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.

References

Alandia, S. 1994. Antecedentes y formación de colecciones germoplasmáticas de tubérculos andinos y quinua en Bolivia. Primera Reunión Internacional de Recursos Genéticos de Papa, Raices y Tubérculos Andinos. 7-10 febrero 1994. Instituto Boliviano de Tecnología Agropecuaria (IBTA), Proyecto de Investigación de la Papa (PROINPA). Convenio IBTA-CIP-COTESU. Cochabamba, Bolivia. p. 46-64

Ames, T. 1997. Enfermedades fungosas y bacterianas de raices y tubérculos andinos. Centro Internacional de la Papa. 172 p.

Arbizu, C. 1981. Catálogo de recursos genéticos de tubérculos y granos andinos de la Universidad de Ayacucho. Departamento Académico de Agronomía y Zootecnia, Programa de Investigación en Cultivos Andinos, Univerisidad Nacional de San Cristóbal de Huamanga. Ayacucho, Perú. 35 p.

Arbizu, C. and E. Robles. 1986. Catálogo de recursos genéticos de raices y tubérculos andinos. Facultad de Ciencias Agrarias, Progr. de Investigación en Cultivos Andinos, Universidad Nacional de San Cristóbal de Huamanga. Ayacucho, Perú. 82 p.

Arbizu, C., R. Blas, M. Holle, F. Vivanco, and M. Ghislain. 1997. Advances in the morphological characterization of oca, ulluco, mashua, and arracacha. *In*: The International Potato Center, Program Report 1995-96. Lima, Perú. p. 110-117.

Bryant, J., M. Jackson, and N. Meléndez. 1981. Técnicas de multiplicación rápida en papa. Centro Internacional de la Papa. Lima, Peru. 20 p.

Bukasov, S.M. 1930. The cultivated plants of Mexico, Guatemala, and Colombia. Bull.Applied Botany, Genetics and Plant Breeding, Suppl. 47, Leningrad.

Cárdenas, M. 1985. II informe sobre trabajos hechos en Bolivia sobre oca, ulluco y mashua. *En*: M. Tapia (ed). Avances en las investigaciones sobre tubérculos alimenticios de los Andes. Serie informes técnicos. Programa Nacional de Sistemas Andinos de Producción Agropecuaria, INIPA-Instituto Nacional de Investigación y Producción Agropecuaria. INIAA-CIID-ACDI (PISA). Lima, Perú. p. 5-21.

Fuentes, S., y C. Chuquillanqui. 2004. Las enfermedades causadas por virus y su control. *En*: G. López y M. Hermann (eds). El cultivo de ulluco en la sierra central del Perú. Serie: conservación y uso de la biodiversidad de raices y tubérculos andinos: una década de investigación para el desarrollo (1993-2003). No.3. Centro Internacional de la Papa, Universidad Nacional del Centro, Instituto Vida en los Andes, Universidad Nacional Agraria La Molina, Agencia Suiza para el Desarrollo y la Cooperación. Lima, Perú. p 13-31

Lizárraga, C., M. Santa Cruz, anf L.F. Salazar. 1997. Progress in identifying virus infecting Andean root and tuber crops. *In*: The International Potato Center, Program Report 1995-1996. Lima, Perú. p. 156-158.

López, G. 2004. Tubérculos – semilla. *En*: G. López y M. Hermann (eds). El cultivo de ullucoen la sierra central del Perú. Serie: conservación y uso de la biodiversidad de raices y tubérculos andinos: una década de investigación para el desarrollo (1993-2003). No.3. Centro Internacional de la Papa, Universidad Nacional del Centro, Instituto Vida en los Andes, Universidad Nacional Agraria La Molina, Agencia Suiza para el Desarrollo y la Cooperación. Lima, Perú. p 83-104

Otazú, V., M. Rodriguez, C. Arbizu, and Ch. Lizárraga. 1998. Arroseramiento del ulluco (*Ullucus tuberosus* Caldas) causado por *Verticillium dahliae*. Fitopatologia 33(3): 153-159.

Panta, A. 2007. Fyfty accessions of the potato core collection cryopreserved and 30 in total sweet potato, oca, and ulluco accessions cryotested (collaboration: Univ of Leuven GPG-Phase 2/SGRP); and one hundred oca (*Oxalis*) and ulluco (*Ullucus*) accessions established under low temperature slow growth. Annual Progress Report 2007, Division 2, International Potato Center. Lima, Perú. Mimeograph 11 p.

Panta, A. and W. Roca. 2008. In vitro multiplication of Andean root and tuber crops. International Potato Center. Mimeograph 2 p.

Panta, A., N. Sato, A. Golmirzaie, and C. Arbizu. 1994. Introducción *in vitro* de yacón (*Polymnia sonchifolia* Poeppig & Enlicher). Agro-Sur 22 (número especial). VIII Congreso Internacional de Sistemas Agropecuarios Andinos. Resúmenes. 21 al 26 de marzo de 1994. Universidad Austral de Chile. Valdivia.Chile. p. 8.

Rojas, E., A. Panta, M. Ruiz, A. Collins, D. Tay, W. Roca, and R. Simon. 2008. Barcoding of CIP's genebank and adopting by other CGIAR centers. Annual Progress Report, Division 2, International Potato Center, Lima, Perú. Mimeograph 1 p.

Sanchez, D.F., A.Panta, D. Tay, and W. Roca. 2008. Cryopreservation of ulluco (*Ullucus tuberosus* Cal.) and oca (*Oxalis tuberosa* Mol.) shoot tips using the PVS2 droplet-vitrification method. International Potato Center. Lima, Perú. Mimeograph 8 p.

Talledo, D., C. Arbizu, J. Estrella, T. Franco, L. Guarino, L. Lizárraga, T. Medina, L. Muñoz, A. Panta, J. Seminario, V. Soto, C. Tapia, M.L. Ugarte, M. Verástegui. 2001. Conservación y uso de la biodiversidad de raices y tubérculos andinos. BIOGEN, CIP, COSUDE, CRIBA-UNSAAC, INIA, INIAP, IPGRI, PROINPA, UNC. Lima, Perú. 42 p.

Toledo, J., C. Arbizu, and M. Hermann. 1994. Colección internacional in vitro de ulluco, oca, mashua y yacón. Agro-Sur 22 (número especial). VIII Congreso Internacional de Sistemas Agropecuarios Andinos. Resúmenes. 21 al 26 de marzo de 1994. Universidad Austral de Chile. Valdivia.Chile. p. 1

Zorrilla, C, J. Nuñez, E. Rojas, and D. Tay. 2007. Five hundred potato, 500 sweet potato, and 500 in total oca (*Oxalis*), ulluco (*Ullucus*), and mashua (*Tropaeolum*) accessions stored as DNA samples. Annual Progress Report, Genetic Resources Conservation and Characterization Division, International Potato Center. Lima, Perú. Mimeograph 3 p.

www.mcknight.org. Collaborative Crop Research Program.