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)					