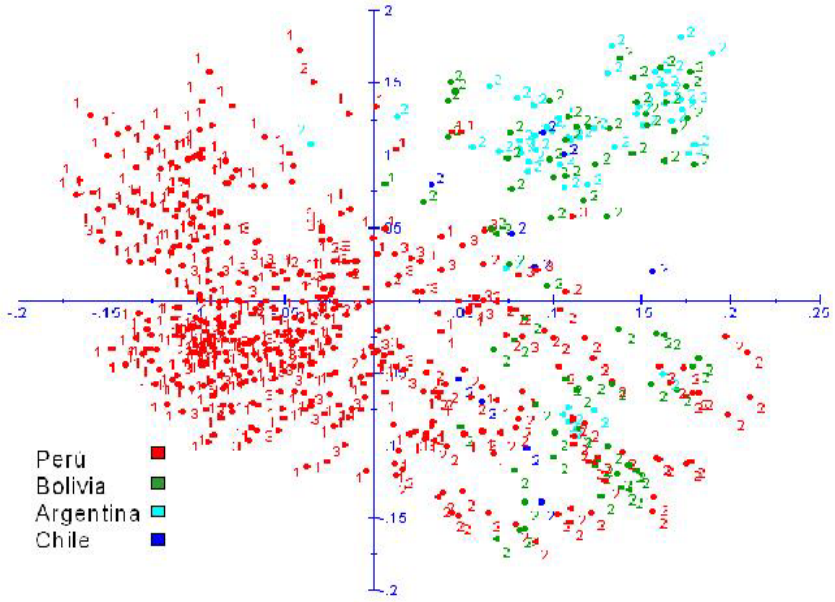

Figure 2. Representation of the two main axes obtained from the Principal Coordinates Analysis of 585 oca accessions. Axes X (6.3%) and axes Y (4.17%) represent the 10.47% of total variability using the software Darwin 4.0

Geographical patterns of genetic diversity.

The Jaccard's disimilarity and the Nei's index were calculated to estimate genetic diversity in every country and the whole collection. The results indicate that Peru is the country with the highest genetic diversity of oca, with the highest values of Jaccard's dissimilarity (0.410) and Nei's index (0.31); and Argentina is the least diverse country with 0.309 and 0.19, respectively (*Table 2).* Also, the Analysis of Molecular Variance (AMOVA) reveals that the genetic variation is 6.77% between countries, which is significant. Although, the main source of genetic variation is individuals within countries (*Table 3).*

Table 2. Jaccard's dissimilarity and Nei's index

País o Región	Jaccard's dissimilarity	Nei's index
Argentina (52 accessions)	0.309	0.19
Bolivia (75 accessions)	0.351	0.22
Chile (10 accessions)	0.359	0.20
Perú (448 accessions)	0.410*	0.31

*This average was calculated as a (1-similarity)

Table 3. Analysis of Molecular Variance (AMOVA) by country

Source of variation	*df.*	Percentage of variation
Between countries	3	6.77
Indivi. within countries (Perú, Bolivia, Argentina y Chile)	582	93.23
Total	**585**	**100.00**

In the study of Pissard *et al.* (2006) in a set of the oca accessions maintained at CIP genebank using another type of marker called ISSR, it was also found that Peruvian ocas are the most diverse compared to the ocas of Bolivia, Chile and Argentina.

Conclusions

In conclusion, the molecular clustering of the 585 accessions from the CIP genebank shows three groups, two of them formed by accessions from Peru and one formed by accessions from Argentina, Bolivia and Chile. Peru is the most diverse country and there is a geographic pattern of genetic diversity due to genetic variation among different countries is significant.

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Preserving biodiversity of Andean roots and tubers: working with women

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PROINPA, CIP-Papa Andina Initiative, and the Bolivian Ministry of Agriculture have worked together in promoting women's participation in producers' associations. Those associations try to increase their member's income through the use and promotion of the biodiversity of Andean roots and tubers, highlighting their nutritional and medicinal properties. Traditional knowledge, especially from women, in relation to the different uses of roots and tubers such as *achira (Canna edulis)* and *arracacha (Arracacia xanthrorriza)* was combined with new information on additional uses of such products. Results were presented at several food fairs and other events, thus disseminating the knowledge to other communities. The project has contributed not only to increase the income of the whole family, but in particular to increase women's income since they were the ones commercializing the products. It has also contributed to improve women's social capital including self esteem and increased recognition from other community members.

Keywords: Rural Women, Biodiversity, Andean Roots and Tubers.

Background

Rural women while pursuing food security for their families have been contributing since ancestral times to the preservation of native roots and tubers. They have passed on to their children their knowledge and skills on resources management, seeds selection and use of several agricultural products (Estrada, 2000; Tapia and de La Torre, 1997).

However in most of the cases, rural women have been performing their duties in silence, without properly recognition. In Andean communities, women's participation in decision-making face barriers imposed by a world predominantly governed by men, in which female members play a subordinate role. Those women can often only communicate in their native language, which limits further their possibilities.

It is therefore a challenge to implement actions with female community members for strengthening the use and conservation of biodiversity. PROINPA and Ministry agriculture Bolivia, with the support of CIP-Papa Andina Initiative have accepted this challenge involving **the participation of women** in various experiences in the area of genetic resources aiming at recovering the important role of Andean roots and tubers in food diet and at generating possibilities of additional family income with these products.

Areas of intervention

Activities were implemented in 3 Bolivian zones: Coroico and Cariquina Grande (in La Paz) and Colomi (in Cochabamba). In total, approximately 700 families have benefitted from PROINPA´s activities in the 3 zones (direct and indirect beneficiaries).

Strategy

Even if biodiversity loss in countries with ancestral cultures such as Bolivia is considered not as dramatic as in other countries (Sevilla, 2006), such loss still takes place. In order to stop this process, the strategy has been based on the development of social and economic incentives for in situ conservation of the agrobiodiversity in microcenters with high biodiversity. Rural women played a key role in this process.

The strategy included following actions:

- Selection of microcenters with high biodiversity of Andean roots and tubers (Coroico and Cariquina Grande in La Paz, Colomi in Cochabamba). Microcenters are geographical areas whose environmental and socio-cultural characteristics contribute to the existence and conservation of a diversity of species and varieties (García et al, 2003a).
- Identification of communities and families, particularly women, with extensive traditional knowledge in the use of Andean roots and tubers.
- Use of participatory methodologies for the characterization of Andean roots and tubers as well as for emphasizing their properties. These methodologies are useful to better understand people's interaction in their own context (Almanza et al, 2003).
- Campaigns (local radio, workshops and lectures) to point out the importance of the use and conservation of Andean roots and tubers both to improve family nutrition and for generating additional family income.
- Workshops with female community members for the recovery of traditional uses and for promoting innovations in the use of Andean roots and tubers. Elaboration and diffusion of food recipes. At this stage emphasis was laid on the role of preserving biodiversity for food security (Terrazas and Iriarte, 2009). According to Fries (1997), training is a key element to improve nutrition and to promote and encourage wide use of edible species.
- Promotion and organization of biodiversity and nutritional fairs with the participation of health and local education representatives. In recent years, local fairs have become an important element to promote the conservation of genetic resources (Tapia and De la Torre, 1997; García et al, 2003b; PROINPA, 2005).
- Promotion of women´s active participation in Producer Associations, in order to open up their market opportunities. Currently there is a large demand for non traditional and organic products, providing a good opportunity for products such as roots and tubers (Hermann and Heller, 1997). As Tapia (2006) points out, market links may provide an incentive for conservation.

Achievements

Andean roots and women in Coroico (Yungas of La Paz)

The Coroico municipality located at 95 kilometers from La Paz has as a tradition production and consumption of several Andean roots, such as the achira (*Canna edulis*), ajipa (*Pachyrhizus tuberosus and P. ahipa*), walusa (*Xanthosoma saggitifolium*), aricoma or yacon (*Smallanthus sonchifolius*) and racacha (*Arracacia xanthorrhiza*) (Figure 1). This tradition has however been neglected in recent years by the widespread of commercial products such as coffee and orange.

Figure 1 Andean roots grown in Coroico

Thanks to the persistence of some farming families in which women play a leading role to maintain their natural resources and to the support of several institutions during this last decade, these roots are being rescued, reincorporated in the diet of the family and generating additional income.

The project identified interest groups (producers associations) and local promoters to work in the rescue of available knowledge on management and use of the roots. Men showed little or no interest in the experience.

Women on the contrary showed a high personal commitment and actively participated in the associations. One of three associations in the area is currently composed exclusively by women.

Work with those women has contributed to recover the traditional uses of those roots and to introduce innovations for culinary purposes.

Participating women told PROINPA, that they are proud of their achievements. They mention that before the project, the use of the roots was limited to a couple of recipes and their families were tired of them. However nowadays, they are more aware of their nutritional value and have learned to use them in different ways. Women participate also actively in local and regional fairs selling their products and thus improving their own income and the family income.

Andean roots and Women in the subtropics Cochabamba

In the subtropics Cochabamba, the town of Tablas Montes in Colomi is located at approximately 100 km from the city. The economic basis is agriculture, particularly the cultivation of locoto and papa, although there are a variety of other Andean crops.

The objective in this area was to promote local use of biodiversity. The strategy was to engage teachers, school students, local health center and the women associations of Tablas Montes. The project developed and implemented biodiversity and nutrition fairs with those actors (Figure 2). Training workshops on traditional and innovative uses of those products were conducted. Women from Coroico came to the area to share their knowledge and experiences performing as trainers. This further contributed to the empowerment of women improving their knowledge and self esteem.

Figure 2. Biodiversity and Nutrition Fair with the participation of the health and education sector in Tablas Montes (2007.)

During the fair, female community members and students gathered together for preparing with their own hands traditional and innovative uses of the roots (Figure 3). They developed own recipe booklets and shared their knowledge to members of neighboring communities.

Figure 3. Women making products out of Andean roots

Participants are currently selling the products, in other regional fairs to raise funds for their organization. At the same time, through the training, they are better endowed to plan and conduct other business, such as provision of snacks for the school breakfast in Colomi.

Andean tubers and women in Cariquina Grande (Highlands of La Paz)

Cariquina Grande is an Aymara community in the northern highlands of La Paz, close to Lake Titicaca. Cariquina has a large variety of native potato and other Andean tubers as the oca (*Oxalis tuberosa*), papalisa (*Ullucus tuberosus*) and isaño (*Tropaeolum tuberosum*), which since ancient times coexist in the community. Their conservation is strongly linked to food security and to cultural relations among people, and between people and nature (mother earth).

PROINPA worked with local women searching for incentives for increased consumption of Andean tubers, especially among younger generations. Traditional, but also innovative, forms of consumption were promoted such as cakes baked from native potatoes, and bread out of oca (Figure 4). Recipes included also new ingredients such as quinoa (*Chenopodium quinoa*) and tarwi (*Lupinus mutabilis*). It is currently being explored the feasibility of delivering such products to local schools as part of the breakfast school.

Figure 4. Native potato cake and oca "qayapalala" bread

The project promoted also women's participation in the producers association. Currently, female members are actively engaged in production and marketing of native potatoes.

Figure 5. Planning the production in the Producers Association at Cariquina Grande

Lessons

- Rural women in the Andes seem to be better informed than men in relation to use and properties of agrobiodiversity.
- Working with rural women to promote the use and conservation of biodiversity seems to be a right approach since they are motivated and interested partners.
- Engaging other sectors in the Project, such as the health sector and the education sector contributes to further promote the use and conservation of Andean roots and tubers.

- Rural women have increased their income trough the marketing of roots and tuber's products. This has contributed to improve women's social capital including self esteem and increased recognition from other community members.
- Rural women appear to be willing to try technological innovations. Through women's participation, it is also possible to call the attention and engage male community members.

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Genetic diversity of arracacha (*Arracacia xanthorrhiza*) in Peru

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Abstract

Arracacha (Umbelliferae family) is a traditional Andean root crop that has been used as a food for thousands of years. It is an important source of calcium and contains a special starch easily digestible. It has become a significant crop in Brazil, where 12,000 ha are grown annually. Peru is the country with the largest gene bank collections, but the diversity is poorly understood. The aim of this work was to study the genetic diversity of 334 accessions from five gene banks collections in Peru using 148 polymorphic AFLP markers, obtained from five primer combinations. The molecular variance analysis (AMOVA) and the genetic dissimilarity (Nei and Shannon-Weaver index) indicated that arracacha in Peru is diverse. It was found that all five collections were significantly different among them and no duplicates were identified among 334 accessions studied. The UPGMA dendogram showed three clusters related to geographical distribution patterns of arracacha diversity: northern, central and southern of Peru. The northern cluster contains a much higher diversity than other clusters.

Keywords: *Arracacia xanthorrhiza*, AFLP, genetic diversity, gene banks.

Introduction

Arracacha is a starchy food and an important source of β-carotene, ascorbic acid and calcium. Several studies point to Andean South America as the place of domestication of arracacha. Although the genus *Arracacia* is particularly diverse in Mexico, the wild species most closely resembling arracacha are known from Peru and especially Ecuador. Arracacha is produced mainly in four countries: Brazil, Colombia, Ecuador and Venezuela (Hermann, 1997). Preliminary work using PCR amplified DNA from random sequence decamer primers has yielded promising results in terms of molecular polymorphism for application in fingerprinting of arracacha cultivars. Thus Blas *et* al. (1997) and Castillo (1997) report occurrence of DNA polymorphism in 48% and 85% of primer assayed, respectively. Castillo, however, concludes from his work that overall molecular diversity in his Ecuadorean material is low. Another work is the study of Blas (2005), where compared cultivated with wild species of arracacha, but there are lot of publications about morphological characterization. The AFLP analysis is considered by now as the most efficient technique to study the DNA in the case of species in which the genome is little known. In Peru there are arracacha's gene banks in different zones, each gene bank collected arracacha mostly from their zones; in the last years some accessions have been lost and then they agreed to make one gene bank with all the accessions. The purpose of the present study is to develop the AFLP technique on the arracacha genome to assess its genetic diversity of this set of gene bank and give information of what is the genetic diversity of arracacha in Peru.

Materials

A total of 334 accessions of arracacha of five institutions of Peru were tested (Table 1).

Table 1. Number of accessions per arracacha gene banks

Name of Institutions that maintain arracacha accessions	Number of accessions
Centro Internacional de la papa (**CIP)**	48
Instituto Nacional de Innovación Agraria (**INIA)**	144
Universidad Nacional de Cajamarca (**UNC)**	121
Universidad Nacional San Cristóbal de Huamanga (**UNSCH)**	20
Universidad Nacional Agraria La Molina (**UNALM)**	1
Total	**334**

Methods

DNA extraction

DNA extraction was made from young leaves using CTAB technique (Doyle and Doyle, 1990) medium scale protocol standardized and reported by CIP (2000). The DNA concentration was calculated in comparison with known concentrations of Phage Lambda DNA digested with *Pst I* restriction enzyme and visualized in agarose gel 1%.

AFLP analysis

AFLP protocols are based on Vos et al. (1995), adapted for use with silver staining of polyacrylamide gels 6%. The protocols of DNA restriction and ligation, PCR amplifications and electrophoresis were used as reported in the manual of the CIP (2000).

Data analysis

The AFLP bands pattern for each accessions were registered in a binary matrix in an excel sheet, where the present bands registered by 1 and the absent bands by 0. The unique genotype recognizing and the identification of unique genotypes and genetic redundancy is achieved through a cluster analysis based in the Jaccard index and UPGMA algorithm. The genetic heterozygosity was estimated using the diversity index of Nei and Shannon, and the genetic structure was estimated with the factorial analysis.

Results

AFLP primers combinations

A screening with 120 primers combination was made for pick up the AFLP primers combination used; these primers were selected due to its high polymorphisms index and good resolution of the bands (Table 2).

Table 2. Primers used for AFLP markers

Laboratory Code	Primer	Total Bands	Polymorphic Bands	Percentage of Polymorphism
EGA-M36	E-GA	36	27	75
	M-ACC			
E32-M52	E-AAC	50	30	60
	M-CCC			
E35-M48	E-ACA	45	38	85
	M-CAC			
E37-M55	E-ACG	40	28	70
	M-CGA			
E38-M60	E-ACT	35	25	72
	M-CTC			
Total		**206**	**148**	**71**

AFLP ,arkers evaluation

AFLP pattern bands were different in all 334 accessions tested. From a total of 148 polymorphic markers obtained by 5 combinations of AFLP primers, 77 are share bands for all collections and only 11 exclusive markers were identified (Table 3).

Table 3. Markers present in each collection

Genebank	No. of present bands	No. of exclusive band	No. of share band
CIP	129	1	77
INIA	139	2	
UNC	140	5	
UNSCH	127	3	
UNALM	78	0	

Cluster analysis

The cluster analysis identified three main molecular groups of diversity. The largest group is formed only for accessions collected from the north of Peru; the other two groups are formed by arracachas from the center and the south, respectively (Figure 1) but some accessions grouped with arracachas from other collections (Figure 2). Also there are 33 accessions that not grouped in any cluster. The factorial analysis showed that these three groups are not very separated between them because there are relations between accessions from north south and center of Peru (Figure 3).

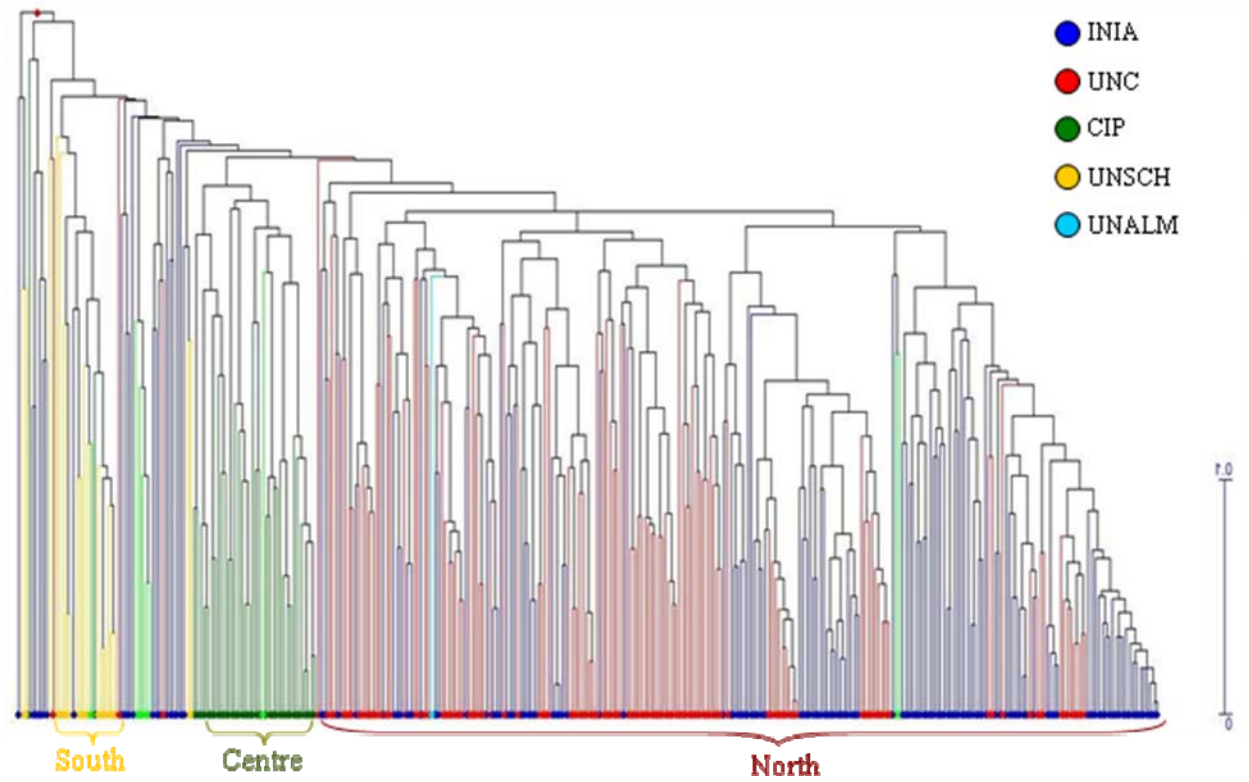

Figure 1. Clusters of the arracacha gene banks, based in 148 AFLP markers using Jaccard index and UPGMA algorithm

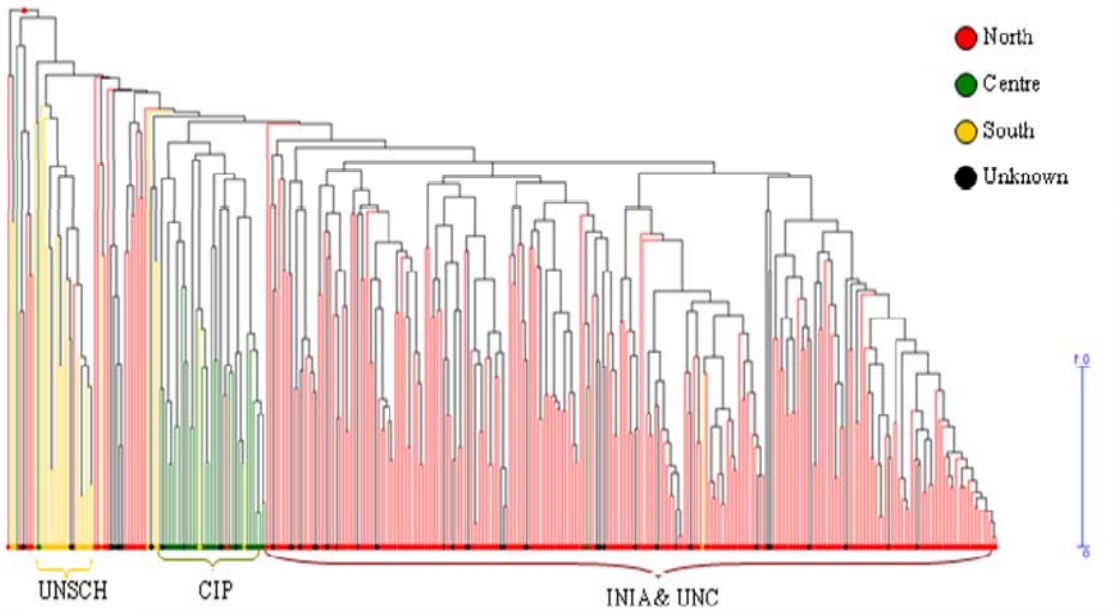

Figure 2. Clusters of the arracacha gene banks per region of origin, based in 148 AFLP markers using Jaccard index and UPGMA algorithm

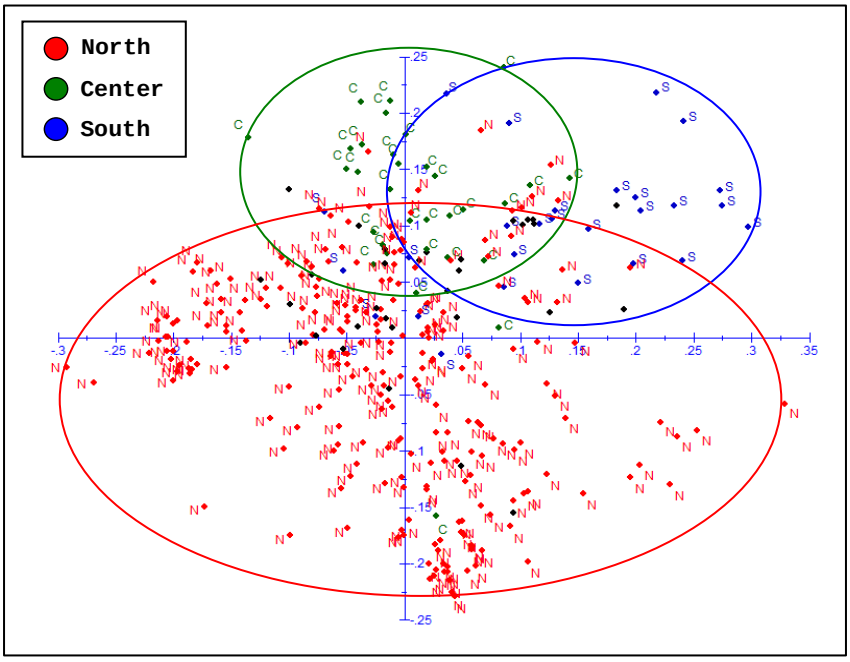

Figure 3. Factorial Analysis of principal coordinates per region of origin

Genetic diversity index

The Shannon-Weaver index (5.59) and the Nei index (0.28) indicate that the genetic diversity is high for all collections (Table 4). The highest values of Nei index were found in INIA and UNC (0.29), which are the largest collections. Although CIP collection is small its genetic diversity resulted high (0.28).

Table 4. Nei and Shannon-Weaver Index for genebank

Genebank	No. of accessions	Nei index	Shannon-Weaver index
CIP	46	0.28	0.849
INIA	145	0.29	2.305
UNC	122	0.29	2.060
UNSCH	20	0.26	0.382
UNALM	1	-	-
Total	**334**	**0.28**	**5.596**

Analysis of Molecular Variance (AMOVA)

The AMOVA shows a significant difference (Table 5) and the genetic distance between collections (Table 6) which shows a significant genetic distance between collections.

Table 5. Percentage of variation per collections

Source of variation	g.l	Percentage of variation
Among collections	4	12.68
Within collections	329	84.32
Fst.		0.12684

Table 6. Genetic distances per collection

	UNSCH	CIP	INIA	UNC
UNSCH	-			
CIP	0.178 (+)	-		
INIA	0.230 (+)	0.154 (+)	-	
UNC	0.239 (+)	0.133 (+)	0.070 (+)	-
Distance average	**0.215**	**0.155**	**0.151**	**0.147**

Also the Table 7 shows a significant difference between regions from Peru and Table 8 shows that there is significant genetic distance between regions.

Table 7. Percentage of variation per regions

Source of variation	g.l	Percentage of variation
Among regions	4	14.93
Within regions	329	85.07
Fst.		0.14928

Table 8. Genetic distances per region

	Centre	North	South
Centre	-		
North	0.139 (+)	-	
South	0.165 (+)	0.159 (+)	-
Distance average	**0.152**	**0.149**	**0.162**

Discussion

Blas (2005) obtained 76 markers with 3 AFLP primer combinations in cultivated arracacha. These primers were not use in the present work because their lack of resolution and high percentage of monomorphic bands. We obtained 148 markers with 5 AFLP primer combinations, which have high resolution and high polymorphism. Although the arracacha has few AFLP markers in comparison with others crops (Kim *et al.,* 1998).

Diversity of arracacha is specially organized by a geographical distribution, which is higher in the north of Peru (Nei index=0.29), where most accessions are from INIA and UNC collection. Besides CIP collection (Nei index=0.28) was more representative from center of Peru because with a low number of accessions has a high index of diversity, probably because the accessions were strategically better collected.

The Analysis of Molecular Variance shows differences among collections, this is possibly because there are genetic differences between the areas of collections, and also, each collection represents nearly one region. Also the analysis shows a high significant genetic distance between collection, being the largest distance between UNSCH (south accession) with INIA and UNC (north accessions) but is closer genetically with CIP collection (principally accessions from center). The INIA and UNC collections are genetically close; this agrees with the shown in the cluster analysis. According with the Fst. Analysis the differences between collections (0.12684) and between regions (0.14928) are moderate.

Conclusion

According to these results, the genetic diversity from Peru seems to be high and the higher genetic diversity is from Northern region. All the 334 accessions analyzed are unique and there are no duplicates in the gene banks. Besides, the genetic differentiation among Northern, Centre and South region was moderate, and accessions from south are more different in relation with the other regions.

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Morpho-agronomic characterization of Lake Victoria Basin taro genotypes

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Abstract

An on-farm experiment was conducted in Kenya to assess the agro-morphological diversity of taro cocoyam accessions based on phenotypic characters. One hundred and seventy accessions of taro collected from the Lake Victoria basin of Kenya and Uganda were evaluated-morphologically using the IPGRI descriptors. 18 qualitative and 17 quantitative characters were studied. Hierarchical cluster analysis identified two major clusters each with various subgroups. Most qualitative and quantitative descriptors displayed considerable variability. Diversified qualitative taro characters were leaf margin color, lower leaf color, petiole color and leaf surface glossiness while growth habit, exterior corm surface texture, corm shape and corm exterior color were monomorphic. Most divergent quantitative parameters were observed in corm weight, plant span and height and petiole length while low variability was seen in the ability to produce sucker above ground, corm length and diameter. Pearson's correlation revealed significant correlation between corm weight, corm length and corm diameter and some qualitative and quantitative taro characters. Euclidean proximity similarity matrix for all the tested taro samples was 82.1% for quantitative parameters. These data indicate significant genetic variation exists within the genotypes used in this study and that the germplasm would be of great value as a genetic resource in breeding programs.

Keywords: Cocoyam; Germplasm; Colocasia esculenta; Descriptors.

Introduction

Cocoyam (*Colocasia esculenta)* a perennial herb with origins in South Central Asia and presently grown widely grown around the world, *is* among the major crops grown in the wetlands and uplands of Lake Victoria basin of East Africa (Talwana et. al., 2009; Serem et al. 2008). Taro belongs to the genus *Colocasia*, within the sub-family Colocasioideae of the monocotyledonous family Araceae. Because of a long history of vegetative propagation, there is considerable confusion in the taxonomy of the genus *Colocasia (*Onwueme, 1999). Cultivated taro is classified as *Colocasia esculenta*, but the species is considered to be polymorphic. There are at least two botanical varieties (Purseglove, 1972):Globally, cocoyam is the 14th most consumed vegetable in the world (FAO, 1998). It is highly nutritious and the leaves are consumed because they are rich in protein and vitamins while the root is rich in carbohydrates and minerals (Dako, 1981; Dura and Uma, 2003). The crop offers a high potential for alleviating food insecurity and can be promoted to contribute to food diversity and improved livelihoods.

In spite of various efforts to achieve food security in East Africa, the status remains unattained and malnutrition is prevalent in many communities around the Lake Victoria basin. While there are concerted efforts to increase the production of high value crops including maize, wheat and rice, contrasting emphasis is placed on indigenous crops including taro and tannia (*Xanthosoma sagittifolia*) cocoyam among other crops. However, for various reasons that include culture, low costs of production and environmental adaptability, small scale rural farmers rely on these marginalized food crops (Chumo, 2007). Opportunities to promote and support the use of alternative food crops can make a major contribution to the food security of farmers in the tropics particularly in East Africa.

In the Lake Victoria basin, taro is popular and successfully grown due to its adaptability to a wide range of soil and climatic conditions (Chumo, 2007). The crop is thus adaptable to varied environmental factors for survival in ecological zones. The interplay between diverse cultivation practices, biotic and abiotic factors may have resulted in the development or modification of taro landraces and inherent allelic frequencies of desirable traits in the region. Since the extent of genetic variability in the crop as well as genotype performance remains unknown indicates a vast and largely untapped potential for research on cocoyam in the region.

Since characterization of germplasm and search for desirable traits are important in crop productivity and breeding, there is therefore, the need to fully investigate, identify and correlate taro landraces to their agronomic and physiological performances. This study is a first step towards understanding the genetic variability of taro in the region. The objective of the study was to identify the extent of phenotypic variability in quantitative and qualitative traits within the Lake Victoria taro landraces.

Material and methods

The plant material was made up of 170 taro cocoyam accessions collected from a wide range of agricultural zones in the Lake Victoria basin. This comprised 153 Kenya (Accession numbers 1-153) and 17 Uganda taro accessions (numbers 154-170) (Data not shown). Corm suckers were planted as upland cocoyam in 2008 and 2009 at the Masinde Muliro University experimental field in Kakamega, Kenya. The cocoyam accessions were planted at a distance of 2m x 2m between rows and plants. A field experiment was conducted at the Makerere University Agricultural Research Institute Kabanyolo (MUARIK), located about 14 km North East of Kampala, at an elevation between 1250m and 1320m above the sea level. The soils at MUARIK are deep, highly weathered with a pH of 5.0 – 6.0; the climate is tropical, with annual rainfall of about 1300mm, divided into two peaks: abroad peak in March to May and a narrow peak in October to November. The daily maximum temperatures vary from 26^0C in July to 28.5^0C in January and daily minimum temperatures vary from 15.5^0 C in July/August to 17.4^0 C in April.

Taro basal stems (the apical 1-2 cm of the corm with the basal 15-20 cm of the petioles attached) collected from neighbouring farmers' fields were planted out in the field in August 2007 and March 2008 and arranged in a split – split – plot design with three replications

Phenotypic descriptors of taro as described by IPGRI (1999) were evaluated at four months after planting wile corm characteristics were recorded after six months. The quantitative characters evaluated were: Plant span; Plant height; No. of sprouting cormel suckers; Cormel sucker length; Leaf lamina length; Leaf lamina width; Petiole length; Leaf sheath length; Midrib length; Denuding angle; Collecting vein number; Number of leaves; Corm weight; Corm length; Corm diameter; No. of cormels/corm and Plant span. Qualitative descriptors recorded were: Growth habit; Interior sheath color; Petiole attachment; Lamina orientation; Leaf shape; Leaf margin color; Leaf sinus denuding; Leaf surface glossy; Leaf margin type; Leaf vein color; Upper leaf color; Lower leaf color; Petiole color; Vein pattern

To illustrate variability among individual accessions descriptive statistics (mean, standard error of mean, variance, standard deviation and range), correlation and cluster analysis were computed. Pearson's correlation bivariate analysis was carried between qualitative and quantitative parameters on one hand and corm weight, corm length and corm diameter on the other at P=0.01 significance level. Correlation distance (proximity similarity matrix) for taro accessions was calculated using binary Euclidean distance for qualitative and quantitative parameters separately. A hierarchical cluster dendogram was generated using proximity matrix (rescaled distance of the average linkage) between accessions. For this matrix, Pearson correlation coefficients were generated from all quantitative morphological data using SPSS Version 11.5 software package programme.

Results and discussions

For the qualitative morphological characters evaluated, considerable morphological variations were observed for most parameters of taro (Table 1). There was uniformity in most qualitative parameters including the leaf shape, lamina position, corm flesh color while the quantitative parameters were highly variable. Slight variation was observed some characters. 97.6% of accessions had a yellow interior leaf sheath color. In most accessions, variability in qualitative characters was significant. For example, leaf attachment was peltate (in 65.3% of accessions), subpeltate (32.4%) or nonpeltate (2.4%) while lamina orientation was either in downward plane

(77.1%), erect plane (5.9%) or cup-shaped (17.1%). Leaf shape was either without basal bodies (1.8%), hastate (22.9%), sagittate with entire leaf (44.2%), sagittate with basal bodies (5.4%). The leaf margin color was concolorous in 39.4%, purple/red (60%) or clear edge (0.6%) of accessions. For leaf sinus, denuding was either absent (15.3%), slightly present (84.1) or totally present (0.6%). Leaf surface was glossy in 87.1% and not glossy (12.6%). The upper leaf color of tested taro accessions ranged from being light green (37.6%), medium green (46.5%), dark green (12.9%) or reddish/purple green (2.9%) while the lower leaf color was light green in 26.5%, medium green (26.5% or green streaked with red/purple (47.1%). Most taro accessions (71.2%) had petiole color as red/purple color while light green and green streaked with red/purple were in 25.9% and 2.9% respectively. All taro accessions had Y-shaped (85.9%) or V-shaped (14.1%) vein pattern or yellow (7.1%), orange (16.5%) or purple (70.6%) colored veins. Leaf margins were entirely smooth (53.5%) or undulate (46.5%). The proportion of taro with purple corm interior color was 89.4% either while those with white were 10.6%. From these results the most diversified qualitative taro characters were leaf margin color, lower leaf colour, petiole colour and leaf surface glossiness. However, all tested accessions had acaulescent growth form, fibrous corm exterior surface/texture, elliptical corm shape and dark brown corm exterior colour.

Table 1. Variability of some qualitative parameters within taro accessions

Parameter	Mean±S.E	Standard deviation	Variance
Interior sheath color	2.05 ±0.023	0.305	0.093
Petiole attachment	1.37±0.041	0.531	0.283
Lamina orientation	2.11±0.036	0.467	0.218
Leaf shape	2.82±0.045	.593	0.351
Leaf margin color	2.25±0.091	1.187	1.409
Leaf sinus denuding	1.850±0.28	0.371	0.138
Leaf surface glossy	0.94±0.065	0.844	0.712
Leaf margin type	1.46±0.038	0.500	0.250
Leaf vein color	3.75±0.051	0.669	0.447
Upper leaf color	1.81±0.059	0.769	0.592
Lower leaf color	2.68±0.100	1.304	1.669
Petiole color	2.54±0.071	0.924	0.854
Vein pattern	2.72±0.054	0.698	0.488
Corm interior color	1.11±0.02	0.309	0.095

N=170

Few taro traits displayed positive correlation with corm weight, diameter and length (Table 2). However, a strong positive correlation was observed between corm weight and leaf margin type, petiole color. As expected the highest correlations were observed between the corm weight and corm dimensions. Significant negative correlation was seen between corm weight and petiole attachment, lamina orientation and leaf surface glossiness. Interestingly, petiole and lamina parameters that were quite divergent also showed very high correlation with corm weight.

Quantitative characters were highly variable for all tested taro accessions (Table 3). Greatest variation was observed in corm weight, plant span and height and petiole length while low variability was observed in ability to produce sucker above ground, corm length and diameter. While 99.8% of the tested accessions did not sprout cormel suckers above the ground, most harvested corms possessed cormels. The corm weight, plant span and denuding angle were the most divergent characters and would be useful parameters in gauging biodiversity variation among cultivated taro.

Correlation analysis revealed that the Euclidean proximity similarity matrix for all the tested taro samples was 82.1% for quantitative parameters. Pearson's correlation revealed significant correlation between corm weight, corm length and corm diameter and some qualitative and quantitative taro characters. After four months of cultivation, significant positive correlation between corm weight/yield and some vegetative characters including plant span, plant height, number of leaves, lamina width and height, midrib length and leaf sheath length

(Table 4). Garcia et al., (2006) and Dwevedi and Sen (1999) showed significant correlations between yield and several vegetative traits. This reinforces the suitability of agronomic characters in selecting genotypes.

Table 2. Pearson-Correlation between taro corm characters and qualitative parameters

Parameters	Corm weight	Corm length	Corm diameter
Interior sheath color	-0.068	-0.189*	0.015
Petiole attachment*	-0.190*	-0.130	-0.180*
Lamina orientation	-0.188*	-0.380**	0.018
Leaf shape*	0.098	0.377**	-0.165*
Leaf margin color*	0.220**	0.111	0.152*
Leaf sinus denuding	0.004	-0.299**	0.009
Leaf surface glossy	-0.172*	0.048	-0.110
Leaf margin type	0.358**	0.123	-0.001
Leaf vein color	0.161*	0.149	-0.046
Upper leaf color	0.054	0.085	0.344**
Lower leaf color	-0.054	0.101	-0.198**
Petiole color	0.221**	0.297*	0.048
Vein pattern	0.124	0.343**	0.030
Corm interior color	0.049	0.223**	0.168*

Computed at P=0.1 except for * where P=0.05

Table 3. Variability of some qualitative parameters of taro cocoyam accessions

Parameter	Mean±S.E	Standard deviation	Min.	Max.	Variance	Range
Plant span (cm)	61.28 ±1.40	18.23	14.00	130.00	332.23	116
Plant height (cm)	42.95 ±0.99	13.04	14.00	83.00	169.95	69
No. of sprouting cormel suckers*[1]	1.50 ± 0.50	0.70	1	2	0.50	1
Cormel sucker length*[2](cm)	22.10±3.90	5.51	18.2	26.00	30.42	7.80
Leaf lamina length (cm)	29.45±0.65	8.50	7.50	50.00	72.39	42.50
Leaf lamina width (cm)	20.65 ± 0.58	7.55	6.20	78.00	57.03	71.80
Petiole length (cm)	34.15 ±0.93	12.07	12.80	72.00	145.81	59.20
Leaf sheath length (cm)	17.17 ±0.64	8.32	2	49.00	69.37	47
Midrib length (cm)	17.93 ±0.38	4.98	5.50	30.20	24.79	24.70
Denuding angle (°)	57.33±1.26	16.47	10	110.00	271.21	100
Collecting vein number	9.8 ±0.55	7.15	5	99.00	51.12	94
Number of leaves	5.72 ±0.09	1.16	2	9.00	1.35	7
Corm weight (g)	198.25±10.78	140.50	19.39	927.00	19741.05	908.35
Corm length (cm)	12.43±0.43	5.56	1	25.50	3.96	24.50
Corm diameter (cm)	3.76±0.09	1.13	1	6.70	1.28	5.70
No. of cormels/corm	3.44±0.22	2.91	0	16.00	8.46	16

*[1] Mean computed with reference to only accessions with cormel suckers present (N=2)
*[2] Computed as the horizontal ground distance between a main corm plant (stem) and its relative sucker sprout (N=2)

Table 4. Pearson-Correlation between taro corm and quantitative characters

Plant characteristics	Corm weight	Corm length	Corm diameter
Plant span	0.444**	0.039	0.398**
Plant height	0.570**	0.149	0.490**
No. of sprouting cormel suckers	1.000**	1.000**	1.000**
Cormel sucker length	-1.000**	-1.000**	1.000**
Leaf lamina length	0.478**	-0.035	0.418**
Leaf lamina width	0.444**	0.135	0.342**
Petiole length	0.559**	-0.294**	0400**
Leaf sheath length	0.560**	0.102	0.541**
Midrib length	0.545**	-0.055	0.475**
Denuding angle	-0.034	-0.381**	0.163*
Collecting vein number	-0.038	-0.197*	0.010
Number of leaves	0.243**	0.189*	0.212**
Corm weight	-	0.199**	0.732**
Corm length	0.199**	-	-
Corm diameter	0.732**	0.030	-
No. of cormels/corm	0.447**	0.152**	0.465**

Computed at P=0.05

On the basis of quantitative traits, the hierarchical classification of the collected taro biodiversity showed similarity between groups. Two major groups were categorized (dendogram not shown). One group comprising only one plant (Acc. 126) was very unique. On the basis of quantitative traits, the hierarchical classification of the collected taro biodiversity showed similarity and differences between groups. Two major groups were categorized. One group comprising only one plant (Acc. 126) was very unique from a clade which had further two major subcategories comprising a smaller group and a larger group. In all, nine distinct taro subgroups were identified. These nine taro subgroups may be cultivar lines within the large taro population present in East Africa. Within the latter group there was a high degree of variability and it is here that the Ugandan taro accessions were located. However, no distinct geographical clades were observed signifying the relatedness of taro germplasm populations in the region. This high variability of intra-specific productivity in cultivated cocoyam has been shown in tannia (Tambong et al., 1997).

All taro accessions in the region had monomorphic trait loci for growth habit, corm exterior surface texture, corm shape and corm exterior color. The corm weight, plant span and height and petiole length were the most divergent quantitative traits and could be used to select agronomically important accessions for breeding and cultivation. The correlation between corm weight and other morphological traits is could be a tool for selecting taro accessions for agronomic purposes. However, there is need to determine genetic variability since it has been shown that morphological variability in taro does not necessarily translate into a variable genetic base (Lebot and Aradhya, 1991).

The genetic variation displayed by the cluster dendogram, highlights an important biodiversity pool for genetic resources that could be harnessed for better agricultural exploitation of cocoyam germplasm in the region. Given the neglected and underutilized status of taro cocoyam in Africa, for a sustainable breeding program whose mandate would be to develop well adapted and cultivars with increased crop productivity, it will be important to build taro conservation strategies in the region. It will be interesting to develop genetic markers linked to the discussed phenotypic traits. This will be important in defining the genetic relatedness and biodiversity of taro cocoyam, and in cloning genes of agronomic importance (quantitative trait loci; QTL). In this study, biodiversity variation was not linked to geographical distribution. There would be need to critically assess and determine the degree of genetic variation in the constituent East African countries.

Conclusion

The analysis of phenotypic variability and agronomic assessment of 170 accessions in the East Africa Lake basin region indicate that significant phenotypic variation exists among taro cocoyam populations and that, traits like corm weight, plant span and denuding angle are quite divergent parameters and would be useful parameters in gauging biodiversity variation among cultivated taro in the region. Our aim is to establish a biological database for the conservation of taro in the region and selection of high yielding genotypes for distribution to farmers. According to the correlation matrix, the most important vegetative characters corresponding to increased productivity (corm weight) we can focus our selection on traits such as plant span and height, and leaf lamina height and size. To maximize utility of taro germplasm resources in the region, however, there will be need to correlate results from the morphological evaluation with a genetic analysis as a strategy towards selecting germplasm for breeding.

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Participatory design of sustainable alternatives for managing and conservation of agro-diversity of Andean tubers in Márquez, department of Boyacá, Colombia

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Abstract

In the municipalities of Tumerqué and Ventaquemada, department of Boyacá, Colombia, were identified approximately 35 small farmers that include Andean tubers on their productive systems: chugua (*Ullucus tuberosum*), ibias (*Oxalis tuberosa*) y cubios (*Tropaeolum tuberosum*), as a main base of the family feeding. However, they express that there haven't been any processes of research and development on the region, related to alternatives of management, conservation, consume and commercialization of these products, which results on the risk of their extinction.

Based on the latest, generation of useful information about agro ecological factors of production, conservation, use and commercialization of Andean tubers in the two municipalities became crucial, especially for providing options, accurate and compatible with the agro ecological, cultural and economic conditions of the region. This process was made through the methodology of "participatory strategic research" that includes the establishment of a prime study about the current condition of the cultivations at a national and local level, together with participatory processes of characterization of the productive systems; and the collection, identification and conservation *in situ* of the native material on the studied zones.

These processes have been implemented as a way to promote the conservation of agro-biodiversity, the exchange of experiences on the growing of these products, the dialogue between different knowledge and the rescue of the local expertise; which results on a significant gain for the small farmers as much as for the technicians who work with them and have a great influence on the studied zones.

Keywords: conservation; agro-diversity; sustainable alternatives; participative research; Andean tubers.

Description and justification of the subject

At the Andean countries, besides the already known species of the Solanum (potatoes) type, it has been achieved the domestication of a group of tubers, morphologically alike, but from different botanic families, that have been less studied and praised in the agronomical world (Tapia y Fries, 2007). The ruba or chugua (*Ullucus tuberosum*), (togheter with some other species like the libia ((*Oxalis tuberosa*) and the cubio(*Tropaeolum tuberosum*) are some of them. They are grown on small areas under traditional production systems, but are indispensable for assuring the feeding diversity and the sustainability of the communities that face a higher risk (Espinosa et al, 1997).

In the particular case of the municipality of Turmequé, located on the province of Marquez of the department of Boyacá, they were identified approximately 35 small farmers, who are currently growing ibias, cubios and rubas, as the main base of the family's feeding. Also, they are considered as a self-consume cultivation so its process involves all the family members. However, based on the testimony of these small farmers, there haven't been enough research projects in the region, so there are no tools for assuming conservation strategies, and sustainable management of the agro diversity; as well as strategies for using, consuming and selling these products. This situation is dangerous for the existence of the products, although their importance "they are healthy cultivations, and a heritage of our elders, a symbol of our culture and the base of our feeding, but the face the risk of remain forgotten" (Melciádes Muñoz, small farmer of Turmequé, 2007).

A participatory process of research took place, based on the latest and with the aim of providing useful information related to the agro ecological elements of production, the identification, conservation of the agro diversity, the alternatives of use and of organizational strengthen for the small farmers of the Andean tubers in Turmequé. This research took into account three components:

5. Historical reconstruction that allows a primary state of art about the different research activities already implemented in Colombia on Andean tubers, particularly, in the department of Boyacá. Also, gathering information about the process of conservation, consume and use on behalf of the small farmers; and about the marketing possibilities for the products.
6. Promote participatory local processes of collection, identification and conservation of native material on the studied zone, through activities of interchange, not only of germplasm between the productive zones in Boyacá, which could promote the identification and conservation of agro diversity; but also an interchange of experiences on agricultural management and alternative ways of use and consume.
7. The research intends to promote a third component that includes the organizational strengthen for the small farmers who conserve and produce these species, in order to start business processes that allow them enhance the development and commercialization of the products and improve their living conditions, as designing strategies of sustainable management.

Geographical location and beneficiary population

The municipality of Turmequé its located at 110 km away from Bogotá D.C. Its population is 12716, distributed on 14 paths. It is located, attitudinally, between 3000 and 3400 musl, and it has medium temperatures of 12ºC a 18ºC, its annual precipitation is from 800 to 2000 mm. The inhabitants of Turmequé have based their economy on agriculture, providing supplies for the local and regional markets. They grow specially potato, beans, curuba, "caducifolios", fruit tomato, uchuva, peas, corn and Andean tubers as ibias, cubios and rubas, but the latest without the production based on performances and competitive characteristics.

The municipality has a smallholding structure, with low technological development and based on the tradition that every son inherits a part of the land when getting married. This has sharpen the problem, because the families don't get to ensure the basic salary for their sustainability, according to the estimations of the Agricultural Familly Unit (AFU). The farmers show a decreasing educational tendency, caused by the low incomes. In Turmequé, a large number of people have moved out from their paths taking their children with them, especially because of the worsening of the productive activities, which has led to improvements on their economical and social conditions. This migrated group is located on rural zones and it has higher proportion of non educated women, 213 women represent a 54% and 181 men represent 46%.

They were identified 35 small farmers in Turmequé that are currently cultivating Andean tubers, located on the following paths: Pozo negro, Teguaneque, Uratá, Matanegra, Volcán Blanco, and in neighboring municipalities as Ventaquemada. The beneficiary population is as follows:

Municipality	Consulted and interested farmers	Direct Beneficiary Families	Indirect beneficiary families
Turmequé	35	80 families	250 families
Ventaquemada	20	60 families	100 families

Objectives

General objective

Design viable alternatives of management, use and conservation of agro diversity through a participatory research of the agro ecological, economical, cultural and market conditions of Andean tubers (*Ullucus tuberosum*, *Oxalis tuberosa* y *Tropaeolum tuberosum)* and the initiation of organizational strengthen processes for the small farmers in the province or Marquez, Department of Boyacá.

Specific objectives

1. Establish the state of art regarding the research processes and the use, production and commercialization of the Andean tubers in Boyacá, focusing on a case study about the municipality of Turmequé.
2. Identify and characterize the production systems of Andean tubers in Turmequé, based on agro ecological, economical and technological criteria; in order to know the implications of this production for the farmers and their families in the zone.
3. Develop participatory alternatives of conservation, use and production within the agro diversity of Andean tubers in the studied zone.
4. Initiate organizational strengthen and individual growing processes for the small farmers, in order to promote self-management, leadership and sustainability of the Andean tubers.

Methodology

This research bases on the Rural Participatory Innovation (RPI) approach, as this is an integral and trans-discipline methodology that seeks for the communities to be involved since the beginning to all the phases of the process, since the planning to the implementation and follow up phase.

Objective 1. For establishing the state of art about the Andean tubers at a scientific and technical level and regarding the use, production and commercialization in Colombia and specially in Boyacá; it was developed a process of gathering information from different sources: Secondary and primarily sources and field work, using the Case Study approach with the small farmers in Turmequé. Finally, the resembled data was organized and systematized, triangulated, analyzed, which led to a written document.

Objective 2. It was created a participatory committee for characterizing the productive systems of Andean tubers in Turmequé, formed by small farmers and researchers. The committee used participatory methodological tools as semi- structured interviews, talking maps, time lines, and future expectations, so on; for gathering information. The most important variables to consider were: Social, agro ecological, technical and economical variables.

Objective 3. Taking to account the aim of promoting sustainable process for using Andean tubers, according the farmer's priorities and necessities, they were held a set of workshops called "training workshops about food safety and nutrition". They combined the use of Andean tubers with some other nourishment. The workshops also tried to rescue the traditional recipes of the zone, to standardize and balance them.

For promoting conservation process *in situ*, it was created and implemented the "**First Andean tubers agro diversity Fair".** The main objective of the Fair was to collect the local germplasm (from Turmequé and other municipalities). This germplasm was subsequently classified and identified by a local morphological characterizing committee. This processes let the establishment of two focus groups of participatory research about conservation *in situ* on small farms. The focus groups are still working.

Objective 4. For the accomplishment of this objective, we took the methodology developed by the PBA Corporation that works transversally in all the projects at the five countries that belong to the Andean Consortium. This methodology is based on promoting the empowerment of its all process, trough the development of a system of training and monitoring, focused on personal growing, organizational development, that tries to enhance individual abilities and capacities, teamwork, self esteem, trust, communication and conflict management.

This methodology also seeks to identify and strengthen local leaders, as well as promoting the creation of participatory local groups that could later multiply and reproduce the ideas of conservation, use and management of the agro diversity.

Preliminary results

Establishment of the first state of art about Andean Tubers (AT) in Colombia

We currently have a complete written document, still unpublished, that joins several research projects about the use, production and commercialization of Andean tubers in Colombia.

The study shows that, although the importance of these species, there are just a few researches about them, in comparison with other countries such as Bolivia, Ecuador and Peru, where they have a greater value to the general population. The lack of sufficient research in Colombia could be produced by the feeding habits of the urban people, (according to the 2005 national census, made by the DANE, 76% of the Colombian population live in urban environments), which have change their dietary dynamics where, despite of the potato, the Andean tubers are absent and have lost their commercial and cultural importance. This explains why the Andean Tubers are not a priority for researchers.

However, due to the unexplored potential of de AT, public and private institutions have implemented several efforts for conserving and researching about AT, toward studies on different fields; but they are not continuous and barely published.

The document is divided on three parts: The first part is a review of general information about the three species of AT, taking into account their origin, morphological description, agro economical management, use and nutritional properties. The second part, shows the characteristics of the current state of research about these species on a regional and national basis. Finally, the third part is and market prospection in Bogotá, Turmequé and Tunja, developed in order to understand the current possibilities for commercialization and consume of these products.

As the main results, we can shift the fact that the researches already done are followed by a strategic interests, personal motivations and very exact subjects as identification and conservation of germplasm *ex situ,* fertilization, chemical composition, market studies and inter specific diversity.

Related to the commercialization and consume of AT in the urban trade centers, we found that the most popular tuber is de ibia and the cubio is the less popular. Also, we found that in general, the level of consume of this products is very low, due to the small accounts that families often buy and the reduced opportunities when they buy them.

By the other hand, another important result is that the merchants on the trade centers perceive a lot of advantages in selling these products, especially for their price and quality.

Characterization of the productive systems

For achieving this goal, we also produced another written document, still unpublished, which, together with the document presented above, are part of a book that will be published next year.

The main objective of this document is the systematization of the agro ecological, economical and technological characteristics of the AT production systems, at the municipalities of Turmequé and Ventaquemada; in order to know and understand the different implication of their production for the pleasant families on the zone. In addition, we also studied the nutritional situation of the producers of AT, as a primary diagnosis that could led future interventions based on global strategies for solving de nutritional vulnerability together with the conservation of agro diversity.

As a methodological basis, we took a theoretical framework based on the “systemic approach” and the “rural participatory diagnostic (RPD)”. Those two components led the process of research.

The research process let us conclude important assertions, such as the fact that the characterized production systems are typically agricultural, highly heterogeneous because of the small size of the farms and the number and variety of the species grown; also, the educative and socio economical level of the producers and their families affects the characteristics of the productive systems.

We identified as well, a broad diversity of vegetable species and some animal species that are normally kept in the studied farms and enhance de nutritional security of the families, allowing the diversification of productive activities, and a broader flexibility for getting economical incomes when the main products as potato, lower their prices on the markets.

The presence of rubas, ibias and cubios was demonstrated on the characterized farms, at different level of grow, since little spots inside the farms till main products on the productive systems. The level of consume is frequent and is closely related with the nutritional culture and traditions on the zone, which have guaranteed their conservation. However, the existence of these species is facing a progressive restriction, due to the nutritional changes of the rural population, the high changes on the environment and the permanent shortage of the products because of the long cycles of production and the pressure of other more suitable and profitable products as the potato.

The AT are closely linked with the rural culture of this zones, so the cultural expressions and the traditional agricultural techniques have been the same since the indigenous ages, and the forms of use are diverse and unique for every municipality, being almost an expression of their identities. This means that the conservation of the agro diversity is not only a matter of economical expectations.

Taking into account that the AT are part of the dietary structures of this social groups and their production is normally low, all the members of the family are involved in the process. The role of women is very important, especially because they care about the nutritional supplies. Also, the children learn their parent's techniques, guaranteeing the generational survival of these species.

The size of the farms has significant technical and economical implications, which is reflected on the level of productivity and brings difficulties for the competiveness of the productive systems. That is why, future interventions for communal development have to take into account this element, and be creative when designing non- exclusive strategies for enhancing bonds within the farmers and offering tools for building capacities, in order to face the market's needs and guarantee the productive viability on a long term.

The AT productive systems are characterized by the utilization of agricultural traditional tools as the mattock, the high use of handmade procedures, the low utilization of external row material as pesticide or fertilizers. These conditions make the production really clean and with an extended exploitation and commercialization potential, focused on a set of markets that seek these types of clean products and with costumers willing to pay an extra price for them.

In terms of family's nutritional security, it could be seen that they have a broad diet based on diverse nourishments that are mostly grown on their own farms. However, it can be analyzed that some products consumed on a daily basis are bought on local markets, which produces that, in low productivity periods or low prices of grown products, the real access to these products is restricted. The exchange of aliments is pretty unusual.

Development of participatory alternatives for conservation, use and production of AT agro diversity

First Andean tubers agro diversity Fair. The Fair occurred on 17th may of 2009. The main objective was to collect seeds of ibias, rubas and cubios, for a later establishment of plots of conservation *in situ* that will be managed by the same farmers. About five hundred people visited the fair, especially agricultures, technicians, students, researchers and local and national political authorities. There were collected about 36 sets from different paths from the municipalities of Ventaquemada y Turmequé, together with genetic material from other municipalities of Boyacá and Cundinamarca.

The farmers were the main key actors in the process of planning and implementing the Fair, and it was a space where they had the opportunity to show the results of the project toward some presentation stands, where they

could spread their local knowledge, the species diversity, the traditional agricultural management and their nutritional culture, showing dishes and preparations that came out from the nutritional security workshops.

Plots for conservation in situ

Until today, there have been established two focus groups of participatory research about conservation *in situ* at small farms, one in the municipality of Ventaquemada and the other in Turmequé. Each focus group is integrated by two farms, in which they were established plots where the seeds recollected from the fair were grown. A certain farmer is in charge of one plot and has to register all the phenological developments and agricultural activities implemented.

Next September, there will be a field trip day when all the farmers will have the opportunity to evaluate and visit other conservation plots, receiving from their partners the reports from the development of the plots. Once the plots are completely grown, the seeds will be distributed among all the farmers of the project, in order to be multiplied and validated regarding their nutritional conditions.

Organizational and personal strengthen processes

Until today, there have been implemented four workshops of personal growing and organizational strengthen. The participation of men and women on the process has shown a strong commitment and motivation. The young participants have been very creative and active, designing the project's logo, writing songs and poems about the AT.

The social capital that is developing around the implementation of the project is one of the most important indicators, because, the productive area and the rescue of the agro diversity are not the only evidences of success; however, the cohesion and committed team work within a community that is currently trying to keep their own cultural and nutritional richness are the most important basis of this process and it sustainability though time.

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Some photographs

Farmers on a Practical Nutrition Workshop

Plots of conservation *in situ*

Agro diversity Fair

Characterization of productive systems

Contribution of standards to developing networks and providing access to plant genetic resources

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Abstract

The world is continually faced with the need to increase crop productivity, and to develop new varieties better adapted to face environmental and biological challenges or to meet the needs of local communities. To meet these needs and challenges, farmers and breeders must have access to a wide range of plant genetic resources (PGR) and to the essential information about those PGR and traits they possess to facilitate their utilization.

The value of PGR is dependent upon the information available. Accurate characterization and evaluation data promote use, especially if it is available in a standard format. Through the production of descriptors in collaboration with National Agricultural Research Systems, CGIAR centres, and crop research institutes and networks, Bioversity aims to stimulate the characterization and evaluation of PGR collections by providing uniform and unambiguous guidelines for the description and exchange of information on germplasm.

Crop descriptors and derived standards are essential for the scientific documentation of PGR. They are an important tool that permits the international PGR community to exchange information in a 'common' language. This in turn helps to develop active PGR Networks because data exchange is easy and enhances collaboration. Furthermore, these networks can be assembled into a global partnership whereby access to information and use of germplasm are further facilitated, which supports the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA).

Keywords: Standards, Descriptors, Characterization, Evaluation, Access, Information, Utilization, Plant Genetic resources.

Introduction

This paper explores how standards contribute to establishing databases and developing networks and in this way, provide access to plant genetic resources information to further its utilization. It also provides and insight of the history of descriptor lists —scientific standards for documenting plant genetic resources— which have been developed by Bioversity International (formerly IPGRI) since 1976.

We can not manage what we do not know. In the same way, we can not use genetic resources if we do not know them, or if we do not know where we can obtain them. Germplasm accessions without information do not have any value, like books without a title.

There is a huge amount of plant genetic resources already conserved in genebanks worldwide, but all the associated information is of limited use because data are maintained in unlinked documentation systems, or simply because documentation is lacking. Although most genebanks have developed their own information system according to their needs, there are just a few examples of linkages among them. These constraints prevent breeders and users from effectively searching for desired crop specific genetic traits within all of the genebanks around the world.

Bioversity has been working since its inception to support the use and conservation of plant genetic resources through the development of scientific standards for crops. The idea for developing standardized methodologies for describing germplasm accessions was debated around 1976, as it was apparent that a universally understood methodology was essential for any global system of conservation and networks of genebanks to operate effectively (Gotor *et al.*, 2008).

Initially, there was not capacity of partners to understand characterization and evaluation data on crops common to them, leading to exchange of information and utilization of plant genetic resources. Furthermore, crop specific descriptors were not available and they were developed through experiences and observations about the crop. Only when many researchers are working on the same crop, they realize that there is a need to standardize the way they describe a characteristic or a trait, so that they can review and compare data from different sources. There are still cases where different genebanks measure the same trait of the same crop in different ways, giving totally different values for the same characteristic.

This lack of compatibility in documentation systems for describing plant genetic resources seriously affected data exchange between genebank collections. This was the reason behind the need to develop a methodology that had international approval and that could be easily used within a country and among different countries as well.

To be effective, the methodology needed to unambiguously and correctly describe an accession in order to discriminate between them within the same collection, thus, nurturing collaboration among scientists working in different countries.

A comprehensive and standardized description of a crop allows better compatibility between documentation systems and facilitates the exchange of information. This also reflects the value attached to traits by plant genetic resources researchers and users.

An accurate characterization and evaluation data promote and increase the use of plant genetic resources, particularly if it is available in a standard format, because information is the link between conservation and use. Conservation and use of plant genetic resources for food and agriculture are crucial for ensuring adequate food supply worldwide. Particularly nowadays, when the continuous improvement of crop plants is essential for agriculture mainly due to climate changes, and relies on the use of genetic variability through breeding.

In collaboration with partners throughout the world, Bioversity aims at building the knowledge base needed to ensure effective use of diversity to increase sustainable agricultural production, improve livelihoods and confront the challenge of climate change. The development of standards is one way to achieve its mission since the value of conserved plant genetic resources is dependent upon the information utilized to promote their use.

Contribution to crops description

The development of standardized descriptors began in 1976, when a universal system was essential for global efforts in plant genetic resources conservation and for crop networks and genebanks to operate effectively. Bioversity in collaboration with CGIAR centres, crop networks, research organizations and national programmes, has developed descriptors for more than 100 crops in different languages.

Each crop descriptor list represents an important methodology and provides an internationally agreed format and universally understood language for plant genetic resources data, particularly regarding characterization and evaluation information. The adoption of this tool (and in some cases, a conversion method to Bioversity format), helped to create an efficient and reliable instrument for information exchange, storage and retrieval to facilitate the utilization of germplasm.

The Bioversity crop descriptors published so far represent 3.5 millions accessions held in genebanks worldwide and cover the 90% of the crops listed in the Annex I of the International Treaty. They also represent a 95% of the CGIAR mandate crops.

Descriptors contribute to increase knowledge and facilitate research not only on mandate crops, but also on crops that have limited attention by the research community but which are often crops preferred by the poor. The 35% of crop descriptors deal with Neglected and Underutilized crops.

In addition to the use of these traditional crop standards made by the majority of plant genetic resources workers, they are being used as 'best practices' and most of them have been adopted by the Crop Genebank Knowledge Base (which is a product of the System-wide Genetic Resources Programme). Furthermore, the current activities on controlled vocabularies, such as the Trait Ontology Consortium, or the Generation

Challenge Programme have adopted most of the text included in the crop standards or use them as data templates.

Impact of passport standards

When germplasm collections were integrated into multicrop collections at the national level, it was evident that common descriptors were required to be more consistent across different crops. As a result, Bioversity jointly with the Food and Agriculture Organization of the United Nations (FAO), with substantial contributions from European countries and CGIAR Centres, published a standard for passport data, the List of Multi-crop Passport descriptors (FAO/IPGRI, 2001).

This subset of passport information provides an international standard across crops to facilitate information exchange on passport data in research institutions worldwide. It constitutes the basis for a standardized documentation system. Each descriptor incluyes suggested field names to assist in a computerized exchange of data and a brief explanation on how to record the information.

This standard has had a huge impact, especially in Europe where the European Search Catalogue of Germplasm Accessions (EURISCO) with passport information on *ex situ* collections maintained in Europe has been developed based on the passport standard. This European Catalogue contains information on around 30 countries representing more than 1.0 million accessions from Europe. Additionally, this standard for passport data is being used as a basis for the development of many of the central crop databases belonging to the European Cooperative Programme for Plant Genetic Resources (ECPGR).

Same applies to the FAO-WIEWS Directory of Germplasm Collections, which includes passport data fields matching this standard. Also, almost all CGIAR centres follow, whenever possible, the format and content proposed, making it easier to retrieve or exchange data.

Outside the CGIAR system, other scientific organizations such as the Crop Scottish Crop Research Institute have adopted the MCPD to create their database (GERMINATE), a software which links phenotypic and genotypic data through passport data. It has been also adopted by the Generation Challenge Program for the creation of 'The Bioversity/FAO MultiCrop Passport Descriptor Ontology' which is an adaptation of the FAO/IPGRI passport standard.

Coping with marker technologies information

With the rapid development of marker technologies and an increased molecular and biochemical characterization of plant genetic resources, the need arose to define common standards for documenting information about genetic markers. In order to address this issue, Bioversity published the 'Descriptors for Genetic Marker Technologies' to complement classical agro-botanical analysis (De Vicente *et al.* 2004). This descriptor list includes a minimum set of standards for documenting information about genetic markers and is targeted at researchers who use genetic marker technologies. This standard is meant to facilitate documentation and exchange of standardized genetic marker data. It also provides descriptions of content and coding schemes that will assist in computerized data exchange.

Integrating farmers' knowledge and science

In an effort to integrate traditional knowledge and science, Bioversity has developed in 2009, a standard for sharing data describing farmers' knowledge about people and plants. This standard has been developed to create a *lingua franca* to capture and share information amongst farmers and scientists and to integrate biology and traditional knowledge.

It provides a standard format for the gathering, storage, retrieval and exchange of farmers' knowledge of plants. It aims to capture key characteristics, uses and values of cultivated and wild plants as described by farmers and other people in farming communities. Many of these descriptors are not included in conventional descriptor lists. Wild and weedy plants are also covered by this list since they often play a significant role in farming communities, being useful from a socio-economic and ecological standpoint.

This standard is a first attempt to combine a documentation system traditionally used in controlled environments (genebanks, breeding institutes) with an approach that involves people and their knowledge 'in the field'. We hope that this list, which is the result of many years of review of fieldwork by scientists and field practitioners, will become an important tool for integrating biology and traditional knowledge (Bioversity and The Christensen Fund, 2009).

Maximizing information through a global portal

Bioversity and its partners are currently developing common information standards to describe the key characteristics of genetic resources that are important for crop improvement, so everyone can communicate effectively, through a project funded by the Global Crop Diversity Trust. These key standards constitute the backbone of the global portal of information on germplasm accessions which aims at making key information about accessions available for breeders and others in order to use the material stored in genebanks. This will increase the utilization of biodiversity, which is the key to agricultural development in a time of climate change.

The goal of the project is to provide access to information by scientists to the material they need and addresses the impediments faced by users who seek hard to find data about germplasm that can provide resistance to biological and abiotic stresses that reduce yields.

The project has three components: the first component will mobilize scientists to agree on initial key sets of characterization and evaluation traits of most interest to users. These standards will be incorporated into the second component, the GRIN-Global genebank datamanagement system being developed by USDA through another project funded by the Trust. This system will be deployed to national programmes and other genebanks in developing countries. Finally, the third component of the project involves the development of a global accession-level information portal for accessing and managing accession data in support of conservation and use of crops for food and agriculture, by linking up national, regional and international genebank databases (see Figure 1).

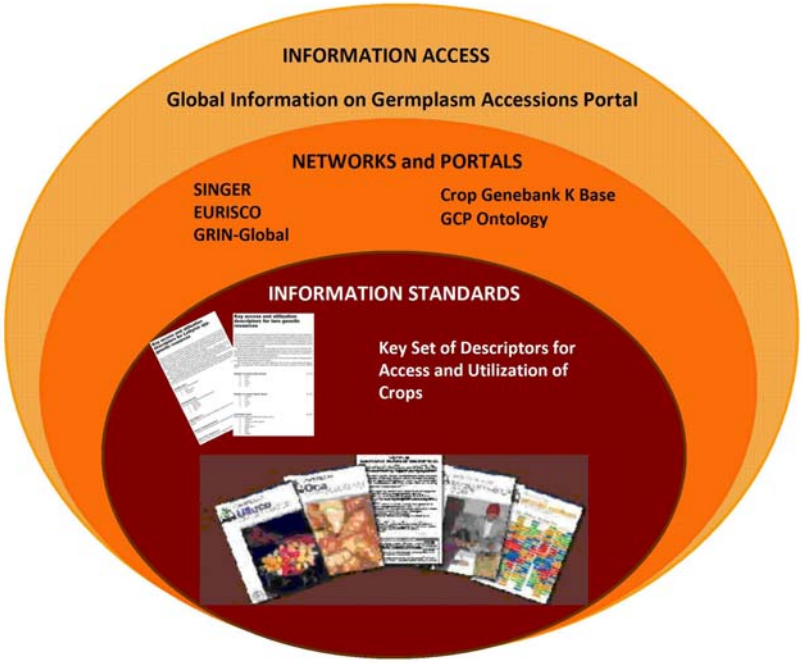

Figure 1. Contribution of standards to developing networks and providing access to PGR utilization

Conclusion

Through the production of standards in collaboration with partners, Bioversity aims to stimulate the characterization and evaluation of plant genetic resources collections by providing uniform and unambiguous methodologies for the description and exchange of information on germplasm. Thus, the adoption of descriptors and derived standards should be integral to genetic resources activities, assuring adequate germplasm conservation and its efficient utilization in crop improvement programmes.

Crop descriptors and derived standards are essential for the scientific documentation of plant genetic resources and are an instrumental tool that permits the international community to exchange information in a common language. This in turn helps to establish databases and to develop crop networks because data exchange is easy, and nurture collaboration globally.

Furthermore, these networks can be assembled into a global portal whereby access to information and use of germplasm are further facilitated, which assures a full implementation of the Convention on Biological Diversity and supports the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA).

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Molecular characterization of potato cultivars using SSR markers

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Abstract

The potato crop has a very narrow genetic base, so the use of molecular markers is a very important tool in the characterization of germplasm banks and in the identification of the most promising choice of parents to be used in plant breeding programs. The objective of this study was to assess, using microsatellite or simple sequence repeat (SSR) markers, 38 accessions of potato from two separate collections of commercial cultivars, aiming at the genetic characterization, identification of duplicates and possible parents for breeding programs. For the molecular characterization 10 primers were used, generating a total of 46 alleles (bands), which were analyzed as binary data. A cluster analyses was performed with the Jaccard´s similarity coefficient and the UPGMA method, using the software NTSYSpc. On average, the number of alleles per locus was 4.6, ranging from two alleles for primers STM1049, STM 1053 and STM 1104 to 12 alleles per locus for primer STM0019a. Of the 46 alleles, only five were monomorphic, therefore presenting 89.1% polymorphism. The polymorphism information content (PIC) varied from 0.13 to 0.86, with an average of 0.54. The Jaccard´s coefficient varied from 0.41 to 0.93, showing a high genetic variability among accessions. Two possible duplicates [Atlantic (Canada) and Atlantic (Chile), and Colorado and Agata (EPAMIG)] were identified. High similarity was also shown by cultivars Chipie and Melodie (EPAMIG), Voyager and Gourmandine (EPAMIG), Eole and Caesar (EPAMIG), and Cupido and Santè (Pedro Hayashi). The genetically most divergent accessions (Lady Rosetta and HPC-7B) were also identified. The high levels of polymorphism observed for *Solanum tuberosum* suggest that microsatellite markers represent a useful tool to detect genetic differences between cultivars and can be used in potato breeding programs.

Keywords: *Solanum tuberosum*, germplasm banks, genetic diversity, microsatellites.

Introduction

Potato (*Solanum tuberosum* L.), in order of economic importance, is the fourth agricultural crop, planted at least in 125 countries and consumed by more than a billion people around the world (Pastorino et al., 2003). Worldwide, this crop is undergoing major changes. Until the beginning of 1990, it was the most cultivated and consumed in Europe, North America and in countries of the former Soviet Union. Since then, there has been an increase in potato production and demand in Asia, Africa and Latin America, where production rose less than 30 million tons in the 60s to over 165 million tons in 2007 (FAO, 2008).

Brazil ranks as a major potato producer in Latin America, with a record harvest in 2006 of around 33.1 million tonnes. The highest yield was observed in regions of southeast and south with 1,962,045 and 1,188,416 tons respectively. Current data indicate a total area of 138,852 hectares and a total production of 3,438,825 tonnes with an average yield of 24.7 tonnes per hectare, giving a positive variation of 3.7% (Brazilian Institute of Geography and Statistics - IBGE , 2009; FNP, 2009).

Despite the great progress in all these years, it is necessary to search for more productive, adapted and resistant material. Molecular characterization is one of the biotechnology tools to help, remarkably, plant breeding programs in a period of time considerably shorter compared to traditional methods of breeding. Microsatellites, also called SSR (Simple Sequence Repeats), are one of the more polymorphic molecular markers available today (Grattapaglia and Ferreira, 1998). Microsatellites also have advantages over other markers based on PCR (Polymerase Chain Reaction), such as RAPD (Random Amplified Polymorphic DNA), because they are co-dominant and easily reproducible, and have a frequent and random distribution, allowing a wide coverage of the genome. The high level of variation detected with microsatellites increases the resolution for genealogy and germplasm genetic diversity studies and reduces the number of markers required to distinguish between genotypes (Borém and Caixeta, 2006).

Rocha (2008), using six RAPD and three SSR primers, identified 16 cultivars of potato. The author observed that SSR markers were more efficient than RAPD markers, since three of the SSR primers allowed the distinction of all cultivars studied, compared with the six primers used for RAPD. Several studies have used SSR markers for the characterization of potato cultivars and accessions, such as Ghislain et al. (2000), using two SSR primers to identify 20 varieties of native potatoes of the Andes, Norero et al. (2002) using four SSR loci for discriminating 37 commercial potato cultivars from INTA (National Institute of Agricultural Technology) in Argentina, Braun and Wenzel (2004) using 26 SSR loci to evaluate 47 genotypes from the potato breeding program in Germany, Braun et al. (2004) evaluating 75 cultivars of North America, Europe and Japan with 15 SSR loci, Chimote et al. (2004) assessing 32 potato cultivars in India with 16 SSR loci, Barandalla et al. (2006) evaluating with 19 SSR loci 41 cultivars from Tenerife Island, Mathias et al. (2007) using 21 SSR loci to evaluate 71 potato genotypes from the Institute of Agricultural Research (INIA) of Chile, Ispizúa et al. (2007) assessing 155 accessions from INTA in Argentina using four SSR loci, and Fu et al. (2009) evaluating 114 Canadian and 55 exotic potato accessions using 36 SSR loci.

Within this context, the aim of this study was to characterize, at the molecular level using microsatellite markers, 38 commercial cultivars of potatoes used in Brazil, originating from two separate collections, identifying possible duplicates and different materials with potential for use as parents, to assist in the breeding programs.

Material and methods

In this study, 38 potato cultivars from two collections, Pedro Hayashi Company, located in Vargem Grande do Sul, SP and Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), were assessed (Table 1). For the DNA extraction, recently expanded leaves were dried in an oven at 45ºC for a period of 24 hours, after which they were macerated and submitted to a 3% CTAB methodology as described by Siqueira et al. (2009). DNA concentration of each individual was estimated by running samples in 0.8% concentration agarose gels. The gels were prepared using 0.8 g of agarose diluted in 1X TBE buffer [100 mL 10X TBE (0.89 M Tris base, 0.89 M boric acid, 20mM EDTA pH 8.0) and 900 mL of distilled water] and stained with 4µL Ethidium bromide.

Ten specific microsatellite primers were used (Ghislain et al., 2006). The total volume for each polymerase chain reaction (PCR) was 10.2 µL, including the following components: 0.2 µL of Taq-Polymerase (5U/µL), 1.0µL of buffer (10x Amplification Buffer), 1.0µL $MgCl_2$ (50mM); 0.5µL of Primer F (5pmoles/µL), 0.5 µL of Primer R (5pmoles/µL); 1.0µL of dNTP's (2.5mM each); 3µL of Milli-Q H_2O and 3µL of DNA in each microtube. For these reactions, the thermocycler MyCycler Thermal Cycler model of BioRad was used. The PCR reactions were conducted in the following sequence: 3 min at 94°C, followed by 30 cycles of 30 sec at 94°C, 1 min at the annealing temperature for each primer set (Table 2) and 1 min at 72°C, and a the final extension of 5 min at 72°C.

The amplification products were separated in 6% polyacrylamide gels under an initial voltage of 60 volts for 30 min, extending it to 120 volts for about two hours in TBE buffer (0.09 M Tris, 0.09 M boric acid, 2 mM EDTA). Standard molecular weight markers of 10 bp and 100 bp were used. The material was stained with silver nitrate (Bassam et al., 1991) to reveal the microsatellite bands and photodocumented.

For the statistical analysis, each SSR locus was characterized as a dominant marker, according to the presence or absence of bands, which were analyzed visually. These data were used in the construction of a binary data matrix, where the value 1 (one) means presence of bands and the value 0 (zero) their absence. With this matrix, the Jaccard similarity coefficients were obtained. Using this coefficient and the cluster method UPGMA (Unweighted pair-group method with arithmetic averages), a cluster analysis was performed using the NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) software (ROHLF, 1992). The accuracy of the groupings was estimated from simulations with resampling, using 10,000 bootstraps, and the BOOD software, version 2.0 (Coelho, 2001).

The polymorphism information content (PIC) was calculated by the formula: $PIC = 1 - \sum_{i-1}^{n} p_i^2$ where:

pi = frequency of the allele (band) in each locus and n = number of alleles observed.

Table 1. Potato (*Solanum tuberosum*) accessions evaluated and origin (source) of the accessions

Nº	Comercial Varieties	Source
1	ATLANTIC	Pedro Hayashi
2	CUPIDO	Pedro Hayashi
3	ASTERIX	Pedro Hayashi
4	SANTÈ	Pedro Hayashi
5	MONDIAL	Pedro Hayashi
6	ATLANTIC Chile	Pedro Hayashi
7	FIANNA	Pedro Hayashi
8	PIRASSU	Pedro Hayashi
9	ITARARÉ	Pedro Hayashi
10	SPUNTA	Pedro Hayashi
11	HPC-7B	Pedro Hayashi
12	LADY ROSETTA	Pedro Hayashi
13	PANDA	Pedro Hayashi
14	AGATA	Pedro Hayashi
15	MONALISA	Pedro Hayashi
16	AGATA	EPAMIG
17	ASTERIX	EPAMIG
18	ATLANTIC	EPAMIG
19	BRS ANA	EPAMIG
20	BRS ELISA	EPAMIG
21	CAESAR	EPAMIG
22	CANELE (FR)	EPAMIG
23	CATUCHA	EPAMIG
24	CHIPIE	EPAMIG
25	COLORADO	EPAMIG
26	EDEN	EPAMIG
27	EMERALDE	EPAMIG
28	EOLE	EPAMIG
29	FLORICE	EPAMIG
30	FONTANE	EPAMIG
31	GOURMANDINE	EPAMIG
32	GREDINE	EPAMIG
33	MELODIE	EPAMIG
34	MONALISA	EPAMIG
35	NATURELLA	EPAMIG
36	OPALINE	EPAMIG
37	SOLÉIA	EPAMIG
38	VOYAGER	EPAMIG

Results and discussion

In this study, all 10 loci used showed polymorphism among the accessions analyzed, producing well-defined and reproducible bands. A total of 46 alleles (bands) were amplified with an average of 4.6 alleles per locus, ranging from two alleles for primers STM1049, STM1053 and STM1104 to 12 for the primer STM0019a (Table 2).

Only five alleles were present in all the varieties evaluated, while 41 alleles were shown to be polymorphic for all the 38 cultivars, therefore showing 89.1% polymorphism. Milbourne et al. (1997), evaluating 14 potato genotypes from northwestern Europe with 17 SSR loci, found 98 alleles (bands) with an average of 5.76 alleles, greater than the value reported in this study, although with a greater number of loci. Braun and Wenzel (2004), evaluating 69 cultivars from Germany with 26 SSR loci, observed 128 alleles (98.4% polymorphism) and a mean number of alleles of 5.12.

Table 2. Potato (*Solanum tuberosum*) primers[1] used in this study, including the number of alleles per locus, the annealing temperature (T°C), the size in bp and the polymorphism information content (PIC) per locus

Locus	Sequence (5' → 3')	Alleles number	T°C	Size pb	PIC
STM0019a	F: AATAGGTGTACTGACTCTCAATG	12	54,3	160-280	0,8583
	R: TTGAAGTAAAAGTCCTAGTATGTG				
STM0037	F: AATTTAACTTAGAAGATTAGTCTC	6	56,1	70-100	0,7149
	R: ATTTGGTTGGGTATGATA				
STM1049	F: CTACCAGTTTGTTGATTGTGGTG	2	61,6	190-200	0,6073
	R: AGGGACTTTAATTTGTTGGACG				
STM1053	F: TCTCCCCATCTTAATGTTTC	2	60	180-190	0,1264
	R: CAACACAGCATSCAGATCATC				
STM1104	F: TGATTCTCTTGCCTACTGTAATCG	2	60	170-180	0,3732
	R: CAAAGTGGTGTGAAGCTGTGA				
STM1106	F: TCCAGCTGATTGGTTAGGTTG	4	60	150-170	0,5554
	R: ATGCGAATCTACTCGTCATGG				
STM2013	F: TTCGGAATTACCCTCTGCC	3	60	145-160	0,5678
	R: AAAAAAAGAACGCGCACG				
STM2022	F: GCGTCAGCGATTTCAGTACTA	6	64	170-230	0,4301
	R: TTCAGTCAACTCCTGTTGCG				
STM3012	F: CAACTCAAACCAGAAGGCAAA	3	66	170-210	0,5039
	R: GAGAAATGGGCACAAAAAACA				
STPoAc58	F: TTGATGAAAGGAATGCAGCTTGTG	6	63	240-285	0,6997
	R: ACGTTAAAGAAGTGAGAGTACGAC				

[1]Ghislain et al. (2006)

Mathias et al. (2007) observed a variation of two to 17 alleles/locus in the evaluation of 71 genotypes from INIA, Chile, with 21 SSR loci, in agreement with Fu et al. (2009) reporting two to 17 alleles per locus in the evaluation of 114 Canadians and 55 exotic potato accessions with 36 SSR loci. The values found in this study, ranging from two to 12 alleles/locus, are therefore consistent with the literature whereas a smaller number of genotypes (38) were evaluated.

The polymorphism information content (PIC) ranged from 0.13 to 0.86, averaging 0.54, with the highest value obtained for primer STM0019a and the lowest value obtained for primer STM1053, showing that the SSR primers in this study presented, on average, a high level of information. Similar values were obtained by Rocha (2008),

with PIC values ranging from 0.21 to 0.97 in the evaluation of 16 potato cultivars with 21 SSR primers, Ghislain et al. (2006), with the PIC values ranging from 0.0 to 0.67 in the evaluation of 170 potato genotypes with 22 SSR loci, and Mathias et al. (2007), with their PIC values ranging from 0.42 to 0.90 for a total of 71 genotypes of potato and 21 SSR loci. Fu et al. (2009), however, observed much lower PIC values, ranging from 0.01 to 0.49, when assessing 114 Canadians and 55 exotic potato accessions with 36 SSR loci. Therefore, the information level depends on the set of primers used and the material evaluated. These authors concluded that the Canadians accessions have a narrow genetic basis, while the exotic accessions showed greater variability.

The Jaccard's similarity coefficient ranged from 0.41 to 0.93 (Figure 1), showing a significant genetic variability for the commercial varieties assessed in this study, higher than the 16 Brazilian cultivars evaluated with 16 SSR loci by Rocha (2008), where the Jaccard's coefficient ranged from 0.57 to 0.73. Braun and Wenzel (2004) found a total of 128 SSR bands and 98.4% polymorphism when assessing 47 genotypes of the potato breeding program in Germany, with the similarity coefficient of Nei and Li (1979) ranging from 0.57 to 0.79, showing less variability than the cultivars used in this study. Similar results to our study were obtained by Barandalla et al. (2006) in the evaluation of 41 cultivars of the Tenerife Island using 19 SSR loci, with the Jaccard's coefficient ranging from 0.57 to 1.00.

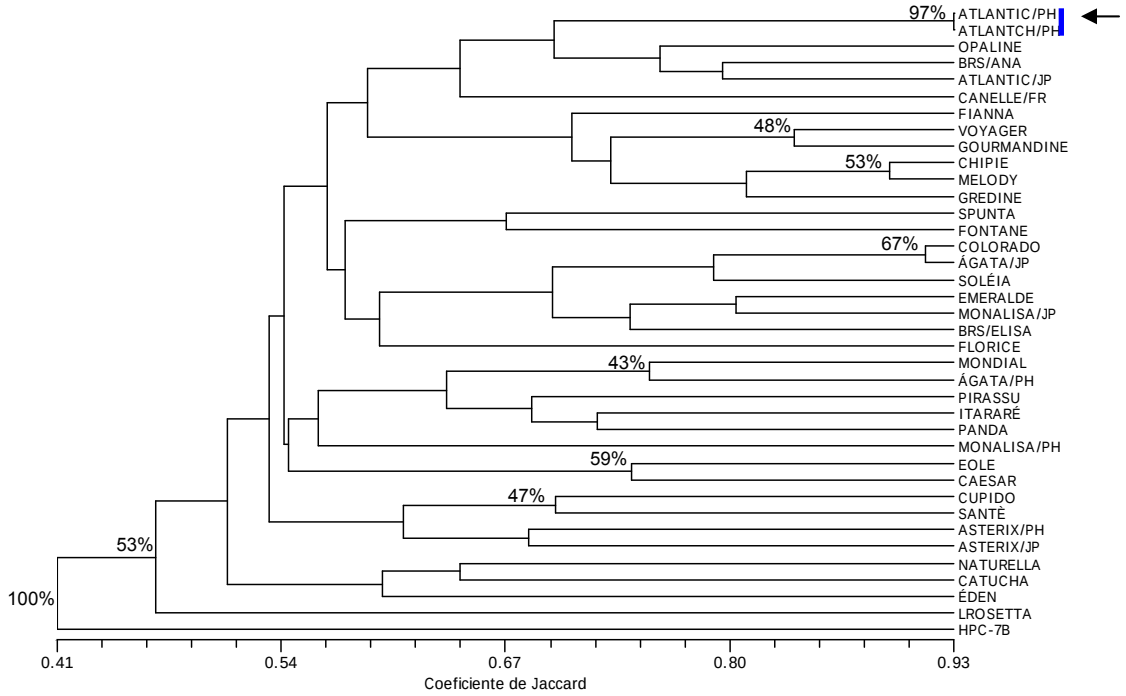

Figure 1. Dendrogram obtained from the Jaccard´s similarity coefficient, the UPGMA cluster method and confidence degree using the Bootstrap method for 38 cultivars of potato (*Solanum tuberosum*)

The 38 cultivars were classified in three groups in the cluster analysis (Figure 1). The first group, with a 53% confidence degree, was composed of all the varieties except for Lady Rosetta and HPC-7B varieties. The second group included Lady Rosetta variety, with a 53% degree of confidence obtained by the Bootstrap method, while the third group classified the HPC-7B variety, with 100% reliability. Within the first group, the data suggests the presence of a duplicate for the cultivars Atlantic (Canada) and Atlantic (Chile), which is the same variety but originating from different places, presenting 93% similarity by Jaccard's coefficient and a confidence degree of 97%, with great possibility of being the same genetic material.

Varieties Colorado and Agata (EPAMIG) also showed high similarity with approximately 91% similarity by the Jaccard's coefficient and a 67% confidence degree. Rocha (2008), using 21 SSR loci, also observed high similarity (69% by the coefficient of Jaccard) between these two cultivars (Agata and Colorado). However, these cultivars do not have parents in common, and also do not have similar morphological and agronomic characteristics, that is, tubers of Colorado present elongated oval shape and skin-red-flat tubers, while Agata tubers show oval shape

and yellow skin. It is interesting to emphasize that the cultivars named Agata, from two collections (EPAMIG and Pedro Hayashi), were genetically distinct, although both of them are part of the large group (group I). Cultivar Agata (Pedro Hayashi) was closer to Mondial cultivar, with about 75% similarity and 47% reliability.

It is worth noting that the greatest similarities tended to occur between accessions from each collection (Pedro Hayashi and EPAMIG). Cultivars Chipie and Melodie (both from EPAMIG) were also very similar, with about 90% similarity and 53% reliability in this grouping, followed by Voyager and cultivars Gourmandine (EPAMIG) with 48% reliability and cultivars Eole and Caesar (EPAMIG), with a 59% confidence degree. These two cultivars (Eole and Caesar) are similar in relation to the tuber characteristics, which are oval, large, with a moderately smooth yellow skin and superficial eyes. Varieties Cupido and Santa (Pedro Hayashi) were also similar, with approximately 70% similarity, but with a degree of reliability below 50% (47%). It is worth considering that there are similarities found in the tubers of these two cultivars, which are large, oval to round-oval and uniform, with a smooth and yellow skin and light yellow flesh.

Variety HPC-7B (Pedro Hayashi), obtained by crossing *Solanum phureja* and *Solanum chacoense*, was the most divergent accession from this collection, followed by Lady Rosetta (Pedro Hayashi). These two varieties differ with respect to some characteristics, especially in relation to disease susceptibility, that is, Lady Rosetta is susceptible to late blight (*Phytophtora infestans*), while HPC-7B shows high resistance.

The polymorphism levels presented in this study are high, considering that in the analyses each allele is considered a unique character and, as potato is a tetraploid species, each individual may present from one to four different alleles in one locus. This contributes to a high level of genetic diversity. Associated with the high reproducibility of the SSR markers, the results obtained in this study support the use of these markers as an important tool in the molecular characterization of potato varieties in germplasm banks and breeding programs.

Conclusions

Although potatoes have a narrow genetic base because of its propagation, in this study the molecular characterization of microsatellites showed a significant genetic variability and high levels of polymorphism.

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