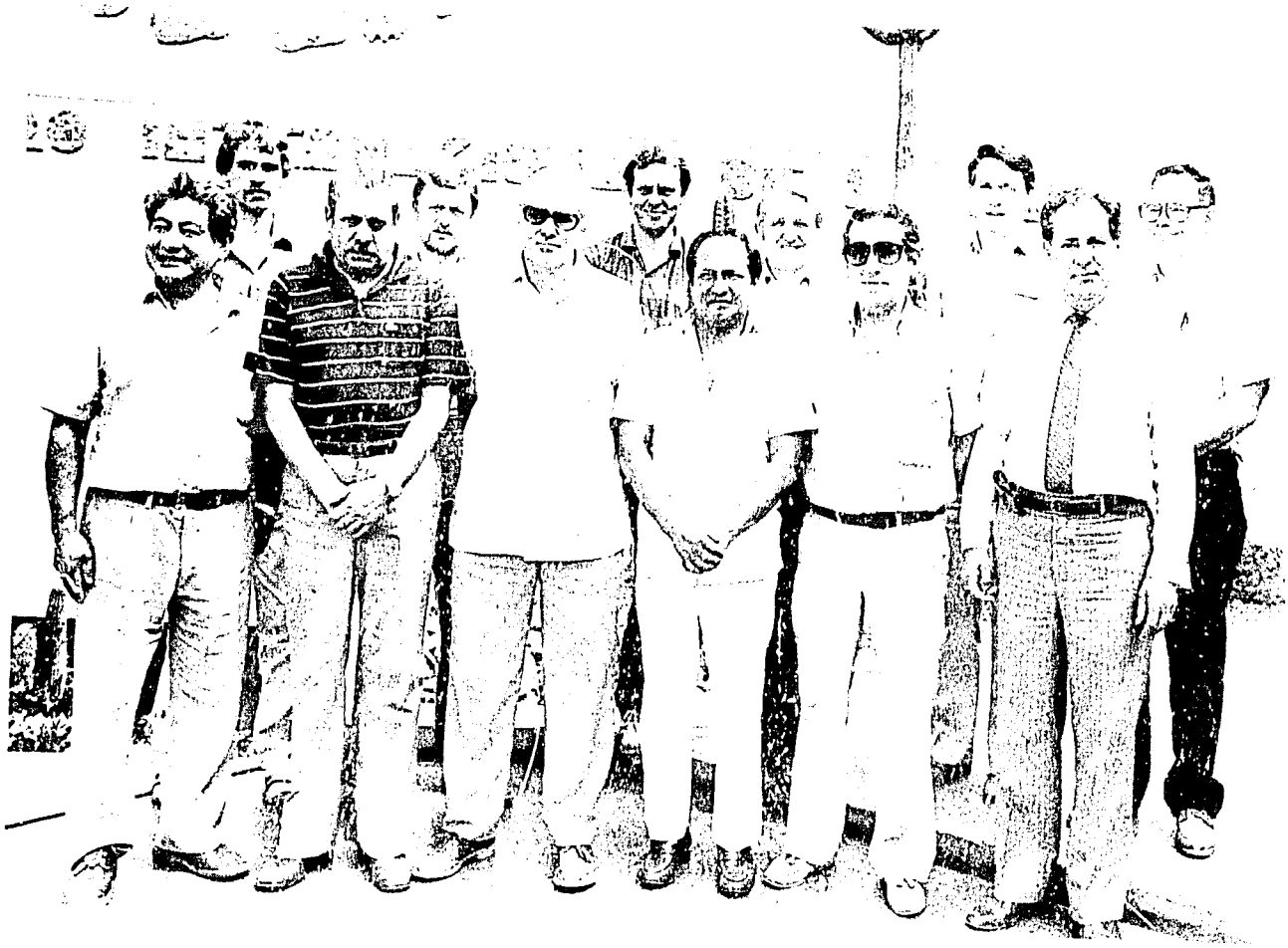
STRATEGIES FOR THE CONSERVATION OF POTATO GENETIC RESOURCES IV



Left to right: Armando Okada, Roel Hoekstra, Carlos Ochoa, Peter Gregory, Miguel Holle, Sjaan Hopmans, Zósimo Huamán, Peter Schmiediche, Humberto Mendoza, John Dodds, Luis López, Robert Hanneman, Jr.

COVER:

A sample of cultivated Andean potato tubers illustrating the genetic variability of this germplasm. This is a reproduction of a colored plate painted by Franz Frey for a future volume on the biosystematics of South American potatoes by Carlos Ochoa.

STRATEGIES FOR THE CONSERVATION OF POTATO GENETIC RESOURCES IV

XXIX

PLANNING CONFERENCE

February 9-13, 1987

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OBJECTIVES

The fourth planning conference on the Maintenance of Potato Germ Plasm will be directed to meet the following objectives:

1. To review the progress made in the collection, maintenance, evaluation, documentation and distribution of potato genetic resources.

2. To determine the best strategies for future seed regeneration of duplicate accessions not maintained clonally.

3. To pursue the development of an inter-gene bank agreement for the conservation of potato genetic resources.

4. To determine interests and distribute responsibilities for exploitation of wild species which appear to have important needed traits.

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STRATEGIES FOR THE CONSERVATION OF POTATO GENETIC RESOURCES

Jose Valle Riestra

Good Morning!

Director General Sawyer is currently out of town and hence I have the privilege and pleasure of welcoming you to this Planning Conference. He will arrive tomorrow from Australia and consequently will have the chance of participating in some of the discussions and listening to your conclusions.

Practically since its begining CIP has followed the strategy of planning its research activities, thrusts or programs through the direct collaboration of the most distinguished scientists in that particular area or discipline. In these exercises, which we call Planning Conferences, scientists from developed and developing countries participate, thus capturing both the views and needs of the developing world, as well as the most advanced views and techniques of academia. We at CIP feel that this strategy has been extremely successful and in a way, has helped in taking the cutting edge of science to farmers fields. We are thus starting today what we consider a very important activity, but, furthermore in one of the most important areas of our research.

In fact it is now well recognized that one of the most important areas of research of the International Centers is the area of collection, maintenance, utilization and distribution of genetic resources. This is the area of research for which the International Centers have most comparative advantages and which will probably justify the continuation of the Centers, when many of the tasks currently covered by them have been passed to the National Programs.

Let me now thank you most sincerely for your important help in this task, for having left your homes and countries and travelled all the way to Peru to spend all this week with us.

Welcome to CIP, and welcome to Peru and my best wishes for a successful, and I hope, intellectually provocative planning conference.

RECOMMENDATIONS

Recommendations for Potato Germplasm Exploration

A. Cultivated Species

The Planning Conference recognized the completeness of the significant holdings of cultivated germplasm that the International Potato Center (CIP) has. Nevertheless, the following recommendations were made:

1. Further collecting is recommended in Guatemala, Mexico, and Colombia (diploids).

2. CIP is encouraged to cooperate with national programs to complete collections in specific areas of Argentina, Bolivia, Chile, Colombia, Ecuador and Guatemala.

B. Wild Species

1. The same criteria used in assessing the need for the collecting of wild species in the 1976 and 1979 planning conference reports were applied here. They are:

   1. Absence from germplasm collections.
   2. Potential usefulness for potato research and improvement.
   3. Threats to habitat and consequent genetic erosion.

In addition, when planning an expedition, collectors should pay special attention to species that have known resistance or other traits of interest for potato improvement.

For each country or region the same priorities were used as in 1976 and 1979. They are:

E = Emergency

A = High priority

B = Medium priority

C = Low priority

These conform with FAO classifications.

The agreed priorities and criteria for countries, regions or sections of countries are as follows:

Country	Priority	Chief Criteria
U.S.A.	B	2, 3
Mexico	A-B	2, 3
Guatemala	B	1, 3
Central America	A-B	1, 3
Venezuela	B	1, 3
Colombia	A-B	1, 3

Country	Priority	Chief Criteria
Ecuador	B	1, 3
Peru (North)	E-A	1, 3
Peru (Central)	B	1, 2
Peru (South)	A-B	1, 2
Bolivia	A-B	1, 2
Chile	B-C	1, 2, 3
Argentina	B-C	1, 2
Uruguay	C	1
Paraguay	B	1
Brazil	B	1, 2

2. High priority should be given to collecting species which are not available in genebanks, for all new recently described species, and for those known on the basis of only a few living collections. A list of material for which no or only a few living collections are available is included in Appendix I.

3. High priority is recommended for the identification of specific areas in which further collections are needed. Provenance data for all materials in genebanks should be scrutinized in order to identify areas within the total distribution of a wild species where further collections would be justified. Joint collecting efforts by foreign and local specialists are encouraged.

4. Collectors are encouraged to use population sampling methods and to take more samples throughout the species range. They should document their collections thoroughly, providing as complete passport data as is possible.

5. Subsamples of all material collected must be offered to the country of origin. Collectors should be encouraged to send subsamples, with complete passport data, to one or more genebanks.

6. The Conference strongly recommends that new accessions obtained in recent collecting expeditions be shared/ exchanged freely.

II. Recommendations for Biosystematic Studies.

A. Cultivated Species

1. It is recommended that CIP continue its efforts to identify duplicates and that it be encouraged to use new techniques when appropriate in this effort.

2. Biosystematic studies to determine the degree of genetic variability should be continued and suitable methods to determine genetic diversity should be established.

B. Wild Species

1. CIP should support, directly or indirectly, collecting and biosystematic research on the wild species to understand species relationships and to promote their utilization in plant breeding.

III. Recommendations for Documentation and Data Management.

1. It is strongly recommended that CIP publish, as soon as possible, complete inventories with appropriate accompanying passport and evaluation data for all wild and cultivated species accessions it holds. CIP is also encouraged to publish a revised list of cultivated accessions with desirable traits for breeding.

2. CIP is encouraged to prepare or promote the preparation of world indexes and inventories of available potato genetic resources.

Recommendations for Germplasm Maintenance.

1. It is recommended that CIP continue to be responsible for the maintenance, regeneration and distribution of cultivated species.

2. Duplicates of diploid and tetraploid cultivated accessions should be converted to true seed by selfing and by geographic pooling. Selfing of duplicates is recommended where possible to preserve genetic fidelity and to preserve gene frequencies. Geographic pooling should be done by crossing among different accessions of similar geographic origin. Geographic pools will aid in maximizing diversity and in avoiding inbreeding depression. All seed derived from selfing or pooling should be stored under long-term conditions for future use.

3. True seed from nonduplicate accessions should be produced by selfing and by crossing accession from the same geographical area; those having common desirable attributes/traits, etc.

4. It is recommended that seeds of odd-numbered polyploids be produced by 3x x bulk 2x or 4x pollen, or by doubling the 3x clones to create 6x clones and then intermating among the 6x derivatives. It is recommended that pentaploids be maintained by making 5x x bulk 4x pollen crosses and/or by selfing the pentaploid.

5. It is recommended that sub-samples of at least 1000 seeds of each accession be shared by each of the major

genebanks as a safeguard against loss due to calamity or political change.

6. Research should be continued on the long term storage of *in vitro* plants and on its effects on genetic stability.

7. Duplicate samples of accessions maintained *in vitro* at CIP should be stored at another/other institution(s) with both the facility for long term storage and the capability to regenerate the samples as needed.

8. The Conference recommends that CIP pursue development of an agreement for inter-genebank cooperation to promote potato genetic resource conservation.

9. CIP is encouraged to cooperate with IBPGR to stimulate countries where potato genetic diversity exists to maintain national collections to safeguard their native germplasm.

10. National programs should be encouraged to participate in training, collecting, and maintaining germplasm whenever possible.

11. The Conference recommends, in keeping with IBPGR recommendations, that a minimum of 4000 seed per accession be maintained for long-term storage. It will be necessary to produce additional seeds for distribution.

12. The Conference recognizes that CIP may need to construct additional screenhouses for true seed and clonal increases due to increased maintenance requirements.

V. Recommendations for Plant Health and Quarantine.

Research on the use of *in vitro* virus erradication techniques should be continued.

VI. General Recommendations.

The Conference recommends that a position paper concerning inter-genebank cooperation be developed. It further recommends the CIP draft this paper and that it be circulated to the participants of this Conference and to other genebanks not represented for their input and approval.

Appendix I.

List of Wild Tuber-bearing *Solanum* Species for Which No or Only a Few Living Accessions Are Available.

Drs. Luis Lopez, Armando Okada and Carlos Ochoa

VENEZUELA:

S. filamentum

S. otites

COLOMBIA:

S. andreanum

S. cuatrecasasii

S. estradae

S. garcia-barrigae

S. lobbianum

S. neovalenzuelae

S. orocense

S. pamplonense

S. sucubunense

ECUADOR:

S. albornozii

S. burtonii

S. chilliasense

S. correllii

S. cyanophyllum

S. regularifolium

PERU:

S. acroglossum

S. antacochense

S. bombycinum

S. burkartii

S. contumazaense

S. hapalosum

S. incahuasinum

S. ingaefolium

S. jaenense

S. lopez-camarenae

S. neovargasii

S. pillahuatense

S. rachialatum

S. sawyeri

S. scabrifolium

S. tapojense

S. trinitense

BOLIVIA:

S. astleyi

S. flavoviridens

S. hoffmani

S. soestii

ARGENTINA: *S. commersonii* subsp. *malmeanum*

S. hannemanii

S. hawkesianum

S. incamayoense

S. kurtzianum

S. maglia

S. oplocense

S. vidaurrei

REVIEW OF RECOMMENDATIONS OF OCTOBER 1979 PLANNING CONFERENCE

Peter Gregory

In the October 1979 Planning Conference on "Exploration, Taxonomy and Maintenance of Potato Germplasm" recommendations were made in the areas of potato germplasm exploration, taxonomic studies, documentation and data management, germplasm maintenance and plant health and quarantine. The purpose of this present paper is to summarize the October 1979 recommendations and the actions CIP has taken as a result of them.

I. Recommendations for Potato Germplasm Exploration

A. Cultivated Species

Further explorative work on cultivated species in Colombia, Chile and Mexico was recommended. Progress has since been made in Colombia and Chile but little has been possible in Mexico. The question remains whether the variability of native cultivated material in Mexico is sufficient to justify concern about its low representation in our collection. Apparently IR-1 has been collecting actively in Mexico. Perhaps it might be possible for them to share material with us.

B. Wild Species

Each of 16 countries, regions or sections of countries in North, Central or South America was assigned a priority for collecting activities. With the exception of the U.S.A. we now have material from all of those locations. We might request U.S. materials from the IR-1 collection.

Solanum calvescens, which was especially cited as a priority species to be collected in Brazil, has been collected.

At present there are 1,450 classified living accessions of wild species in our collection. Two hundred more living accessions are yet to be classified.

A high priority recommendation was made to pay particular attention to species which are unrepresented (or poorly represented) in gene banks. Since 1979 5 new wild species have been described and 10 species that were not available anywhere in the living state have been rediscovered and added to the CIP collection.

The recommendation to use population sampling methods was vigorously acted upon, especially in the case of species known for their genetic variability.

We have also followed up on a recommendation to deposit subsamples of materials in other gene banks but this area still needs improvement with respect to wild species.

II. Recommendations for Taxonomic Studies

A. Cultivated Species

Top priority was assigned to further identification of duplicates in the CIP collection. This has been achieved through use of electrophoretic techniques.

The recommendation to study the taxonomic status of the cultivated potatoes of Mexico and Chile has not been followed up by CIP. However, the Universidad Austral at Valdivia, Chile has done some IBPGR - sponsored work on the subject.

We have not done the recommended work on the possible wild ancestors of cultivated species. Our perspective on this problem has changed since 1979. There has been such rapid progress in overcoming all known crossability barriers, and thus in wild species utilization, that studies on ancestry of cultivated potatoes is no longer a practical issue at CIP.

There was a recommendation that we should develop descriptions, classifications and distribution maps of primitive cultivar groups within each species and describe levels of ploidy. This recommendation will be partially satisfied with the shortly to be published first Volume of a series of books by Carlos Ochoa.

B. Wild Species

As recommended, a taxonomic monograph on the wild species is in preparation.

The recommended biosystematic studies on Series Etuberosa (in relation to Series Tuberosa), Series Acaulia and Series Tuberosa were carried out but little or no work has been done on the five Mexican and Central American Series which were cited.

Parts of the Atlas of the wild species recommended by the Conference is included in Carlos Ochoa's first book of the series: "The Potatoes of South America: I. Bolivia." This book should be published by the end of 1987.

III. Recommendations for Documentation and Data Management

After considering the recommendations, the following actions were taken: (1) publication of up-to-date inventories of the gene-bank holdings (in preparation) (2) submission of full information on the collections to gene bank managers, (3) continued evaluation of cultivated and wild materials followed by timely transmittal of results to data banks. However, preparation of world indexes and inventories of potato genetic resources has not yet been promoted and this should be discussed during this conference.

The recommendation that gene banks should be encouraged to develop appropriate data systems for seed storage germination tasks and regeneration management is being implemented.

IV. Recommendations for Germplasm Maintenance

Following the recommendations, CIP has continued to be responsible for the maintenance, regeneration and distribution of cultivated species and has obtained true seed for duplicate clones before discarding them. When converting material into true seed, crosses have been made among different accessions of similar geographic origin. Up to 3 accessions have been obtained where many duplicates of a clone were identified.

Duplicate long term storage of germplasm in Europe was recommended. *In vitro* germplasm is being sent to Dr. Wenzel at the Institut für Resistenzgenetik in West Germany. Also, duplicates of the germplasm collection are being stored in Ecuador and Colombia.

As recommended, seed regeneration will be conducted when germination has fallen 10-15% below the level at which it was when first placed in storage.

High levels of success have been achieved in the recommended research on long-term *in vitro* plant storage methods and on methods of their distribution, as well as methods of long-term tuber storage.

As recommended, certain clonal materials which were at risk, have been put into *in vitro* culture as a precaution against total loss.

It was recommended that there should be development of a cooperative exchange scheme between CIP, IR-1 and BGRC at Braunschweig and others, for the regeneration and exchange of true seed of cultivated and wild species. This has not yet been implemented and will be discussed at this Conference.

V. Recommendations on Plant Health and Quarantine

In accordance with the recommendations, studies have been continued on the adaptation of tissue culture techniques to problems of pathogen elimination from the germplasm collection. Also, the quarantine facilities at CIP were reviewed. CIP's high standards for quarantine have won the respect of authorities throughout the world.

Conclusion

Thus the recommendations of the 1979 Planning Conference were considered very seriously by CIP and in almost every case the recommended actions were taken. Now it is time to review the work since 1979 and to plan for the future in this top priority area of CIP's research. Our goal is still to continue the development of a comprehensive, fully documented, secure and healthy potato germplasm collection which can be utilized by scientists throughout the world. With the unique combination of experience and expertise present here, I am optimistic that this goal will be achieved in the most efficient way.

STATUS OF THE COLLECTION AT CIP: WILD SPECIES

Carlos Ochoa and Peter Schmiediche

Introduction

Since the last Planning Conference on potato genetic resources in 1979, CIP has continued to expand its germplasm collection of wild as well as cultivated species. The collection and evaluation activities of these past seven years were, of course, principally guided by the recommendations of the 1979 Planning Conference, and CIP's achievements in the light of these recommendations are specifically and in detail considered elsewhere in this report and so is the present condition of the cultivated collection. This chapter will therefore concentrate on the current status of the wild species collection.

The collection of wild species from 1979 to 1989

Since 1979, 50 germplasm collecting expeditions visited the countries of Peru, Bolivia, Ecuador, Colombia, Brazil, Paraguay, Uruguay, Colombia, Mexico and Chile and collected a total of 1010 new accessions the majority of which were wild species. Among those collections were six new wild species, two from Bolivia (**S. neovavilovii**, **S. bombicinum**), three from Peru (**S. peloquinianum**, **S. nemorosum**, **S. sawyeri**) and one from Colombia (**S. donachui**). In Peru alone, 43 collecting expeditions went to more than 70 collecting sites which is a clear indication of the fact that the greatest genetic variability of the wild as well as cultivated potato genepool is concentrated in that country. Two of the expeditions to collecting sites in Southern Peru went also across the border into Bolivia which represents a continuation of the Peruvian center of genetic diversity. One expedition went to Colombia in combination with Mexico, two to Ecuador, two to Brazil, one to Paraguay and one to Uruguay. The expedition that went to Chile collected exclusively cultivated material.

CIP now has living material from virtually all the countries listed in the recommendations of the 1979 Planning conference. The only country in which no new collections were made is the United Stated, which is no doubt a reflection of the fact that hardly any wild potato genetic re-

sources are found there. The species S.calvescens, which the 1979 Planning Conference mentioned specifically as a priority species to be collected in Brazil, has been collected and is now part of CIP's living collection.

Of the 36 species listed in the 1979 recommendations as not available in the living state in any gene bank, the following five species have been found to be botanical varieties or synonyms of species which were already represented in CIP's germplasm collection:

S. hawkesii	syn. of S. bukasovii
S. colominense	syn. of S. brevicaule
S. mollepujroense	syn. of S. brevicaule
S. orophilum	var. of S. ambosinum
S. torrecillasense	syn. of S. alandiae

The following ten species from this list of 36 have been re-discovered since 1979 and have become part of CIP's collection:

S. calvescens
S. ayacuchoense
S. buesii
S. tundalomense
S. villuspetalum
S. olmosense
S. amabile
S. contumazaense
S. suffrutescens
S. virgultorum

The twenty-one species listed below have, however, still not been found again, or if they were re-discovered, as some were, the collectors got to the old collection sites when neither seed nor tubers were available:

S. burkartii
S. garcía-barrigae
S. multiflorum
S. neovargasii
S. pillahuatense
S. woodsonii
S. anamatophilum
S. palustre
S. subandinum
S. x bruecherii
S. trinitense
S. chavinense
S. jaenense
S. hintonii
S. nayaritense
S. stenophyllidium
S. cyanophyllum
S. pascoense
S. pumilum
S. regularifolium
S. scabrifolium

Population sampling has been further improved since 1979, as recommended by the Conference. More accessions of species already represented in the collection have been obtained from a diversity of ecological niches. This was particularly observed for those species that are well-known for their genetic variability as, for instance, **S. bukasovii** or **S. acaule**.

Sub-samples were deposited in other gene banks whenever possible. However, this practice needs to be improved for the wild species in order to become as routine as in the cultivated species.

The number of classified accessions of wild potato species from South America at present in CIP's germplasm collection is summarized in Table 1 along with the countries of origin of this material as well as the number of species found in each country. Table 2 shows the taxonomic and geographic distribution of the South American as well as the Central and North American wild potato germplasm maintained at CIP. The 1387 South American accessions represent 105 species from 12 taxonomic series, and the 63 Central and North American accessions represent 14 species from seven taxonomic series. Apart from the total of 1450 classified accessions of wild potato germplasm there are still about 200 accessions of as yet undetermined material.

Table 1. Number of classified accessions of wild potato species from South Ameria, Central America and Mexico in the germplasm collection of CIP.

Country of origin	Number of species	Number of accessions
Bolivia	28	344
Brazil	1	2
Chile	5	7
Colombia	6	7
Ecuador	10	35
Paraguay	1	19
Perú	50	937
Uruguay	1	32
Venezuela	3	4
Mexico & Central America	14	63
Total	119	1450

Table 2. Classification according to taxonomic series and geographic distribution of the wild South, Central and North American potato germplasm maintained at CIP.

Taxonomic series	Number of species	Number of accessions	Geographic distribution
SOUTH AMERICA			
Acaulia	2	300	Arg,Per,Bol
Circaeifolia	1	10	North Bol
Commersoniana	9	121	S. America
Conicibaccata	27	131	S. America
Cuneolata	3	12	Arg,Chl
Etuberosa	1	1	Chl
Ingaefolia	3	12	Per
Juglandifolia	2	3	S. America
Megistacroloba	6	100	Arg,Bol,Per
Olmosiana	1	1	Per
Piurana	6	22	Col,Ecu,Per
Tuberosa	44	674	Ven to Arg and Chl
Total	105	1387	
CENTRAL AMERICA, MEXICO, U.S.A.			
Bulbocastana	1	6	Gua,Mex
Conicibaccata	2	2	Mex
Demissa	3	23	Gua,Mex
Longipedicellata	3	20	Mex,South U.S.A.
Morelliformia	1	3	Gua,Mex
Pinnatisecta	3	7	Mex,South U.S.A.
Polyadenia	1	2	Mex
Total	14	63	

Biosystematic Research in the Wild Species Collection

Particular biosystematic and phylogenetic studies were carried out with the help of two special projects. The taxonomic series specifically investigated were the series TUBEROSA, OLMOSIANA, INGAEFOLIA, CIRCAEIFOLIA and CONICIBACCATA. The study in series TUBEROSA showed that the two species **S. canasense** and **S. multidissectum** were synonyms of **S. bukasovii** and that **S. gourlayi** and **S. spegazzini** were synonyms of **S. leptophyes.** On the basis of crossability results, the species **S. jalcae**, the biosystematic position of which was not clear, was determined to belong to the taxonomic series INGAEFOLIA and not OLMOSIANA, the other series into which it could have been included if morphological characters alone had been used as criteria for classification. Crossability data obtained from crosses between members of series CONICIBACCATA and CIRCAEIFOLIA demonstrated that these two series should be kept as distinct biosystematic entities, and that they should not be fused into one taxonomic series, something which had been under consideration in the past. These results were obtained on the basis of approximately 4000 pollinations that have yielded about 200,000 seeds.

Research on the use of wide crosses in potato breeding has shown that every known crossability barrier between tuber-bearing and even some non tuber-bearing Solanum species can be overcome if a series of bridging species and **in vitro** techniques, such as embryo culture, are used (Chavez, 1984; Spack et al., 1987). This research has demonstrated that even a species as remotely related to the tuber-bearing germplasm as **S. etuberosa** can be utilized in breeding if necessary. Some of the results obtained in this research are discussed in the paper: "The utilization of wild potato species in breeding" which is also included in this report.

Apart from these exotic wide crosses, thousands of conventional inter-specific crosses have been performed to study the phylogenetics of a wide range of species. The major part of the results obtained in these studies will shortly be published by Cambridge University Press in a monograph by Carlos Ochoa entitled "The Potatoes of South America, Volume I: Bolivia".

The search for resistance to pests and diseases in the gene-pool of wild potato germplasm at CIP

The three wild species **S. microdontum**, **S. sparsipilum** and **S. chacoense** have been used successfully in a breeding program for resistance to bacterial wilt (**Pseudomonas solanacearum**), and some of the implications that have resulted from the results of this research are considered in the paper: "The utilization of wild potato species in breeding" elsewhere in this report. However, in spite of some success with wild species in breeding for bacterial wilt resistance, more sources of resistance have constantly been sought within CIP's wild potato germplasm. The results of this large-scale search are summarized in Table 3.

Apart from resistance to bacterial wilt, the wild germplasm was further screened for resistance to Late Blight (**Phytophthora infestans**), the Potato Cyst Nematode (**Globodera pallida**),the Potato Tuber Moth (**Phthorimaea operculella**), the Potato Spindle Tuber Viroid (PSTV), and the Potato Leave Roll Virus (PLRV). A summary of the results of these screening and evaluation efforts of several years are also presented in Table 3. Further details of this research are found in CIP's Annual Reports from 1981 to 1987 as well as in several papers published in learned journals (Chavez et al.,1987a; Chavez et al., 1987b; Chavez et al.,1987c).

Table 3. Wild species with resistance to pests and diseases in CIP's potato germplasm collection.

Pathogen or pest	Immune species	Resistant species
Phytophthora infestans		CHM
Pseudomonas solanacearu		ACL,CHC,BUK,MCD,SPL,SGR BLV,LPH,MGA,PLQ
Globodera pallida		CRC,BUK,LPH,SPL
PSTV	ACL,ABN,CHM, BLV	CRC,COL,CND,SPL
PLRV	ACL,ABN,CND, LGL,TND	BRD,CHC,CHN,HCH,HCB,SPL MGA
Phthorimaea operculella		SPL,CHC,CMM,TAR,PNT,LGL

Wild species of Solanum:

ACL acaule	ABN abancayense	BLV boliviense
BRD brevidens	BUK bukasovii	CHC chacoense
CHM chomatophilum	CHN chancayense	CND candolleanum
COL colombianum	CRC circaeifolium	HCB huancabambense
HCH huarochiriense	LGL lignicaule	LPH leptophyes
MCD microdontum	MGA megistacrolobum	PLQ peloquinianum
SGR sogarandinum	SPL sparsipilum	TDM tundalomense
CMM commersonii	TAR tarijense	PNT pinnatisectum

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STATUS OF THE NATIVE ANDEAN CULTIVATED POTATO COLLECTION MAINTAINED AT CIP

Zosimo Huaman

Since the last Planning Conference on Potato Genetic Resources in 1979, a great deal of progress has been made in all of the different activities related to the maintenance of the Collection of Native Andean Potato Cultivars at CIP. Our strategy of conserving this collection by annual propagation of tubers in the field, although very costly, requiring large field space and very labor intensive, has facilitated considerable progress in its evaluation for desirable attributes and in the identification of duplicate genotypes.

CONTENT OF THE CULTIVATED COLLECTION

The total number of cultivated accessions assembled in the collection up to 1979 was 12,421. From 1980 to 1986, a total of 3,131 accessions has been added to the collection from countries whose cultivated potato genetic resources were considered to be inadequately represented in CIP's base collection (Table 1). A large portion of these new accessions were donated to CIP by institutional or National Potato Programs in Peru, Bolivia, Colombia, Chile, and Mexico. Other new accessions were obtained by collecting expeditions as follows:

1) The 1983 CIP-IBPGR-IBTA (T. Johns, Z. Huaman and I. Aviles) expedition to the Altiplano region of Bolivia to collect frost tolerant cultivars [11].

2) The 1984 Universidad Austral de Chile-CIP (A. Contreras, P. Montaldo and Z. Huaman) expedition to the Archipielago de los Chonos and las Guaitecas of Chile to collect salt tolerant cultivars [1].

3) The ongoing INIAP Ecuador-IBPGR-CIP (R. Castillo, M. Sola and Z. Huaman) collecting expeditions along the Inter-Andean Valleys of Ecuador initiated in 1985 [16].

Table 1 - Origin of cultivated accessions maintained clonally.

Country of origin	Number of Accessions Collected up to 1979	Collected 1980-86	Total collected	Total * maintained
Peru	10,407	1,143	11,550	3,481
Bolivia	849	977	1,826	767
Ecuador	184	495	679	273
Colombia	464	256	720	323
Chile	188	250	438	260
Argentina	171	0	171	62
Venezuela	141	7	148	59
Guatemala	17	0	17	12
Mexico	0	3	3	3
T O T A L	12,421	3,131	15,552	5,240

* Reduced by duplicate identification

Table 1 shows the number of accessions that at present are maintained from each of the 9 countries represented in the collection. From a total of 15,552 cultivated accessions collected, 5,240 are still being maintained clonally. The rest has been converted to true seed since these accessions were identified as duplicates of cultivars represented in the clonal collection.

Field collections are still needed in Guatemala and Mexico to obtain a more representative sample of the total genetic diversity from those countries.

DUPLICATE IDENTIFICATION

Emphasis to identify accessions of the collection that correspond to identical native Andean cultivars has continued. The collection has been reduced from more than 15,000 accessions to about 5,000 by using: 1) computer assisted techniques to record and perform multiple comparisons of morphological characters; 2) detailed morphological comparisons conducted among morphologically similar groups of accessions identified through the years; and 3) electrophoretic analyses to verify the patterns produced by morphologically alike groups. Table 2 shows

that 11,156 electrophoretic comparisons have so far been made in 3,111 groups. Most of these analyses were made in the Laboratory of Professor H. Stegemann at the Institut für Biochemie, Biologische Bundesanstalt, Braunschweig, West Germany. Proteins and enzymes extracted from freshly harvested tubers were separated by their molecular size, shape, and charge by standard polyacrilamide (PAA) gel electrophoresis with 6% PAA in Tris-borate buffer pH 7.9 and 8.9 [17].

Table 2 - Data on duplicate cultivated aceessions compared by electrophoretic of proteins and enzymes.

Year	Electrophoretic series	Number of groups compared	Number of accessions included
1976	1,000	74	489
	2,000	94	964
1977	3,000	123	495
	4,000	144	969
1978	5,000	229	1,026
	6,000	340	1,035
1979	7,000	346	908
1980	8,000	411	638
1981	9,000	429	1,622
1982	10,000	447	1,512
1983	11,000	312	1,031
1986	12,000	162	467
T O T A L		3,111	11,156

In general, the larger the number of identical morphological characters used to determine duplicate accessions, the higher was the percentage of identical electrophoretic patterns obtained. Experience has shown that groups made only on the basis of tuber characters have about a 40% chance to identify duplicates. Many cultivars produce morphologically alike tubers which, when grown, show variation in other characters such as stems, leaves and flowers.

Synonym group numbers have been assigned to 2,290 groups of two or more duplicate accessions. This represents about 74% of the 3,111 compared. However, through the years a total of 558 synonym groups have been found to

be identical of others and therefore, discontinued in their use. Furthermore, 199 groups include 95 cultivars whose geographic distribution comprises 2 countries, and 5 cultivars are distributed in 3 countries. Therefore, the number of potentially different synonym groups to date is 1,635. However, there are several hundreds of accessions which appear to be unique and so far no duplicates of them have been found in the collection. This will undoubtedly increase the number of genetically different Andean cultivars present in the cultivated gene pool available at CIP.

Example of reduction of synonym groups through continuous morphologic comparisons and electrophoretic analyses.

Electroph. Group	Syn. Gr.	Electroph. Group	Syn. Gr.	Electroph. Group	Syn. Gr.
1014 BB*	153	9279 AA	153	11152 AA	153
3075 AA	681	9279 AA	153		
5087 AA	291	9279 AA	153		
7142 AA	519	9279 AA	153		
1012 AA	151	9280 AA	151	11152 AA	153
4090 AA	992	9280 AA	151		
		3059 AA	517	11152 AA	153
		10219 AA	10	11152 AA	153

* Each letter corresponds to protein and enzyme patters respectively.

The use of protein and enzyme patterns alone to identify duplicates in a large number of samples has some practical limitations. Accurate comparisons of similar patterns are very difficult when duplicates are located in several gels. However, the arrangement of groups to be electrophoretically compared according to similarities in some tuber characters helps to identify identical electrophoretic patterns which, in turn, should be verified by more detailed morphological comparisons.

There have been many instances where slight differences were noticed in the electrophoretic patterns of duplicates. In the beginning it was decided that groups showing this type of variations should be

assigned different synonym group numbers. However, in most of these cases repeated analyses in extracts from new tubers showed that the differences were not consistent.

Example of repeated analyses of electrophoretic patterns showing minor differences.

Electroph. Group		Syn. Gr.	Electroph. Group	Syn. Gr.
10412	AA	586	11197 AA	586
10412	A1A	1033	11197 AA	586
10412	A2A	0	11197 AA	586

In other cases, the electrophoretic results showed different patterns in groups which no doubt were morphologically alike. However, in most of these cases the differences were due to human error mixing either the tuber samples or the extracts.

Example of repeated verification of morphologically alike groups producing different electrophoretic patterns.

Electroph. Group		Syn. Gr.	Electroph. Group	Syn. Gr.
10107	BB	1494	11104 AA	311
10107	CC	0	11104 AA	311
10107	DD	311	11104 AA	311

Finally, it has been possible to document cases in which closely related cultivars of the same species showing differences in tuber skin color, stem color or flower color produce identical protein and enzyme patterns. Thus, in S. x curtilobum there are 3 cultivars producing white, purple and two-colored tubers as well as some minor differences in the distribution of anthocyanin pigmentation throughout the plant. However, these cultivars produce identical electrophoretic patterns. The same has been noticed in some cultivars of S. x juzepczukii, S. x ajanhuiri, and S. x chaucha. A possible explanation to this is that the phenotypic variation in these cultivars might be due to somatic mutations whose effects in the genetic architecture of the plant is concentrated only to genes affecting color expression and therefore, do not affect their electrophoretic patterns.

GENETIC DIVERSITY OF CULTIVATED SPECIES

The identification of duplicate accessions in the collection has contributed to a better understanding of the intra-specific variability within each of the cultivated potato species and has helped to determine the geographical distribution of each cultivar.

Most of the species identifications has been made by CIP's taxonomist Professor Carlos Ochoa. The system of classification put forward by J.G. Hawkes in 1956 has been adopted. This system recognizes as valid two sub-species within the tetraploid potato S. tuberosum and seven other species of other ploidy levels [3].

Table 3 shows the number of accessions within each species presently maintained in the cultivated collection at CIP. Although the exact number of cultivars within each species is still not completely determined, the extent of their intra- specific variation can be estimated on the basis of the number of synonym groups assigned within each species. Data in this table, however, does not reflect the actual number of different genotypes within each species. Replicate samples of the same cultivar have been retained purposely to reduce the risk of losses until each cultivar is safely conserved in the gene bank. Furthermore, we are also following the recommendations of previous Planning Conferences that state that up to three representative accessions of different parts of the geographic distribution of a given cultivar should be retained from cultivars with many duplicates.

Table 3 - Duplicate identification within cultivated species.

Solanum species	Number of Accessions Planted	Unique or new access.	Grouped	Number of synonym Groups *
Diploid 2n=2x=24				
stenotomum	449	205	244	161
goniocalyx	99	47	52	38
phureja	118	98	20	20
ajanhuiri	26	4	22	16
Inter-specific hybrids	147	43	104	49
Triploid 2n=3x=36				
chaucha	235	59	176	61
juzepczukii	75	7	68	31
Tetraploid 2n=4x=48				
andigena	3,789	1,751	2,038	1,292
tuberosum	264	195	69	54
Pentaploid 2n=5x=60				
curtilobum	38	4	34	10
T O T A L	5,240	2,413	2,827	1,732

* Duplicate groups from different countries retain different synonym group numbers. Groups from the same country might still contain duplicates.

The work of duplicate identification in the diploid species has been slow because of difficulties in maintaining these accessions in the field for several years. Their susceptibility to viruses and other diseases made their transfer to and further maintenance in *in vitro* culture urgent.

S. stenotomum Juz. et Buk. is the most variable of all diploid species, and it is considered to be the ancestor of many other cultivated species. The intra-specific variation of this species comprises several hundreds of cultivars. This species is cultivated along the Andes between northern Peru and central Bolivia. C.S.Tay in 1979 [18] studied 606 diploid accessions in the collection classified as S. stenotomum, S. goniocalyx, S. phureja and natural inter-specific hybrids. He found that species boundaries are not clear and diagnostic characters of one species overlap with those of another. Those showing intermediate characters are considered to be hybrids between one and another. He proposed that all materials studied should be classified within the species S. stenotomum subdivided in the sub-species stenotomum, goniocalyx and phureja.

S. goniocalyx Juz. et Buk. comprises cultivars producing deep yellow flesh tubers which have good palatability and high dry matter content. Originally it was considered to have a narrow geographic distribution confined to Northern Peru. However, accessions from this species in CIP's collection were found widely distributed in several highland regions troughout Peru and northern Bolivia. There are several cultivars with white flowers and deep yellow tuber flesh that are different in other morphological characters and electrophoretic patterns. The intra-specific variation includes also some other cultivars with various flower colors.

S. phureja Juz. et Buk. is characterized by its production of tubers that lack a resting period i.e. tubers are already sprouting at the time of harvest. This feature makes the maintenance of accessions of this species in the field extremely difficult. Most of the samples of this species are maintained in vitro culture. The geographic distribution of this species comprises the warmer valleys of Venezuela, Colombia, Ecuador, Peru and Bolivia.

S. x ajanhuiri Juz. et Buk. appear to be the least variable of the diploid cultivated species. Although seven different cultivars have been reported in this species [6], new collections made in the Department of Oruro in Bolivia will certainly increase this number. The geographical distribution of this species is

confined to the high Andean Altiplano region of Bolivia and Southern Peru.

A number of diploid accessions in the collection are classified as natural inter-specific diploid hybrids involving S. stenotomum with either S. goniocalyx or S. phureja. The true nature of these cultivars remains to be determined in order to confirm if they are indeed natural inter-specific hybrids or just part of the total variation of polymorphic S. stenotomum.

The triploid species S. x juzepczukii Buk. is the most tolerant to frost damage among all cultivated species. Preliminary studies using a large number of accessions of this species in the collection indicated that its variation could involve 14 cultivars [15]. However, very detailed morphological comparisons showed that some of these cultivars are almost identical to others except that they have different tuber skin colors. They produce identical protein and enzyme patterns. Some new accessions collected in Bolivia might include some new cultivars. The geographical distribution of this species is almost exclusively confined to the highlands from central Peru to central Bolivia were frosts are expected to occur during the growing period.

S. x chaucha is another triploid species that has been extensively studied at CIP. However, there is a disagreement in the number of different cultivars distinguished in this species. Thus, Ochoa in 1975 [12] described 30 different cultivars whereas Jackson in 1977 [10] included only 20. The area of cultivation of this species is located between northern Peru and central Bolivia.

Within the tetraploid species, S. tuberosum L. involves two sub- species. The sub-species andigena Hawkes is the most variable of all cultivated species and comprises about 73% of the accessions in the collection. The variation is great not only in many morphological characters of tubers, stems, leaves and flowers but also in physiological and biochemical properties, and in reaction to biotic and abiotic stresses. There is no doubt that this sub-species comprises more than one thousand different cultivars. The andigena cultivars are still widely distributed in the Andean zones of Venezuela,

Colombia, Ecuador, Peru, Bolivia and northern Argentina. It is also cultivated in some areas of Guatemala and Mexico. However, the area of greater genetic variation occurs between central Peru and central Bolivia.

The sub-species tuberosum Hawkes is the only cultivated species grown worldwide. However, native cultivars of this sub-species occur only in Southern Chile where they have been cultivated for many centuries. Collections from Chile show a number of cultivars with morphological characters similar to those of cultivars from the northern hemisphere. However, there are others that show great variation in tuber shapes and colors and other plant characters resembling some of those frequently found in the sub-species andigena.

Finally, the pentaploid S. x curtilobum Juz. et Buk. shows the narrowest variation of all cultivated species. Three phenotypes appears to have evolved by somatic mutations of a single cultivar. However, a large number of natural hybrids between S. x curtilobum and the tetraploid andigena are likely to exist within the total variation of andigena. This is so because of the relative ease with which numerous open pollinated fruits are formed in S. x curtilobum when grown mixed with andigena in some native fields. The geographic distribution of this pentaploid species is from central Peru to northern Argentina.

CLONAL MAINTENANCE IN VITRO CULTURE

Since 1983 increasing emphasis has been placed in CIP's in-vitro laboratory, under the direction of Dr. J. Dodds, to transfer to in vitro culture different cultivars of each species maintained in the collection. This transfer has been made to prevent genetic losses, and it is being implemented according to the following priorities:

1) Cultivars which do not set seeds and can therefore only be maintained by clonal propagation, such as the cultivars of triploid species.

2) Cultivars that are extremely susceptible to viruses and other diseases and therefore, are at high risk when maintained in the field for many years. This includes all the diploid species.

3) Tetraploid cultivars showing desirable attributes for use in breeding.

4) Tetraploid accessions which represent numerous duplicates or are considered to be unique in the collection.

5) Representative samples of potentially different cultivars selected on the basis of tuber characters from new collections.

The current number of samples of native cultivars transferred to in vitro culture is 2,192. A total of 108 cultivars representing all different species have been cleaned of pathogens at the Research Institute for Plant Protection in Wageningen, and 64 are in the process of virus eradication. Another 2,020 accessions are being maintained in vitro free of systemic fungi, bacteria, and mycoplasms (Table 4).

Table 4 - Number of native cultivars transferred to *in-vitro* culture.

Solanum species	Pathogen free	Being cleaned	Non-pathogen free *	Total
ajanhuiri	0	5	15	20
goniocalyx	6	9	45	60
phureja	16	7	95	118
stenotomum	20	15	211	246
2x hybrids	0	1	64	65
chaucha	22	8	86	116
juzepczukii	10	3	20	33
andigena	30	16	1,371	1,417
tuberosum	2	0	101	103
curtilobum	2	0	12	14
T O T A L	108	64	2,020	2,192

* Some cultivars from a specie might include duplicates from new collections.

The large number of accessions now maintained in vitro culture requires an efficient system to monitor the status of each accession in the laboratory. A computerized data management system has been developed to facilitate the selection of accessions on the dates when they should be re-subcultured.

SEED CONSERVATION

Since true potato seed is the most recommendable form for long- term storage of genetic resources, the following seed stocks of cultivated germplasm are maintained at CIP:

1) OPEN-POLLINATED SEED. True seed from all accessions in the cultivated collection has been obtained from open-pollinated berries produced by plants grown in the field as soon as they have passed through quarantine. Exception to this are the accessions of the triploid species S. x chaucha and S. x juzepczukii which are sterile, and those of the sub-species tuberosum which under Peruvian conditions do not flower.

2) GEOGRAPHIC SEED POOLS. These seed stocks have been produced along with the process of duplicate identification before duplicates were eliminated from clonal maintenance. Each duplicate accession is used as a female parent in crosses with bulk pollen from other nonduplicate accessions of the same species collected in the same geographic area.

3) TRAIT-SPECIFIC SEED STOCKS. Since a number of desirable traits have been found widespread in many accessions of the cultivated collection, seed stocks for each desirable attribute are being obtained from genetically different accessions. Cultivars posessing the same desirable character are intercrossed using single and bulk crosses.

All three types of seed stocks are cleaned, counted, dried to about 5% moisture content and stored in laminated aluminum foil packets in a cold room at 0 C or in frostless freezers at -15° C [9].

EVALUATION

The role of a gene bank is not only to make sure that the genetic resources are available in the living state but also to identify sources of desirable traits that could be used for the genetic improvement of the crop. Consequently, pathologists, nematologists, entomologists, physiologists and geneticists at CIP have conducted a systematic evaluation of the Andean cultivated gene pool since 1972. A total of 40,715 evaluations on clonal accessions have been made to date using 12 fungi, 2 bacteria, 3 viruses, 1 viroid, 1 insect, 3 nematodes, 2 environmental stresses, and 2 nutritive factors (Table 5). The frequencies of resistant genotypes to pests and diseases ranged from 2% to 17%. In general, the frequency of moderately resistant genotypes is much higher than those with high levels of resistance.

DOCUMENTATION

The data accumulated for each accession in the collection has been of such a magnitude that it could only be efficiently handled by means of a computerized data management system. Most of the data has been recorded following a standardized list of descriptors [5]. A total of 65 descriptors are being used for each accession: 11 on the collection site, 16 on maintenance,14 on taxonomic and morphological data, 17 on disease and pest reaction and 7 on other miscellaneous evaluations.

UTILIZATION

The importance of the breeding potential of the Andean cultivated gene pool has been shown by many authors [2, 4, 8, 13, 14]. However, the extent of the use of these genetic resources is still limited because of the difficulties in eliminating undesirable agronomic characters in their segregating populations.

Table 5 - Evaluation of the collection for desirable traits.

Traits evaluated		Number of accessions up to 1986
Fungi		
Phytophthora	Vine blight	964
Phytophthora	Tuber rot	522
Phytophthora	Pink rot	2,968
Streptomyces	Scab	927
Synchytrium	Wart	1,622
Angiosorus	Smut	329
Macrophomina	Charcoal rot	2,383
Phoma	Gangrene	741
Phoma	Leaf spot	48
Verticillium	Wilt	552
Fusarium	Dry rot	957
Alternaria	Blight	422
Bacteria		
Erwinia	Soft rot	752
Pseudomonas	Wilt	29
Viruses		
PVX		697
PVY		601
PLRV		1,112
Viroids		
PSTV		5,424
Insects		
Phthorimaea	Tuber moth	2,871
Nematodes		
Globodera pallida		5,264
Globodera rostochiensis		1,252
Meloidogyne incognita		3,938
Tuber quality		
Dry matter content		629
Protein content		632
Other traits		
2n pollen production		806
Frost		341
Hail		3,932
T O T A L		40,715

The pedigree records of the different CIP breeding programs show that in 1817 crosses, native Andean cultivars were used as male, female or both parents. They were primarely used as sources of resistance to frost, late blight, golden nematode, PVY, PLRV and wart.

In order to promote a more intensive use of the cultivated gene pool available in the collection, CIP has initiated a program of genetic improvement of Andean cultivars. This consists of producing populations from different Andean cultivars with the same desirable traits or a combination of various traits widespread in the collection. The resulting progenies will then be screened for these traits, and the surviving genotypes will be further selected to remove undesirable agronomic characters. Selected genotypes will be crossed with long day adapted germplasm to produce seed stocks for distribution to interested breeders [7, 9].

At present CIP is developing this type of populations using Andean cultivars highly resistant to Erwinia soft rot.

DISTRIBUTION

The genetic resources maintained at CIP are freely available for distribution worlwide in two forms. First, as clonal materials, either as in vitro plantlets or as tubers produced under quarantine conditions from pathogen-tested stocks. Second, as true seed from any of the stocks conserved in the gene bank.

Considering that most of the requests are for seed samples, testing for potato spindle tuber viroid infection in representative samples of seed stocks of 1180 Andean cultivars has been made using the nucleic acid spot hybridization (NASH) technique.

An inventory of nonduplicate native Andean cultivars with resistances to some pests and diseases and other desirable traits has been produced and will be distributed to breeders. Hopefully this inventory will promote a wider utilization of CIP's collection.

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STATUS OF THE INTER-REGIONAL POTATO INTRODUCTION PROJECT

Robert E. Hanneman

The Inter-Regional Potato Introduction Project (IR-1), since its inception in 1948, has been charged with responsibility for the introduction, classification, maintenance, distribution and preliminary evaluation of the tuber-bearing Solanum's and their close relatives for the United States of America. How IR-1 has attempted to meet this responsibility is the subject of this brief report.

Introduction of new accessions to the United States is accomplished by donations, by direct request to other genebanks, collectors, institutions and scientists, as well as by plant exploration. IR-1 has been actively involved in collecting since the late 1970's, having obtained funding in support of joint collecting expeditions to New Mexico and Arizona (1978), Mexico (1982, 1983, & 1984), Argentina (1983) and Bolivia (1986, 1987). The goal of each of these expeditions has been to collect in areas not explored before or which were noted by previous expeditions/collectors as worthy of more intense exploration.

Through all introduction efforts, IR-1 has added 1,102 accessions in the last 10 years or about 110 accessions per year. The majority of these introductions came from Argentina, Bolivia, Chile, Colombia, Mexico, Netherlands, Peru, Scotland, the USSR and the USA.

Classification of introduced accessions has traditionally been accomplished by bringing recognized Solanum taxonomists to IR-1 to evaluate samples of the collections in the field at Sturgeon Bay as well as to annotate herbarium specimens. Dr. J. G. Hawkes, Dr. J. P. Hjerting, Dr. C. M. Ochoa, Dr. K. A. Okada and Dr. T. R. Tarn have assisted IR-1 with classification in recent years. As a matter of policy, IR-1 personnel do not make taxonomic decisions, no matter how confident they are of their determination. That responsibility fully rests with a formally trained, experienced taxonomist knowledgeable of the tuber-bearing Solanums.

Maintenance of accessions is met through true seed, tuber and in vitro increases of IR-1 stocks. True seed increases typically are accomplished in greenhouses during the spring (February - June) taking advantage of the natural lengthening of the days. Presently, increases of about 250 accessions of 20 plants each can be accomplished. Tuber increases are generally done in the fall of the year (September - January) in greenhouses, though more adapted material, such as the foreign varieties, is increased

in the summer (June - September) in screenhouses. IR-1 is presently nearing the limits of its capacity for true seed maintenance using only a spring seed increase, so alternatives are being explored including fall greenhouse increases using supplemental lighting, summer field increases using cut-stems, and also summer screenhouse increases. With the options available, IR-1 could double its capacity for maintenance by going to fall and spring seed increase cycles. It could expand even more, if needed, by including the other two seed increase alternatives.

IR-1 presently holds about 3500 accessions of which at least 90% are kept as true seed and the remainder as clones. In addition, it maintains a significant number of clonal genetic stocks including about 700 haploids, 100 species-haploid hybrids 20 bacterial wilt resistant clones, 20 trisomic lines, 400 foreign cultivars/breeding stocks and perhaps another 200 miscellaneous stocks.

The University of Wisconsin, Department of Plant Pathology supplies pathological support services to IR-1 under contract. These services include virus and viroid testing, virus freeing, meristem culture, in vitro maintenance and increase of clonal stocks. Through this contract, all plants used in seed increases and a sample of all seed lots produced, are tested for

PSTV. Also all clonal stocks are and have been tested for PSTV as well as for viral diseases. Two-hundred and forty-eight cultivars are now termed "free" of PVA, M, S, X, Y, PLRV and PSTV. Clonal and seed accessions in potential danger of being lost are placed into shoot-tip culture as a safeguard against such loss. This contract support has made a significant contribution to the well-being (health) of the IR-1 collection and to preserving IR-1's reputation for delivering high quality seeds and tubers upon request.

The distribution of stocks has been met through the shipment of seeds, tubers and most recently in vitro stocks, and occasionally pollen. IR-1 has enjoyed a steady increase in demand for accessions as the user community expands its use of wild and cultivated species. During the four year period of 1982-85, IR-1 distributed an average of 4530 accessions/year. In 1986, it distributed about 8900 accessions. IR-1 expects to publish a current inventory of wild and cultivated species by early April, 1987. It will contain a summary of all new information and accessions since the publication of the last inventory.

Preliminary evaluation has generally been met by receipt of voluntary information concerning the accessions from the user community, as they share reports, publications and/or summaries of

their screening efforts. More recently, IR-1 has been able to obtain additional funding to support screening of the accessions for traits determined to be of high priority by the Potato Crop Advisory Committee. With this funding, screening of some or all of the collection has been done for potato leafroll virus (6 contracts), Colorado potato beetle (9 contracts), foliar early blight (3 contracts), Verticillium wilt (6 contracts), PSTV (1 contract), leaf hopper and flea beetle (2 contracts), rootknot nematode (6 contracts), bacterial ring rot (1 contract), bacterial wilt (1 contract), Erwinia (blackleg) complex (1 contract), glycoalkaloids (1 contract), heat and drought stress (2 contracts), and 2n gametes (1 contract). From 1983-86, $463,420 has been spent under contract in support of this screening effort. This organized screening effort should make the IR-1 collection more useful to those involved in potato improvement and research, and will thereby make a lasting contribution to U.S. and world agriculture.

IR-1 has enjoyed stable financial base support which has allowed for modest expansion of staff and upgrading of facilities. Additional outside support has permitted expansion of activities to include support of collecting expeditions and for screening/evaluations of IR-1 accessions for high priority traits needed by the user community for potato improvement/research. The stability of funding, the stability of staff and their dedication

to IR-1, and the cooperation of associates at the national and international levels has helped IR-1 to continue to contribute effectively in serving the needs of U.S. and foreign colleagues in search of useful germplasm for incorporation into their potato improvement/enhancement and/or research programs. IR-1 looks forward to continuing to serve the potato community at large in the best manner possible.

PRESENT STATE OF MAINTENANCE AND EVALUATION OF THE GERMAN-DUTCH POTATO COLLECTION

Roel Hoekstra

Introduction

More than 12 years ago a cooperation between the Federal Republic of Germany and the Netherlands was started at the Institute of Crop Science and Plant Breeding in the FAL, Brunswick, concerning potato genetic resources. The Dutch partners of the cooperation are the Centre for Genetic Resources, Netherlands (CGN) established in 1985 and the Foundation for Plant Breeding (SVP). Objectives and details of this bilaterally financed project were outlined by Lange (1976) at the second planning conference.

Since 1984 this cooperation is a project of the German-Dutch Board for Plant Genetic Resources, which replaced the Programme Committee. This new board consists of 12 members: 4 scientists and 2 representatives from the ministry of each country and a secretary. Its task is to set up new projects in the field of plant genetic resources and to coordinate and support the existing projects. In the new agreement the possibility of participation of other institutions in Europe was left open and other European countries are invited to participate, to form a joint European genebank for potato, eventually.

The German-Dutch Potato Collection

The collection in Brunswick was initially build up by assembling both national collections: EBS (Erwin-Bauer Sortiment, Cologne; curator Prof. Ross) and WAC (Wageningse Aardappel Collectie, Wageningen; curator Prof. Hermsen), material from the Dutch-English Andes expedition and by seed exchange in particular with the INTA collection (Balcarce, Argentina; curator Okada). An expedition in Bolivia (1980) was organised to improve the low representation of Bolivian potato germ plasm in genebanks, as previously pointed out by van Soest (1979) at the third Planning Conference. Thereafter, the collection was expanded with 392 accessions of wild species and 100 accessions of primitive species from Bolivia (van Soest & Hondelmann, 1984). In this way a collection of more than

2.900 accessions of wild and primitive species was assembled. Taxonomic identifications of material from Bolivia were carried out by Hawkes and Hjerting, who described 7 new Bolivian species.
Because three different collections supplied the starting material, duplicates exist inside the German-Dutch potato collection. The same original collection can be present under several BGRC numbers. In total 234 duplicates were excluded from further rejuvenation and evaluation and their data were transferred to a different computer file. These accessions will not be included in publications on evaluation data to prevent confusion at the breeders side. Moreover, intra-specific hybrids were excluded from which both parents were already available in the collection or represented in other hybrids; this especially concerns the species S. infundibuliforme and S. megistacrolobum. By elucidating duplicates and superfluous hybrids the maintenance and evaluation work on the collection can be carried out as efficiently as possible The collection now consists of 2.537 accessions of 90 wild species and 6 primitive species.

Apart from duplicates inside a collection there exist of course quite a few duplicated accessions between collections. By means of the passport data 532 duplicates with the American Potato Collection can be identified and 40 with CIP. These numbers will probably rise, when the collections are more intensively compared. This will especially be the case for the number of duplicates with the collection of primitive species at CIP.

Maintenance programme

In the last 12 years about 1.200 accessions have been multiplied successfully. By the present maintenance programme 180 accessions are rejuvenated per year. This ist achieved by growing 100 accessions in the greenhouse and increasing 50 accessions of self-incompatible diploid species in the field plots in Brunswick and 30 in field plots in Wageningen. In isolated plots located in barley 24 plants from each accession are grown with a distance of at least 50 meters between the accessions. The success of rejuvenation in the field is dependant on the weather. a sunny season guarantees abundant flowering and sufficient pollination by bees, whereas in a rainy summer the flowering is worse and late blight often infects the plants. If fruit setting of a certain

accession is too low, cuttings with flower buds are collected and placed in bottles in the greenhouse. In the temperature controlled greenhouse of 20 - 22 °C the, self compatible or difficult to rejuvenate species are grown with 20 plants per accession.

In the European Community quarantine regulations exist for the import and use of non-European potato material (Anon., 1984). These phytosanitairy regulations hamper the utilization of material from the German-Dutch potato collection. Requested samples have to pass through a quarantine station before they can be used in breeding. As the collection is maintained in the form of true seed only, the seed transmitted quarantine diseases have to be noticed (table 1).

Table 1: Survey of true seed transmitted potato diseases

- Potato spindle tuber viroid (PSTV)
- Andean potato latent virus (APLV)
- Potato virus T (PVT)
- Tobacco ringspot virus, Andean potato calico strain (TRSV-Ca)
  synonym: Potato black ringspot virus (PBRsV)
- Arracacha virus B, oca strain (AVB-O).

The plants used for rejuvenation have been tested from 1986 onwards on these so called quarantine diseases, in order to supply tested material. As yet, these tests are carried out at the Dutch Plant Protection Service (PD) in Wageningen, until a quarantine station in Germany (Hannover) takes over the testings in 1988. PSTV is tested by polyacrylamidegelelectrophoresis (PAGE), APLV and PBRsV by latex-agglutination, and further on *Chenopodium quinoa* *Chen. amaranticolor* and *Nicotiana clevelandii* are inoculated as testplants. For PSTV bidirectional electrophoresis will be used, which allows to test without the intermediate inoculation of tomato plants. For the other seed transmitted viruses the ELISA method ist being developed at the "Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA) in Brunswick.

Because of the high costs for testing, a more selective approach will be needed when selecting accessions for rejuvenation. Therefore, the structure of the collection has to be reconsidered.

Evaluation programme

An important prerequisite for the utilization of genetic resources is their evaluation for desired properties. Evaluation of its potato germ plasm is one of the main objectives of the German-Dutch potato genebank. Evaluation of resistance for important European potato diseases are carried out by specialists in several research institutes in both cooperating countries, as summarized in table 2.

Table 2: Current evaluation programme and institute involved

Characteristic	Institute/ Research worker
Blackleg (*Erwinia caratovora* var. *atroseptica*)	BLBP (Dr. Munzert)
Late blight (*Phytophthora infestans*)	
- laboratory test	BBA (Dr. Schöber)
- field test	SVP (Ir. Colon)
Gangreen (*Phoma exigua* var. *foveata*)	BBA (Dr. Langerfeld)
Wart (*Synchytrium endobioticum*)	BBA (Dr. Langerfeld)
White potato cyst-nematode (*Globodera pallida*)	
- Pa2	BBA (Dr. Rumpenhorst)
- Pa3	SVP (Dr. Dellaert)

BBA = Federal Biological Research Centre for Agriculture, Brunswick and Münster, FRG

BLBP = Bavarian Governmental Institute for Soil Cultivation and Plant Production, Freising, FRG

SVP = Foundation for Plant Breeding, Wageningen, Netherlands.

In addition evaluation data are available for *G. rostochiensis* (Ro1, 2, 3, 5), *G. pallida* (Pa1), Viruses (X, Y, M), Dry-rot and Vitamin-C content. In the case of late blight a mixture of the most complex pathotypes is used for inoculation of leaves or plants, to screen for high levels of race non-specific resistance, which could be determined in well-known Mexican species as *S. demissum*, *S. stoloniferum*, *S. bulbocastanum* and *S. verrucosum*, but also in Andean species like *S. berthaultii*, *S. polyadenium*, *S. microdontum* and some accessions of *S. sparsipilum*, *S. vernei* and *S. mochicense*. In the case of *Globodera pallida*, resistance has been detected in species such as *S. brevicaule*, *S. gourlayi*, *S. circaeifolium*, *S. spegazzinii* and *S. vernei*.

The evaluation data were analysed and discussed in several publications (van Soest, 1983; van Soest et al., 1983, 1984). All available data were published in 1981 (van Soest & Seidewitz, 1981). This year the second edition was completed (Hoekstra & Seidewitz, 1987), which also includes evaluation data of the Bolivian material. In total more than 6.600 evaluation data are available.
It must be mentioned, that the screenings have been conducted on populations, and the results must be considered as preliminary results.

Distribution of samples

Excluding the samples used for evaluation work, more than 40 % of the 1.700 samples that were requested in the last 6 years, were distributed to potato breeders. Almost 90 % of these samples were demanded by government breeding institutes, indicating that private breeders are somewhat reluctant to use wild species in their breeding programmes. Besides late blight resistance and drought tolerance the kind of species distributed (for example S. gourlayi, S. spegazzinii and S. sucrense) reflect in particular the current emphasis on breeding pallida resistant varieties. The remaining 60 % were used by taxonomists and for research on protoplasts, unreduced gametes and resistance systems.

Concluding remarks

Our evaluation strategy has proved to be successful. In the evaluation programme the accessions with desired properties for potato breeders were identified, which, in turn, resulted in demand for breeding purposes. The need to satisfy the quarantine requirements of material complicates the maintenance of the collection and forces a reconsideration of its structure. A more uniform representation of the species is being pursued and the number of species represented in the collection will be raised. A cycle of rejuvenation and testing of the accessions will meet the quarantine regulations for the distribution of this germplasm within the EC.

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THE ROLE OF INTERNATIONAL COOPERATION IN THE DEVELOPMENT OF THE BALCARCE POTATO GERMPLASM BANK

Katsuo A. Okada

HISTORICAL BACKGROUND

This is to give a brief account about the Balcarce Potato Germplasm Bank, and on the role international cooperation played in its development. The germplasm bank is located at the Balcarce Agricultural Experiment Station in the Southeast of Buenos Aires Province in Argentina, and this station is the main place for potato breeding in the country.

In 1966, Professor Hawkes from the University of Birmingham, U.K. and Mr. Peter Hjerting from Denmark had visited Argentina to collect potatoes. In those days I was working as a pasture botanist and knew nothing about potato germplasm but was invited to join the expedition as a representative of the "Instituto Nacional de Tecnología Agropecuaria" (INTA).

To be fair, I should admit that I was not too excited going about collecting potatoes, but somehow, Professor Hawkes managed to persuade me to follow him. As a result of this association, I headed for Birmingham in July of 1967 thanks to a scholarship from the Ministry of Overseas Development of the U.K. I stayed in Birmingham until November, 1970. The training I received there under the guidance of Professor Hawkes was decisive for the fact that henceforth I was dedicated to potato germplasm work. Many years later, my associate, Miss Andrea Clausen, attended the Birmingham course on the Conservation and Utilisation of Plant Genetic Resources, and she obtained her M.Sc. degree in 1979.

The potato germplasm project was initiated in 1970 with the assistance of the Federal Republic of Germany, through the German Agency for Technical Cooperation (GTZ), as part of a comprehensive scheme destined to strengthen our capability to breed new varieties of potatoes as well as to assess their quality and to devise better methods of disease control. For this germplasm work, GTZ provided transportation and laboratory equipment and also sent an expert plant collector, Mr. Werner Hoffmann, to act as my counterpart. Mr. Hoffman made many valuable collections of both wild and native cultivated potatoes between 1970 and 1971. He had the hard task of setting up a potato introduction garden

at Hornillos (2,400 m altitude), at Quebrada de Humahuaca in the province of Jujuy 2,000 Km. away from Balcarce. The purpose of this garden was to maintain clonally native varieties of **S. tuberosum** ssp. **andigena** which had been collected in northwestern Argentina, and to study wild potato species under conditions similar to their natural habitat.
Special mention should be made of Professor Hans Ross, the scientific adviser of the Argentine-German project. He was not only a staunch supporter of the germplasm work but also travelled extensively collecting potatoes in many remote regions of northwestern Argentina. The Argentine-German Project ended in 1974.
In 1978, the International Board of Plant Genetic Resources (IBPGR) made a very important contribution to potato germplasm work in Argentina; it helped to finance the construction of 400 m^2 of screen house for the seed increase of the collected material.

ACTIVITIES OF COLLECTING AND THE DISTRIBUTION OF POTATO GERMPLASM

With the training given by the University of Birmingham, the initial assistance provided by GTZ, and with the funds provided by INTA, IBPGR and the United States Department of Agriculture (USDA), we managed to assemble a fairly large sample of the tuber-bearing **Solanums** native to Argentina. The number of potato collecting expeditions carried out by the Balcarce team** from 1967 onwards are listed in Table 1. As can be seen, 67% of the total number of days of field collecting as well as 70% of all samples obtained were funded by INTA.
We have also participated in international expeditions in Bolivia, as can be seen in Table 2.
At present, we have 825 seed samples of tuber-bearing Solanums in store at minus 15°C. From the beginning, it has been our policy to share duplicate samples of all our collections with the IR-1 USDA and the German-Netherlands (BGRC) Potato Collection. The significance of the Balcarce contribution to both, the American and European Potato gene banks are shown in Table 3.

** Scientists who have participated in potato collecting missions were: K.A. Okada, A.M. Clausen, W. Hoffmann, F. Vargas, C. Monti, A. Mendiburu, L. Montes, E. Bellos, O. Garcìa, O. Gerpes, J. Cassino and M. Castaño. Prof. J.G. Hawkes, Prof. H. Ross, Prof. J.G. Hermsen, Dr. A.M. van Harten, Ir.I. Haisma and Miss S. Saufferer have also participated in some expeditions.

FUTURE PROSPECTS

This forum gives us an opportunity to raise topics that preoccupy us. The argument is often heard that by now so many samples of potato germplasm have been accumulated that there is no more need for further collecting and that further collecting work should be reduced in favour of evaluation and utilization. Although it is true that the more advanced stages of germplasm work are lagging behind, I do not believe that progress in the areas of evaluation and utilization should be achieved by cutting down on exploration activities and field collections. After all, many areas of the mighty Andean mountains are still waiting for the potato explorer, and in many other areas we have just only skimmed the surface of potato genetic resources. I am quite convinced that there is still a wide scope for the potato collector and the taxonomist, and that the results of his search will certainly provide positive benefits to the community of potato scientists.

We all know that populations of wild potato species are disappearing at an accelerated rate in most of the Andean regions of South America. Can we confidently say that we have collected most of the relevant potato germplasm?

Although INTA is well aware of the importance of a potato germplasm project, its resources are now spread so thinly that a project like this will only be viable if some sort of international assistance is secured.

Table 1. POTATO COLLECTING EXPEDITIONS IN ARGENTINA ORGANIZED BY THE BALCARCE AGRICULTURAL EXPERIMENT STATION (INTA), 1967-1983

Source of Funds	Number of Expeditions	Duration (days)	Number of Samples Obtained[1]
INTA	12	457 (67)[2]	2039 (70.5)[3]
GTZ	1	60 (9)	253 (8.7)
IBPGR	1	60 (9)	300 (10.4)
IBPGR/USDA	1	103 (15)	299 (10.3)
TOTAL	15	680 (100)	2891 (100)

[1] Tuber and seed samples
[2] Percentage of total collecting days
[3] Percentage of total samples

Table 2. PARTICIPATION OF K.A. OKADA IN INTERNATIONAL EXPEDITIONS TO BOLIVIA

Source of Funds	Number of Expeditions	Number of Days
CIP	1	30
USDA	1	41

Table 3. SEED SAMPLES OF TUBER-BEARING **SOLANUMS** SENT FROM THE BALCARCE POTATO GERMPLASM COLLECTION TO INTERNATIONAL BANKS, 1970-1983

Int. Potato Germplasm Bank	Total Number of Samples	Samples Sent from Balcarce	Percentage of Total Samples
IR-1 USDA[1]	1402	796	56.7
GERMAN-NETHERLANDS (BGRC)[2]	2366	977	41.4

[1] IR-1 Unpublished Seed Inventory of tuber-bearing **Solanum** species, September 1986.

[2] Index Seminum of tuber-bearing **Solanum** species, FAL, Braunschweig, 1980.

GERMPLASM EXPLORATION AND COLLECTION IN COLOMBIA WITH IBPGR FUNDING

Luis E. Lopez J.

INTRODUCTION

Plant genetic resources are limited and perishable natural resources which provide the raw material or the genes to create new genotypes. Those genes are spread throughout local crops and natural wild populations. Crop plants have been selected by farmers throughout centuries on the basis of their ecological adaptation, disease resistance and yield.

Unfortunately, these genetic resources are in danger of extinction almost everywhere. Conscious of the disaster which would occur if those resources disappeared completely, several projects have been submitted to the International Board for Plant Genetic Resources (IBPGR) requesting financial support for the exploration, collection and conservation of plant genetic resources in Colombia.

This paper will briefly describe what kind of projects have been submitted by the "Instituto Colombiano Agropecuario" (ICA) and were eventually supported by IBPGR.

PROJECTS

a) Exploration and collection of wild potatoes in Colombia

This project was initiated in 1980, with the following aims:

1) To collect the wild potato species of Colombia.

2) To classify taxonomically the material obtained.

3) To evaluate all the collections in field and laboratory.

4) To maintain duplicates of the samples in other centers such as Sturgeon Bay in Wisconsin, USA.

5) To determine the extent to which genetic erosion has occurred, in wild potato species.

In order to carry out these objectives, the exploring and collecting routes were defined, and almost all of the so called "Paramos" (areas ranging from 3,000 to 4,000 m.a.s.l.) were included. Other areas which are not considered as paramos but where wild potatoes are thought to exist, were also included.

ITINERARY

August 15/80	Bogota-Armenia
August 26	Armenia-Pasto
August 27	Pasto-Ipiales
August 28	Ipiales-La Victoria-Ipiales
August 29	Ipiales-Cumbal-Laguna Negra-Ipiales
August 30	Ipiales-Tuquerres-Chiles-Tuquerres
August 31	Ipiales
September 1	Tuquerres-Pasto
September 2	Pasto-Obonuco-Rio Bobo-Pasto
September 3	Pasto-Sibundoy
September 4	El Encano-La Cocha
September 5	El Encano-Pasto-Buesaco-Pasto
September 6	Pasto-El Bordo
September 7	El Bordo-Bolivar-San Sebastian
September 8	San Sebastian-Venecia
September 9	Venecia-Santa Rosa-El Bordo
September 10	El Bordo-Popayan-Coconuco
September 11	Coconuco-Paletara-Pilimbala
September 12	Pilimbala-Purace-La Plata
September 13	La Plata-Silvia-Palmira
September 14	Palmira
September 15	Palmira-La Diana via a Herrera (Tol)
September 16	Palmira via a Ataco
September 17	Palmira-Calarca
September 18	Calarca
September 19	Calarca-Pijao-Calarco
September 20	Calarca-La Linea
September 21	Armenia
September 22	Armenia-Manizales
September 23	Manizales-La Enea-Termales-La Esperanza-Manizales
September 24	Manizales-Bogota
September 25	Bogota
September 29	Bogota-Usme-Paramo de Chusaca-Sumapaz
September 30	Sumapaz-Bogota
February 10/81	Bogota-Zipaquira-Pacho
February 11	Pacho-Paramo de Guerrero-San Cayetano
February 12	San Cayetano-Tausa-Ubate
February 13	Ubate-Bogota
May 20	Bogota-Zipaquira-Granja Don Benito
May 21	Granja Don Benito-Bogota
May 22	Bogota-Bucaramanga
July 14	Bucaramanga-Ocaña
July 15	Ocaña-Cerro de Oroque-Paramo de Jurisdicciones
July 16	Paramo de Jurisdicciones-Abrego-Ocaña
July 17	Ocaña-Cucuta

July 18	Cucuta-Pamplona-Cerro Oriente
July 19	Cerro Oriente
July 20	Pamplona-Paramo de San Turban-Pamplona
July 21	Pamplona-Paramo de Presidente y Almorzadero-Malaga
July 22	Malaga-Soata-San Mateo-El Cocuy
July 23	El Cocuy-La Cueva-El Cocuy
July 24	El Cocuy-Cerro Mahoma-Carrizal-El Cocuy
July 25	El Cocuy-Guican-Sierra Nevada-El Cocuy
July 26	El Cocuy-Guacamayas-San Mateo-La Uvita
July 27	La Uvita-Chita-Paramo-La Caña-Socha-Duitama
July 28	Duitama-Mongua-Paramo de Llano Grande-Duitama
July 29	Duitama-Paramo de Guantiva-Duitama
July 30	Duitama-Sogamoso-Aquitania-Duitama
July 31	Duitama
August 1/81	Duitama-Tunja-Bogota
August 3	Bogota-Une-Gutierrez
August 4	Gutierrez-Une
August 5	Une-Bogota
August 6	Bogota-Soacha-San Jorge

PARTICIPANTS

The project was conducted by the author who was assisted by students from two universities, agronomists and assistants working in ICA. A list of 17 persons, who assisted partially in the execution of this project is found in a report presented to the "Division de Agronomia", ICA, Tibaitata at the end of the project by the author.

Routes and maps

In maps 1 to 9, the routes and explored areas are shown. The thickest lines in map 1 indicate the Departments where exploration and collections was carried out. These Departments are: Norte de Santander, Santander, Boyaca, Cundinamarca, Caldas, Risaralda, Quindio, Tolima, Valle, Huila, Cauca y Nariño.

Samples collected

During the expedition, 141 accessions of tuber bearing potatoes were collected. The samples were represented by seeds, tubers, seedlings, seeds and tubers, as well as herbarium specimens whenever possible.

Taxonomic study

As a result of the study of the collected material, it was possible to identify the following previously described species:

Solanum andreanum

Solanum colombianum var trianae

Solanum colombianum var guambianum

Solanum colombianum var pubescens

Solanum colombianum var zipaquiranum

Solanum flahaultii

Solanum lobbianum

Solanum tuquerrense

Solanum garcía-barrigae

Solanum ochranthum

S. andreanum, **S. flahaultii**, and **S. tuquerrense** have a certain degree of variation at least in the flower colour. New wild species were also found.
Two of them **S. estradae** and **S. pamploense** were published in number 55 of the Journal "Mutisia". A third species called **S. neovalenzuelae**, will shortly be published in the journal "Caldasia", 14 # 6870.

A rather strange wild sample is currently being studied. Cytological observations indicated that some root tips have only cells with 14 chromosomes, and other root tips have cells with 36 chromosomes.

It is likely that there are some more wild potato species in Colombia which are in danger of extinction because of the rapid destruction of the ecosystems where they are found.

Genetic erosion

Wild species which have previously been described for Colombia are still found today. They are not always found in the places were they were originally described but are present in other areas.

b) **Exploration and collection of vegetable plants in Colombia**

This project was carried out between 1981 and 1982 throughout 10 department of the country. The exploration lasted for 60 days.

A total of 572 accessions was collected, and located in ICA, "Programa de Hortalizas. The largest collections were made in genera **Capsicum, Allium** and **Cucurbita.**

c) **Exploration and collection of native cotton in Colombia**

Short expeditions to cover almost all of the country, except the Amazonian area, were carried out in 1983, 1984, and 1985. More than 600 accessions with great intra- and interspecific variation were obtained in diverse ecosystems and ecological niches.

The collection was deposited in the Cotton Programme of ICA in Palmira and in the Genetic Programme in Bogota.

d) **Exploration and collection of native cocoa trees in Colombia**

This project is still in progress. However, 95% of it has already been accomplished.

The Pacific coast, parts of the Amazon Basin, the Departments of Huila and Tolima, and the Sierra Nevada de Santa Marta have already been explored, and about 400 accessions of cocoa trees have already been collected. The material collected includes a great diversity of native criollo and calabacillo types. This project is expected to end in April of 1987. All the samples collected, wild as well as primitive cultivated forms, have been deposited in the "Programa de Cacao" of ICA at Palmira.

e) **Collection of sweet potato and yam in the Colombian Atlantic Coast**

This project was submitted to IBPGR in 1984, it was approved in 1985 and started in 1986. In March of this year, the project should be finished.

Although in the beginning it was planned to explore and collect sweet potato and yam germplasm only on the Colombian Atlantic coast, it was decided later on to cover the center and south of Colombia as well. When it became known that CIP was also interested in collecting wild and cultivated species of **Ipomoea**, Dr. Fermin de la Puente was invited to take part in this collection, and he accepted this invitation. He was

of great help to us in this work, due to his wide knowledge of the **Ipomoea** species.

Thanks to Dr. de la Puente's enthusiasm and specialist knowledge this expedition was highly successful, not only for the exploration of the Atlantic Coast but also for the center and south of Colombia.

The cultivated samples of **Ipomoea** and **Dioscorea** (yam) were deposited in the Experimental Research Station "El Carmen" at Bolivar, Colombia. Through these expeditions the Colombian germplasm bank of **Ipomoea** and **Dioscorea** was enriched with nearly 400 samples.

f) **Conservation and transport facilities**

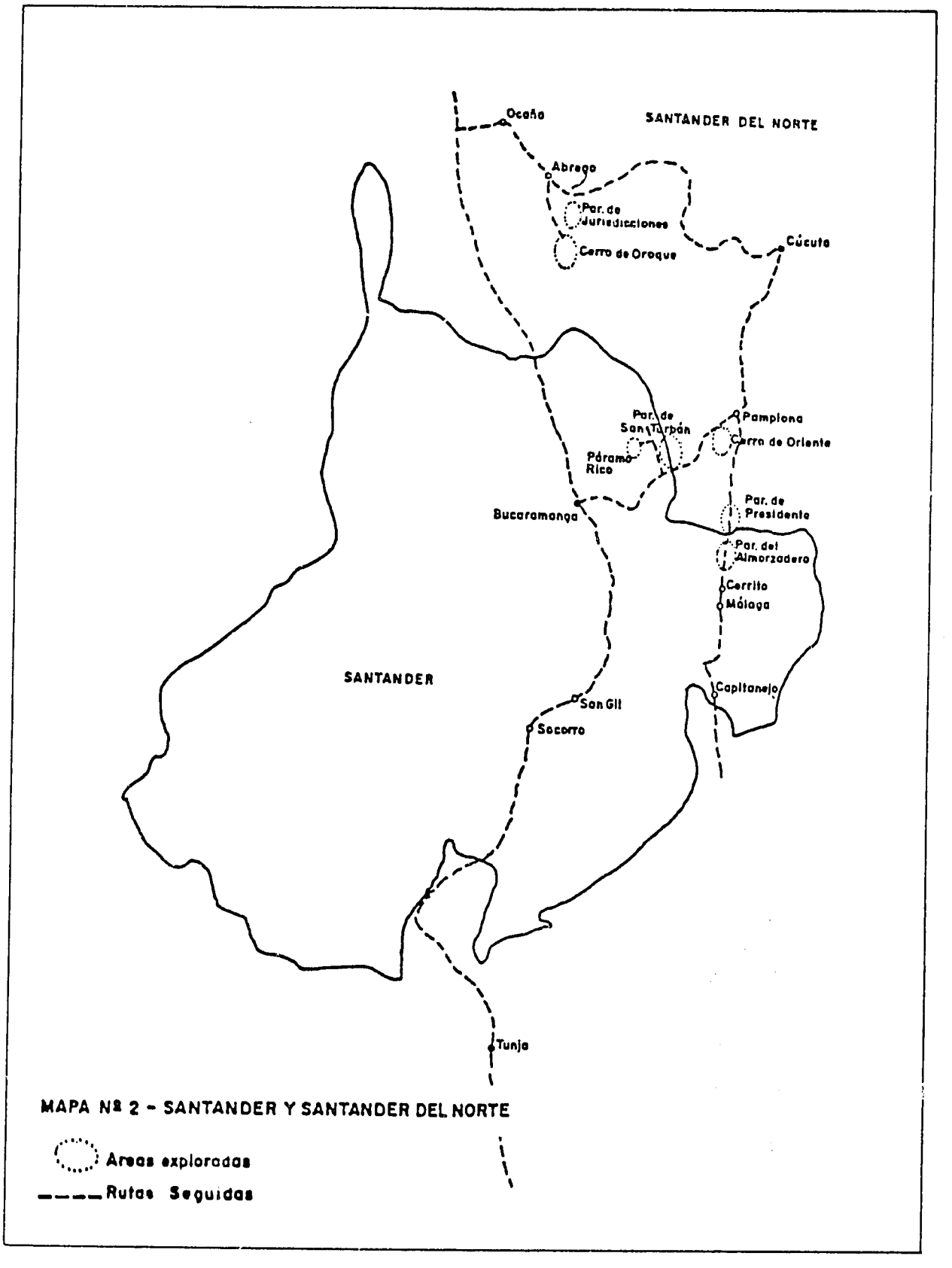
In 1983, a cold room with controlled temperature was provided by IBPGR to ICA. The room has a capacity of 100 m^3, and is equipped with 30 seed containers. Each container has room for 600 accessions, each accession weighing one pound. The room temperature is maintained at 0°. IBPGR also provided a four-wheel drive jeep to ICA's Genetic Programme in 1983 for use in germplasm activities,

RECOMMENDATIONS

Of the two crops in which CIP is interested, potato as well as sweet potato, the wild potatoes in the Paramos of the departments of Norte de Santander, Santander and Boyaca should as soon as possible be explored. Those Paramos were explored during the execution of the project mentioned above, however, this exploration was too rapid because of lack of time. It is believed that wild potato species new to science still exist in those areas. Regarding **Ipomoea** species, it is necessary to explore the Department of Choco, the oriental planes as well as those areas visited in November when no ripe seeds were found on the wild species of **Ipomoea** which occur in that area.

MAPA Nº 1 COLOMBIA

Las líneas gruesas indican los Deptos. donde se exploró y colectó material.


Ocaña
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Abrego
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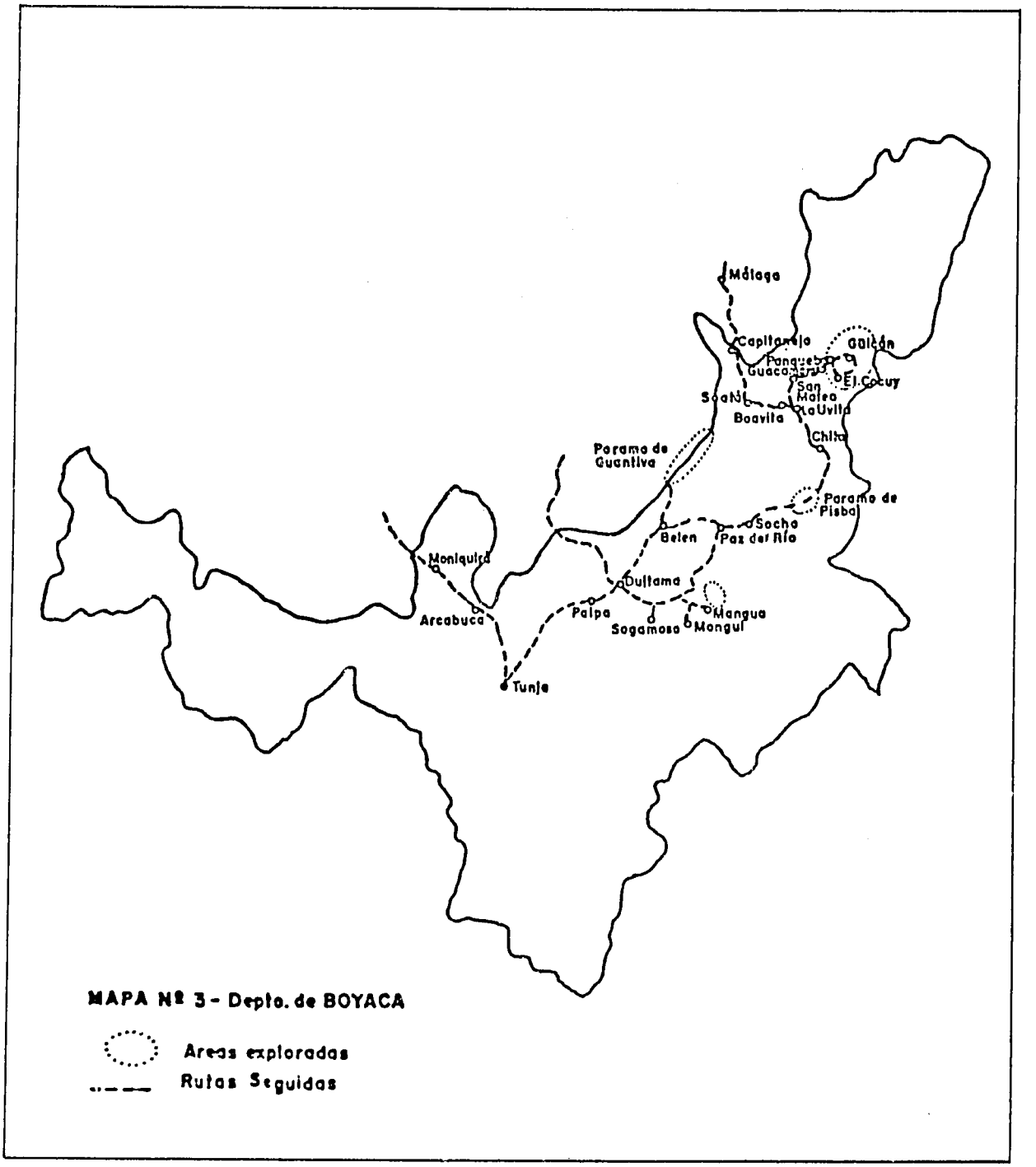
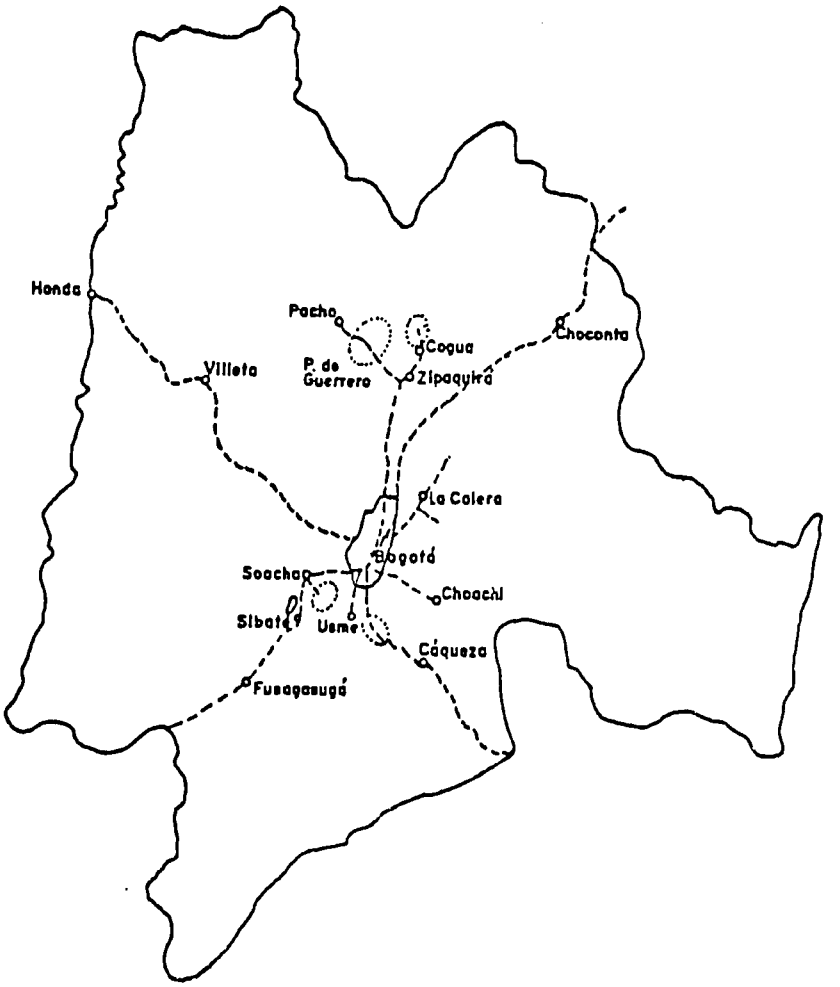
Pamplona
Par. de San Turbán
Cerro de Oriente
Páramo Rico
Bucaramanga
Par. de Presidente
Par. del Almorzadero
Cerrito
Málaga
SANTANDER
Capitanejo
San Gil
Socorro
Tunja
MAPA Nº 2 - SANTANDER Y SANTANDER DEL NORTE
Areas exploradas
Rutas Seguidas



MAPA Nº 3 - Depto. de BOYACA

Areas exploradas

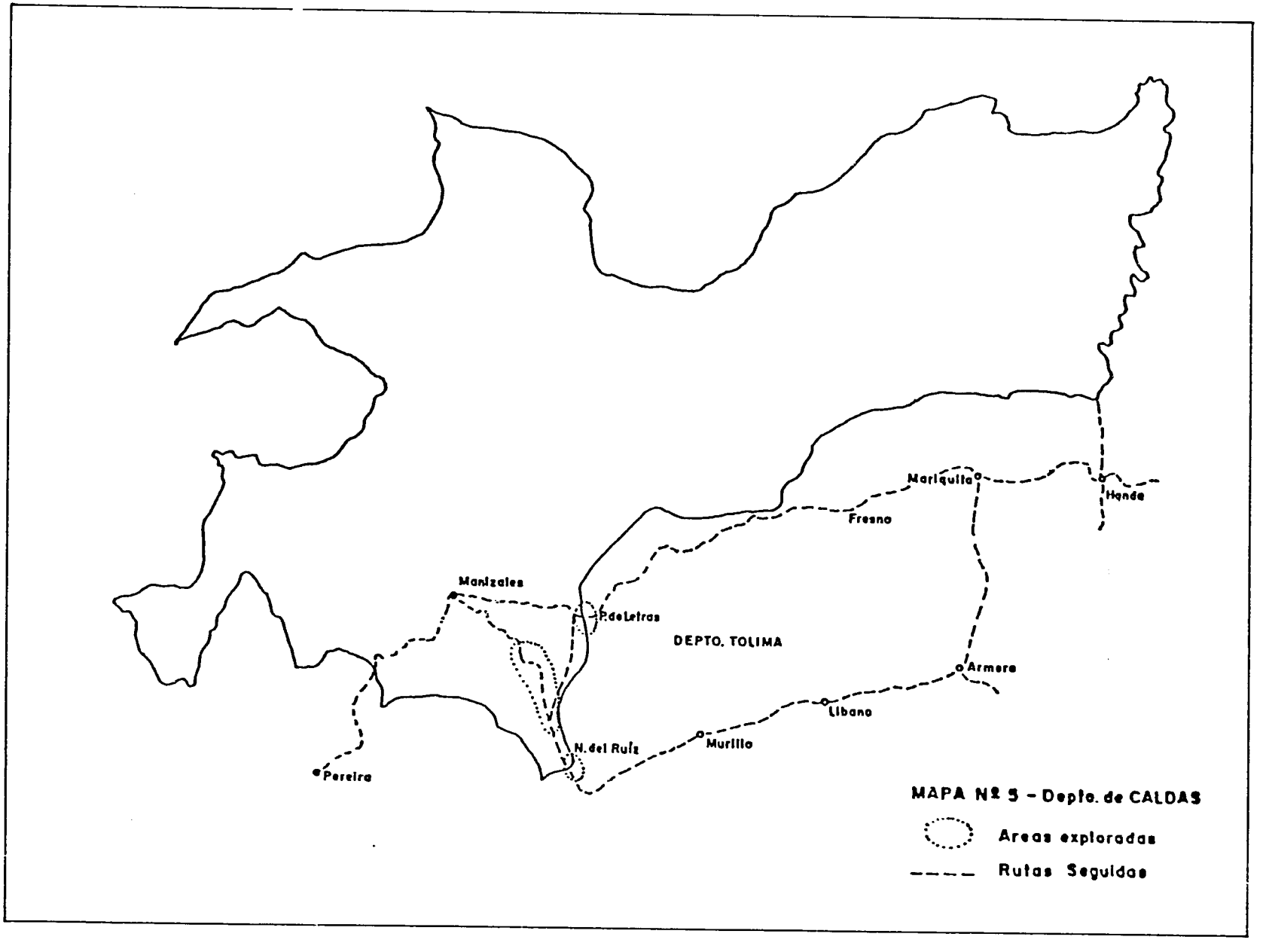
Rutas Seguidas



MAPA Nº 4 - Depto. de CUNDINAMARCA

Areas exploradas

Rutas Seguidas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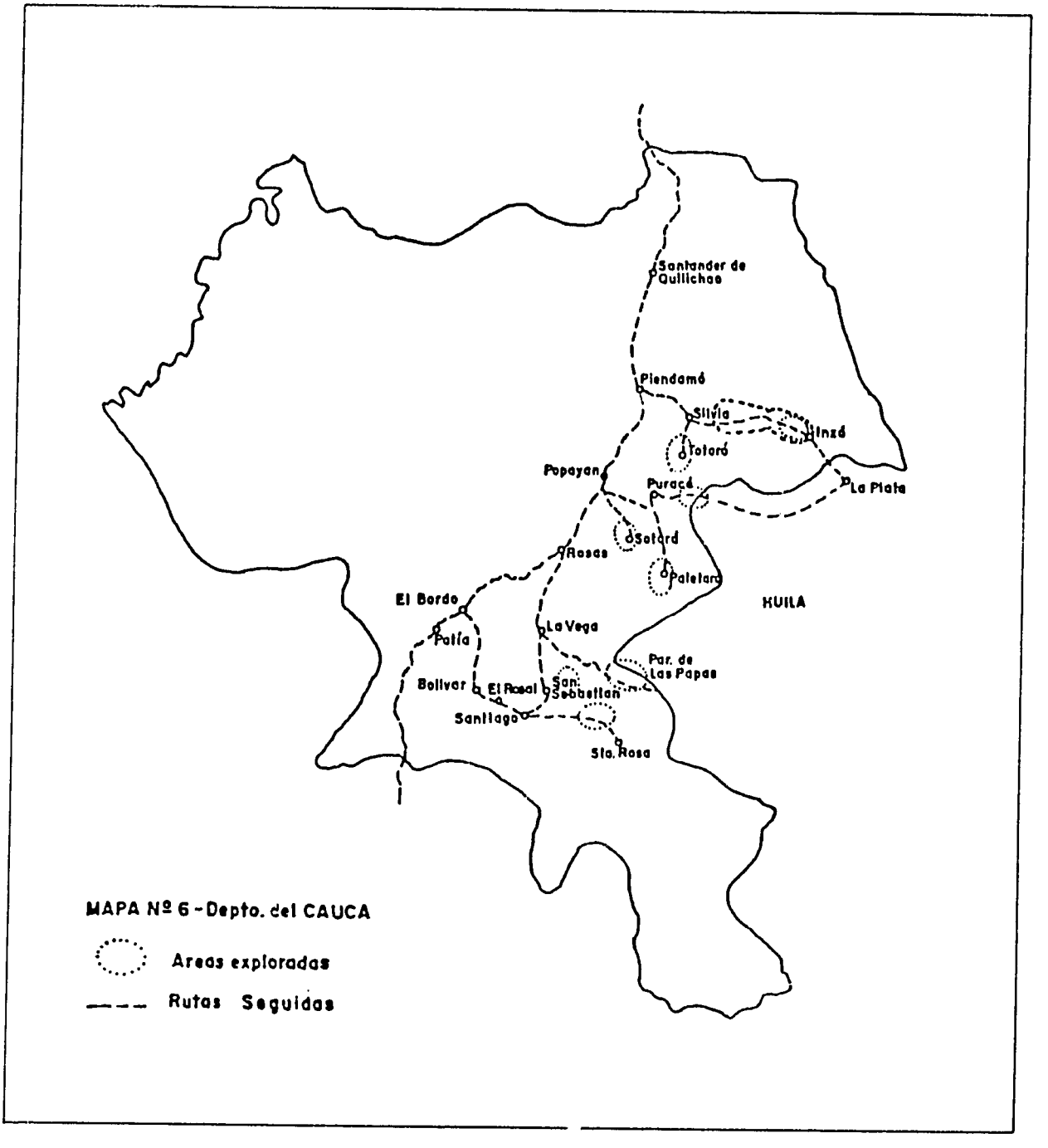
Areas exploradas
Rutas Seguidas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Sotará
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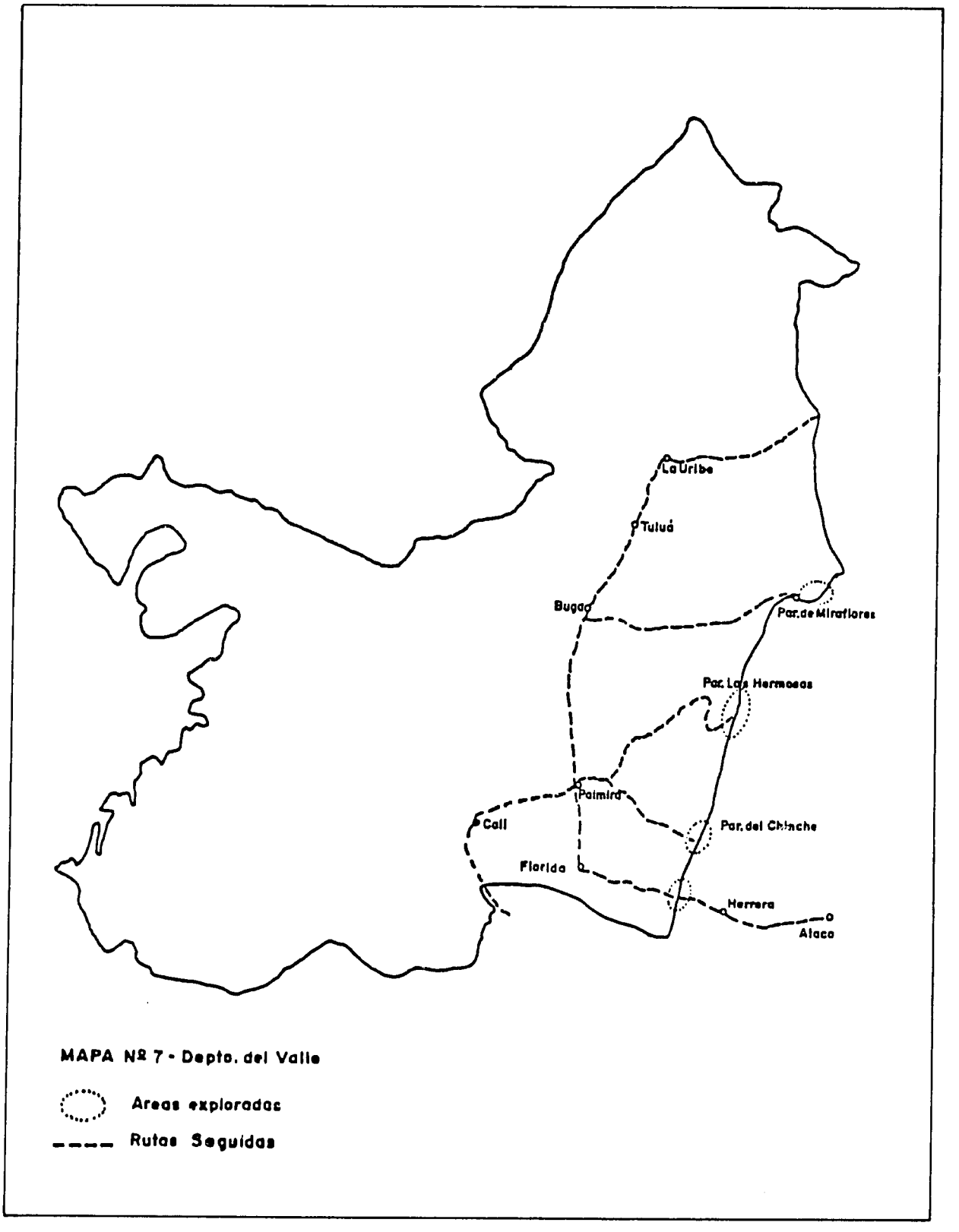
Paletará
HUILA
El Bordo
La Vega
Patía
Par. de Las Papas
Bolivar
El Rosal
San. Sebastian
Santiago
Sta. Rosa
MAPA Nº 6 - Depto. del CAUCA
Areas exploradas
Rutas Seguidas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Alaca
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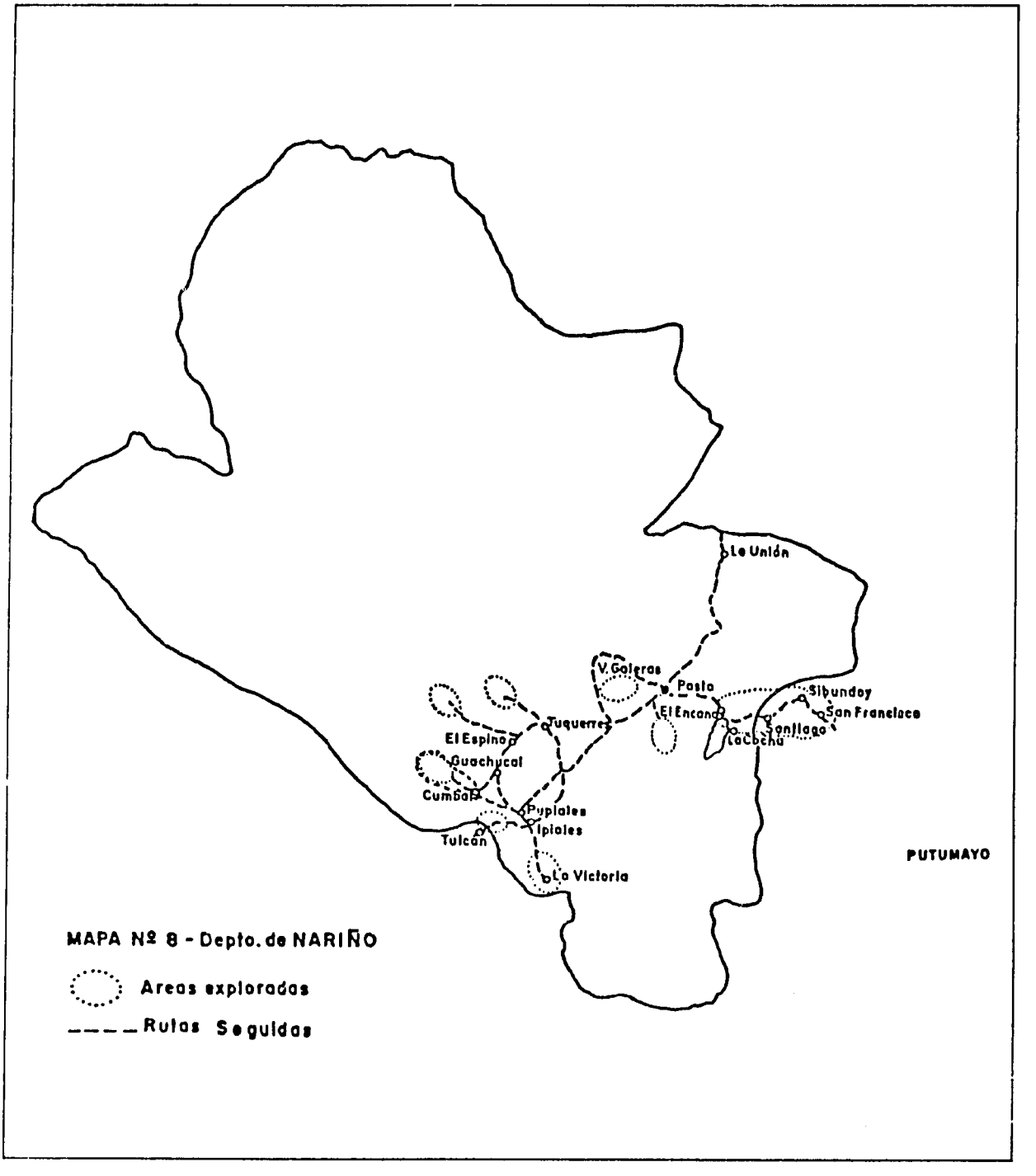
Areas exploradas
Rutas Seguidas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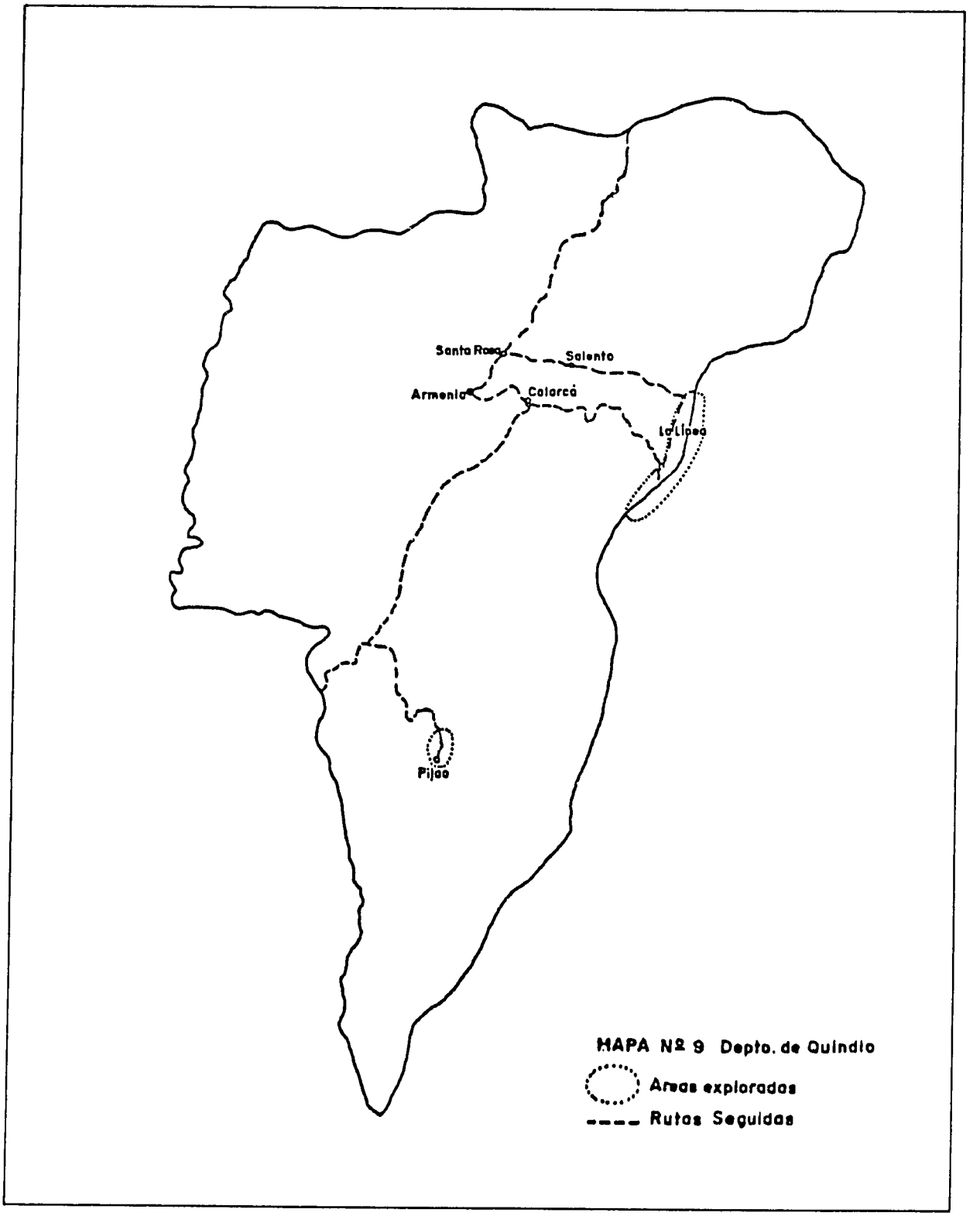
Pasto
El Encano
Sibundoy
San Francisco
Santiago
La Cocha
Tuquerres
El Espino
Guachucal
Cumbal
Pupiales
Ipiales
Tulcán
La Victoria
PUTUMAYO
MAPA Nº 8 - Depto. de NARIÑO
Areas exploradas
Rutas Seguidas


Santa Rosa
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Armenia
Calarcá
La Línea
Pijao
MAPA Nº 9 Depto. de Quindío
Areas exploradas
Rutas Seguidas

STATUS OF THE IN VITRO POTATO COLLECTION AT CIP AND NEW APPROACHES FOR LONG TERM CONSERVATION

John H. Dodds

Introduction

The use of in vitro methods for germplasm conservation has received the attention of many authors [1,2,3]. It is a relatively simple operation to list the advantages of an in vitro germplasm collection over a field maintained clonal collection. In the case of potato a number of special reasons exist for storing clonal germplasm in vitro, these are:

(1) True seed storage does not maintain specific gene combinations, this can only be achieved by vegetative maintenance of the material.

(2) The field maintained collection is known to contain a large amount of viral contamination, by transferring the collection to in vitro the possibility of further cross infection in the collection is removed. If or when the collection is no longer grown in the field this will prevent virus spread to other experimental plots.

Tissue culture methods for potato conservation by a number of distinct methods have been studied over recent years [4,5,6]. CIP has adopted a standard method of in vitro introduction and maintenance using a limited number of culture media. The number of genotypes being handled in the collection is so great that it is impracticable to devise optimal media and methods for each one. We have therefore taken an approach of using methods and media which may not be optimal for a given genotype but function in all genotypes studied to date. The standard method is as follows.

Standard method currently used for in vitro introduction and maintenance of potato germplasm at CIP

Figure 1 shows a flow diagram describing the different steps involved in introducing and maintaining in vitro a clonal potato accession at CIP. The preferred starting material is a sprouting tuber, but it is possible to work from stem cuttings.

The plant material is surface sterilized using Ca hypochlorite and nodal cuttings are excised. These cuttings can then be transferred onto an introduction medium often containing antibiotics to restrict any bacterial growth. Once growth begins nodal cuttings are transferred to standard propagation media [7] until bacteria free status is detected. The material is also cultured on specific bacterial growth media to ensure bacterial free status of the plantlets. These plantlets are now free from bacteria/fungi/mycoplasm but it should be remembered that none of the above mentioned methods eradicate viruses or viroids.

Once the introduction is growing well on propagation media single node cuttings are excised and transferred to conservation media containing mannitol (4%) as a growth inhibitor. The tubes containing these freshly inoculated plantlets can then be handled in two different ways. Firstly, they can simply be maintained at 22°C 16 hours light/8 hours dark; under these conditions the plantlets will need to be re subcultured about once every 12 months. Secondly, the tubes can be kept at 22°C, 16 hours light/8 hours dark daily for 2 weeks. After this time the tubes are moved to reduced temperature storage (LTS) at 8°C 16 hours light/8 hours dark. Under reduced temperature conditions the cultures need to be transferred once every 2 to 3 years.

Current status of CIP in vitro collection

The CIP potato collection is still being introduced to in vitro conditions. The priority listings for introduction are received from the genetic resources department and are followed according to availability of plant material. The speed of introduction has increased since the opening of the new Japanese funded in vitro germplasm facility (see Fig. 2). The genetic diversity of the introduced materials is shown in the presentation of Dr. Z. Huaman (these proceedings).

It is envisaged that new introductions will continue at the present rate in which case a few years more should see the complete clonal collection introduced to in vitro conditions.

Given that all genotypes are introduced and maintained under the same conditions it is to be expected that genotypes will respond in different ways to the culture conditions. Currently a detailed analyses is being made of growth response of the in vitro collection. Data is collected monthly and information entered to the computer database. Predictive analysis is made for the sub cultured period of each clone, this is vital information since some clones need subculture every 8 months and others every 36 months. It is envisaged that monthly listings of materials for subculture will be possible.

Pathogen status of the collection(s)

CIP currently maintains two separate in vitro collections in LTS. The first is the pathogen tested collection, this being materials that have been through thermotherapy, meristem culture and extensive pathogen testing [8,9]. This material is distributed in vitro to national programs for seed programs and research. The second collection is the in vitro germplasm collection, this is known to be free from all known bacterial, fungal and mycoplasma infections. This collection is currently being screened for PSTV and all positive samples will be discarded and the clone reintroduced from clean material. The virus status of this collection is largely unknown. It is hoped that a US-AID funded project on in vitro virus elimination may give rise to methods that could be applied on a large scale to increase the phytosanitary quality of the clonal in vitro germplasm collection.

Duplicate samples

The time, labour and cost involved in introducing the germplasm collection to in vitro conditions is significant. An in vitro collection although preventing certain risks common to field maintained collections is not risk free. It is desirable therefore to maintain a duplicate in vitro

sample at a site distant to the original. CIP has already established agreements for maintenance of in vitro duplicate collections outside of Peru. The duplicate pathogen tested in vitro collection is currently maintained by Prof. G. Wenzel at the Institute of Resistance Breeding in West Germany. The duplicate CIP potato germplasm in vitro collection (the part currently in vitro) is being maintained by INIAP (National Agriculture Research Institute) Ecuador. In both cases CIP received regular status reports of the collections and replaces any accessions lost by the duplicate repository (and visa versa).

Under the current arrangement a new complete duplicate of the germplasm collection is sent to Ecuador annually and the existing material destroyed. It is hoped that arrangements can be made for INIAP or some other institution to assume responsibility for in vitro maintenance and sub culture of the duplicate collection.

Genetic stability of in vitro clones

The basic purpose of an in vitro clonal potato germplasm collection is to maintain specific gene combinations. It follows therefore that any method used for long or medium term conservation should be genetically stable. In this context CIP has given careful consideration to the methods that can be applied to characterize genetic stability. The determination of genetic stability is only as good as the testing method used.

The current evaluation procedure at CIP is as follows. Clones from the pathogen tested collection have been removed from LTS at different time intervals (up to approx. 8 years), plants were grown in pots and tubers were analyzed for morphological characteristics. Soluble tubers protein electrophoresis and isoenzyme analysis was performed and the gel patterns were compared with original genotypes. To date no differences have been identified between original samples and samples after LTS. The basic technique of analysis does, however, have certain limitations, i.e.

(1) It does not study complete genome only specific parts of it i.e. tuber proteins.

(2) It looks at a gene product and not the gene itself. Also, storage protein genes are generally 'conservative' proteins.

For these reasons CIP has recently initiated a research contract with Rothamsted Experimental Station, U. K. (Dr. M.G.K. Jones) to look at the efficiency of these detection methods. In this contract analysis will be made of morphological characters, protein electrophoresis, isoenzyme analysis and restriction length polymorphism (RLPM) to determine the most sensitive testing method. On the basis of results from this contract changes may be made to CIP's standard evaluation method.

Management of CIP's In Vitro Collections

The total in vitro potato collection will contain approx. 4500 accessions or a total of 45,000 culture tubes. It is obvious that to manage a collection of this size a flexible data managing system is required. It is envisaged that through CIP's VAX minicomputer it will be possible to maintain details on in vitro growth characteristics of a given accession and predict its date of subculture. We should also be fully aware of other existence and status of any duplicate samples held outside of Peru. The basis for this system is already in place and I believe given time the system will be successfully implemented.

New Approaches to Long Term Conservation In Vitro of Potato Germplasm

The existing methodology used at CIP has been adopted since we believe it to be the only satisfactory method available at the present time. However, a number of other methods are currently under investigation that in time may supplement or replace the methods currently in use at CIP. They are:

(A) In vitro tuberisation

We have already proven that in vitro tubers can be induced in a wide range of genotypes [10,11]. It may be possible to use these tubers as a way of extending the current in vitro storage period. Preliminary experiment indicate that in vitro tubers inoculated onto storage media can add up to 1 year to the subculture period. However extra labor is involved in the induction of the tubers. The possible use of in vitro tuberisation as a supplement to

existing methods is already under investigation at CIP.

(B) In vitro embryogenesis

If somatic embryos could be routinely induced in a broad range of genotypes then it may be possible to store embryos either on restricted growth medium or by cryopreservation. A great deal of research will be needed before methods can be developed for routine somatic embryogenesis in an incredibly broad range of genotypes.

(C) Cryopreservation

Although successful results have been obtained for cryopreservation of potato meristems [12,13] the system is still not ready for adoption by CIP. The % recovery of genotypes is low and only a restricted number of genotypes have been studied. Further work is needed to optimise freezing regimes for a wide range of genotypes, there is also a need to analyze the effects of freeze/thaw damage of genetic stability of the regenerated plantlets. Although routinely used in animal cell cultures the presence of an insulating cell wall and the need to freeze a multicellular structure greatly limits the direct application of this technique to plant systems. It is understood that several laboratories in Europe and the USA continues to study these problems. CIP should be ready to adopt this technology should it become sufficiently reproducible.

Summary

CIP continues to transfer the potato germplasm collection to restricted growth/reduced temperature storage. Currently over 2000 genotypes are stored in this way. Routine electrophoretic analysis of the material is performed and duplicate in vitro samples are stored in West Germany and Ecuador. While CIP will continue to introduce further accessions using a standard in vitro introduction and maintenance method flexibility must be maintained to use new improved methods if and when they become available.

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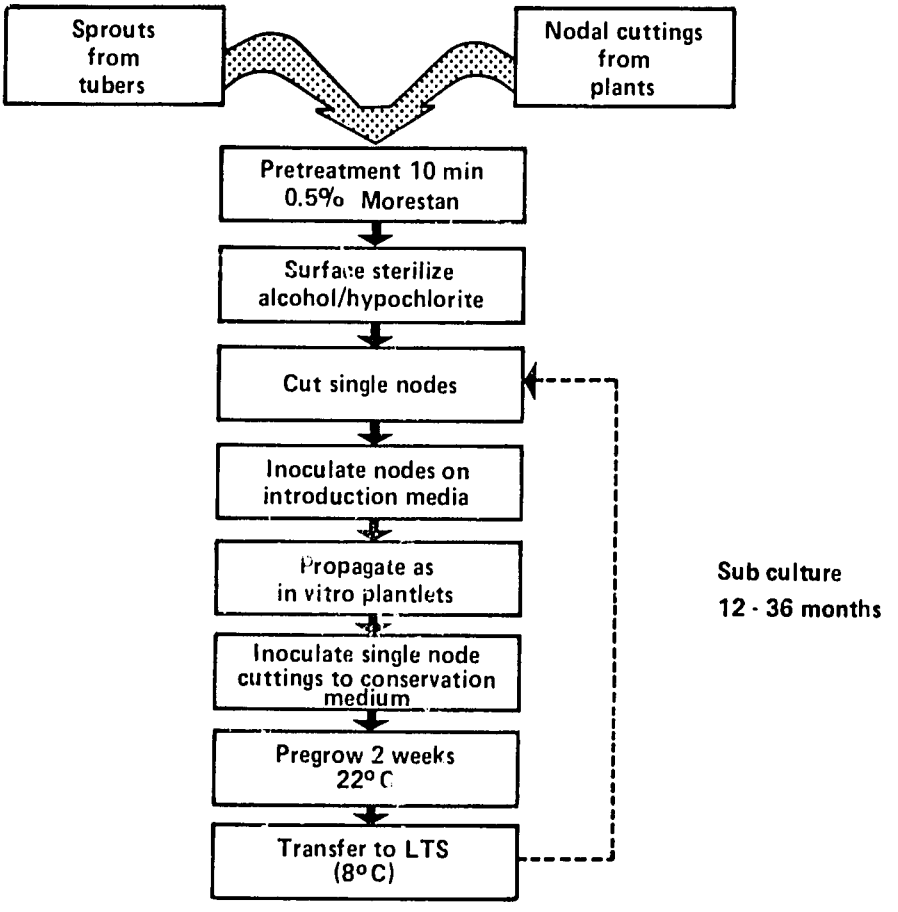
Sprouts from tubers
Nodal cuttings from plants
Pretreatment 10 min 0.5% Morestan
Surface sterilize alcohol/hypochlorite
Cut single nodes
Inoculate nodes on introduction media
Propagate as in vitro plantlets
Inoculate single node cuttings to conservation medium
Pregrow 2 weeks 22° C
Transfer to LTS (8° C)
Sub culture
12 - 36 months

STATUS OF THE IN VITRO COLLECTION IN IR-1 USDA

Robert E. Hanneman

The Inter-Regional Potato Introduction Project (IR-1) has had a vested interest in *in vitro* maintenance, since the USDA, Agricultural Research Service (ARS) added a plant physiologist, Dr. L. E. Towill, to the ARS research group associated with IR-1. Among his responsibilities was the evaluation of the potential for meristem culture and virus-freeing of IR-1 material and the development of long-term storage procedures for maintenance of IR-1 stocks. In 1978, it was decided that IR-1 should hire a full-time person to do meristem culture, virus-freeing and virus testing, after Dr. Towill had demonstrated the feasibility and utility of meristem culture and virus-freeing among clonal IR-1 stocks. IR-1 hired Mr. M. D. Peterson for the position and initially charged him with the responsibility to free the late blight differentials from virus. The program was located at Sturgeon Bay, with technical and research support provided by Dr. L. E. Towill.

In 1983, Dr. Towill was transferred to the National Seed Storage Laboratory at Fort Collins, Colorado and with that transfer it was decided to move the meristem culture and virus-freeing program from Sturgeon Bay to Madison. The program was placed under the supervision of Dr. S. A. Slack, Dept. of Plant Pathology, University of Wisconsin-Madison, who manages the Wisconsin Seed Certification Program. A contract has been in place with Dr. Slack since 1983 to provide pathological support services to IR-1.

In 1986, 134 accessions/clones were placed into culture, the majority of which were species material. In the same time period, 528 Elisa tests were done for PVA, PVM, PVS, PVX, PVY and PLRV, and 986 PSTV dot blot tests were made primarily on parental plants used in seed increases and the resultant seed. Only 10 PSTV positives were found. From 1984-86, 2544 PSTV tests were made with a total of only 19 PSTV positives found. While this percentage is very low, IR-1 will continue to carefully monitor its materials for the presence of PSTV. If material is found to be infected with PSTV, it is discarded. The only exceptions are for rare material which would be held as seed or in culture until individual tests could be made on each plant, or until such time as means are available to free stocks of PSTV.

Presently, 129 stocks are undergoing thermotherapy, and meristem culture for virus-freeing. Six hundred and ninety-eight meristems were cultured in 1986; 440 plants were regenerated, and 108 new virus-free stocks were identified. Virus elimination has been successful for PVA, M, S, X, Y and PLRV both individually and in combination in clonal materials. Consequently, 248 stocks have been freed of the above viruses and viroids. IR-1 is presently switching from clonal maintenance by tubers to <u>in vitro</u> maintenance. It is anticipated that this change will be fully implemented within the year. During 1986, 99 accessions were distributed as <u>in vitro</u> stocks.

IR-1 has been very pleased with the support provided to it by Dr Slack's program. This support has given IR-1 better control over viral and viroid diseases in the collection and permitted it to move closer to freedom from these pests - a point we hold to be critical for a genebank. IR-1 is grateful for the new funding that has permitted it the opportunity to contract for these services.

THE POTATO IN VITRO COLLECTION IN BRUNSWICK AND THE ACTIVITIES OF THE EC WORKING GROUP POTATO

R. Hoekstra and G. Mix

Introduction

The maintenance of certain heterozygous genotypes (clones) can be realized by vegetative reproduction. In vitro storage offers an alternative to the traditional maintenance method of annual tuber propagation of varieties. This method meets the prerequisite of leaving the genotype unchanged, as the cells of shoot-tissue are genetically stable and regenerations from nodal segments give identical progenies. Until cryopreservation becomes practicable, in vitro storage offers the best conservation method.

In the frame of its activities in the field of conservation of plant genetic resources, the Institute of Crop Science and Plant Breeding in the Federal Research Centre of Agriculture in Brunswick (FAL) started to establish a collection of old potato varieties in 1976. Because of the high virus disease pressure in Brunswick and the cost of annual cultivation, the collection was transferred into tubes from 1978 onwards.

Storage method

From 5 germinated tubers per variety nodal segments are taken as starting material. They are surface sterilized and placed in test-tubes on filter paper bridges with a liquid medium of Murashige & Skoog (1962), 2 % sacharose and 50 mg/l Alar (with the active substance daminozide), and a growth inhibitor to keep the stems short. After 3 - 4 weeks at 20 - 22 °C under 4000 lux at a 16 hours day, the plantlets are cut again into nodal segments and cultivated for 4 weeks. For each variety 10 plants of the size of 3 - 4 cm are transferred to 10 °C under 2000 lux at a 16 hours day. After about 2 years segments are cut, cultivated at 20 °C and transferred to 10 °C again. Varietal differences in response to the storage conditions occur. A few exceptions like "Eersteling" and "Voran" suffer from growth depressions due to the low

temperature, and intermediate propagation has to be undertaken after a year. Therefore, the in vitro collection is periodically checked (Mix, 1984).

Status of the in vitro collection

In the autumn of 1985 the maintenance of the potato in vitro collection became a project of the German-Netherlands Board for Plant Genetic Resources. This means that the costs are now shared by both countries. 100 old European varieties annually cultivated by RIVRO in the Netherlands were put into tubes because plant breeders' demand for these varieties was very low. At the moment the collection contains 289 varieties such as "Industrie", "Flava" and "Irmgard" and 27 older breeding lines from the Max-Planck-Institute. The health status of the material in the collection is mostly unknown. Only those varieties whose breeders' rights have expired are available for distribution. The demand for material from the in vitro collection is very low: on the average 1 request per year for 3 or 4 varieties is received. The collection contributes in the first place to the general effort of the conservation of potato varieties.

EC Working Group Potato

The potato variety collection in Brunswick is not the only one and must be regarded together with other collections. Within the European Community the Working Group Potato coordinates the activities on the conservation of varieties. The Working Group Potato was set up by the EC "Programme Committee on Plant Resistance and Use of Gene Banks" in 1981 and concentrated its activities on the preparation of a descriptor list for potato varieties in close cooperation with the IBPGR and an inventory of potato variety collections in the 10 member states of the European Community.

The publication of the descriptor list for potato varieties (Mackay et al., 1985) was delayed by the inconsistency between the IBPGR standard of scoring increasing susceptibility on a 1 - 9 scale and the common practice of scoring increasing resistance on a 1 - 9 scale. The list was prepared for the benefit of the potato breeders and therefore agrees with previous and present practice. In this list the taxonomic traits, used for identification of

different species, have been reduced to a bare minimum. The descriptor list might also form the basis of a more extensive list to include primitive cultivars and wild species.

The Inventory of Potato Variety Collections, compiled by Kehoe (1986), contains the location and health state of about 1 550 different varieties, in vivo and/or in vitro. This inventory shows very clearly two problems: 50 % of the varieties are only present in 1 collection and 37 % of the varieties are not at all available in a definite healthy state.

The working group therefore suggests:

a. to make infected cultivars virus free
b. to enter cultivars in tissue culture for conservation
c. to maintain each cultivar at least at two sites.

Conservation is the most important objective and the transfer to in vitro culture should not be delayed for virus infected material, as infection generally does not affect in vitro culture as such. Because of the high costs of making the material virus free and the low demand for old varieties little energy is put into this work by the member states. It is realized that the low demand is probably caused by the fact that little is known about the characteristics of the old varieties. Therefore, an evaluation programme would be useful, but can of course only be effective for healthy material. Furthermore, funds are not available at present for such a programme. Only when sufficient data are available, the varieties with useful characteristics can be determined and decisions about further maintenance can be made. The establishment of a European data base system is suggested by the working group to compile characterization and evaluation data and to stimulate information access by the plant breeders.

The FAL in Brunswick accepts disease free material for long term in vitro storage and will serve as a centre fur duplicate storage. The varieties only available in the FAL need still to be stored elsewhere.

The remaining problem concerns the exchange of material. Due to quarantine regulations, which also include in vitro cultured material, the exchange is very difficult. A free exchange of certified disease free in vitro material is needed within Europe. The working group will discuss this topic with the Commission of the EC and work on a solution.

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CIP'S ROLE IN THE DEVELOPMENT OF AN INTER-GENE BANK COOPERATION FOR POTATO GENETIC RESOURCES CONSERVATION

Zosimo Huaman

One of the recommendations of the Second Planning conference on the Exploration and Maintenance of Germplasm Resources in Potato, held at CIP in 1976, was that CIP should promote an inter-gene bank collaboration for the conservation of potato genetic resources. This recommendation was endorsed by the 1979 Planning Conference.

A CIP proposal entitled "A cooperative system for the regeneration and interchange of seeds of wild potato species" was sent in 1977 to the U.S. Potato Introduction Station at Sturgeon Bay, Wisconsin (IR-1) and to the German-Dutch Potato Collection at Braunschweig, West Germany (BGRC). The objectives of this proposal were:

1) To establish effective international cooperation for the conservation of the genetic resources of the potato.

2) To utilize more efficiently the funding, physical facilities, and staff available to the potato gene banks, and to eliminate duplication of effort.

3) To safeguard the genetic diversity of the wild relatives of the potato against losses by natural or accidental disasters.

4) To secure the conservation of numerous new accessions of wild potato species that have been collected by CIP and other gene banks.

Basically this proposal suggested that each participating gene bank would be responsible for the maintenance and the regeneration of all wild species present in a determined area of geographical distribution of the tuber-bearing Solanums. Accordingly, the U.S. Collection would be responsible for the regeneration of wild species from all countries of South America, except Peru, the German-Dutch collection for those from all North and Central American countries; and the CIP collection for all wild species from Peru. In addition, CIP will continue to be responsible for the maintenance of the cultivated species.

Although this proposal was submitted to the boards of each of those gene banks and received endorsement in principle, no further progress was made for reasons other than technical or scientific disagreement.

There is a need to revise the possibility of implementing some kind of agreement between the most important potato gene banks. A large number of collecting expeditions have been organized since 1977. Thousands of new accessions of wild species have been collected by CIP throughout Latin America, by the Braunschweig Genebank in Bolivia , and by the U.S. Genebank in Mexico and Bolivia. Numerous accessions of each wild species have been obtained from ecological niches throughout the geographic range of distribution of these species. Additional sources of germplasm have also become available through the rediscovery of many of the wild species known previously only as herbarium specimens. Furthermore, almost 50 new species have been described from materials obtained by these new collecting expeditions.

It appears that there is some reluctancy among potato gene banks to accept a distribution of responsabilities for the regeneration of wild species on a geographic or taxonomic basis. Therefore, there is need to look for areas within the genetic resources activities where the three gene banks could cooperate and benefit from this international effort.

The following are some possible areas for cooperation:

First, exchange of accessions obtained through recent collecting expeditions. This could be for the purposes of duplicating seed stocks in another gene bank to avoid genetic losses due to natural or other disasters. In this case, there are two alternatives related to the usage of the stocks recibed by each gene bank:

1) The recipient gene bank should act only as a seed repository and therefore, have restrictions for the distribution.

2) That the recipient gene bank could incorporate the seed stocks as part of its collection and have freedom to distribute it.

A practical solution to this dilemma could be that if the recipient gene bank decides to include these seed stocks in its inventory for distribution, it should produce enough seed so that it could return a large number of seeds back to the gene bank from which it obtained the material. This would compensate for the efforts of the institution which made the collections, and complement the work of other gene banks increasing the total number of seeds.

Second, collaboration to increase seed stocks when a gene bank has some limitations to insure the availability of enough seed from new collections or when local environmental conditions prevents flowering or seed setting.

Third, implementing joint collecting expeditions to countries or areas of a country considered to be inadequately represented in the gene banks or to areas not previously explored. All gene banks should make an effort to participate and share the costs, equipment and expertise. The samples obtained in this way would be shared by all gene banks.

Fourth, cooperation in the systematic evaluation of the wild gene pool to identify new sources of resistance to tests and diseases and other environmental stresses. Unnecessary duplication of efforts to evaluate populations of wild species from similar eco-geographical niches to the same organism should be avoided. Priorities for the evaluation of collections should take into consideration the relative importance of organisms affecting the potato crop in all countries where the gene banks are located or in their areas of influence. Furthermore, the exchange of selected materials or the data obtained should be promoted. It appears quite feasible that more accessions or more species could be evaluated if the responsabilities for this systematic evaluation are devided among the gene banks.

Fifth, provision of space for the storage of in vitro cultures of valuable genotypes. The recipient gene bank should have storage facilities with standardized environmental conditions to secure a minimum growth in the cultures. The gene bank suppliying these type of materials would be fully responsible for re-subculturing and renovating the in vitro collection.

Sixth, establishment of standardized procedures for the introduction, propagation, conservation and regeneration of potato genetic resources. These procedures should be documented and revised periodically to include newly developed techniques and research breakthroughs.

Seventh, determination of duplicate accessions among the gene banks. This could be made using two types of data generally readily available in gene banks:

1) On the basis of the original collector's numbers since many times the same collector may have deposited the same accession in different gene bank.

2) On the basis of the data on the locality of collection of each accession of wild species since it is widely known that several collecting expeditions visited the same sites where previous collectors had reported findings.

Eight, publication of a joint inventory of potato genetic resources. This could be of enormous benefit for the users of these collections since they could find all available information for all accessions of each species available in these gene banks. A thorough analysis of the data of all these stocks could determine an updated estimate of the number of species represented in the collections; the extent of sampling throughout the area of distribution of each tuber-bearing Solanum species; the extent of common accessions maintained in all potato gene banks; and the frequency of desirable traits among different species.

POTENTIAL AREAS FOR INTER-GENE BANK COOPERATION

Robert E. Hanneman

Inter-genebank cooperation could take a number of forms ranging from simply better communications to actually sharing responsibilities for maintenance, evaluation, etc. The degree to which various genebanks can cooperate will depend on their mandate and its source. Most genebanks were established by their nation's government and have a national responsibility to preserve their nation's germplasm resources or to maintain introduced germplasm for use by scientists within their country. The origin of that mandate indicates to whom that genebank's first allegiance must be given, and to whom and how it is to serve the national or international interests.

A second area that must be recognized in considering formal cooperation is recognition that each genebank must justify its existence to the agency or government(s) supporting it. Usually this takes the form of accounting for the number of accessions shipped and tracking their contributions through research reports, etc. citing their use as well as identifying their presence in the pedigree of released cultivars. Any cooperative effort that would jeopardize a genebank's recognition of accomplishments for materials distributed, should be considered carefully, because that could jeopardize the existence of the cooperating genebank itself.

Let us look at how genebanks could cooperate in the introduction, classification, maintenance, distribution and evaluation of wild and cultivated species. In the introduction of new accessions, joint collecting priorities could be established. Each bank's priorities could be very different. One may focus on broadening the known genetic base of a species by trying to obtain collections from broad and perhaps new geographical areas. Another bank may be interested in only certain species of known usefulness in breeding. A third bank might have a priority to collect germplasm of species known to exist but not available in genebanks or to concentrate on finding new species. Some genebanks may lack collections from certain countries or areas and feel a need to strengthen their holdings by expanding collections from those areas. Other banks may wish to thoroughly collect species and accessions from their country to have as complete a collection of native germplasm as they possibly can. Although their goals may be different, genebanks could still share their goals, cooperate and assist each other as opportunities to collect may arise. Certainly introductions could be shared freely among genebanks.

Assistance with classification of accessions is a more difficult area at present because most of the taxonomists relied upon for these services are now retiring and are not being replaced by their parent institutions. This is true for Dr. J. G. Hawkes, Birmingham University, Dr. C. M. Ochoa, International Potato Center, and apparently will be true for Dr. J. P. Hjerting, Copenhagen University. While these scientists are still available to assist, there presently is only one full-time Solanum taxonomist actively working in the area and that is Dr.

K. A. Okada, INTA, Balcarce, Argentina. The USDA, Agricultural Research Service, recognizing this dangerous scientific gap, is in the process of recruiting a taxonomist to assist the Inter-Regional Potato Introduction Project (IR-1) with its taxonomic needs. It would appear then that classification needs will have to be served by INTA and USDA, ARS taxonomists.

In terms of maintenance of accessions as true seed or as clones (tubers or in vitro), each genebank could share its expertise and assist with specific maintenance problems. Each of the banks probably has gained significant expertise in the maintenance of certain difficult to handle species/stocks (clonally or seed). If a bank is experiencing similar problems, it would be expedient for a bank having success to share its methodology with the one having difficulty. Perhaps a bank enjoying success could even offer to do those increases for its sister bank. Any genebank can be overwhelmed by being inundated with large numbers of accessions for maintenance from recent expeditions or introductions. This would be particularly true for new or more recent genebanks, but holds as well as for older established genebanks. It would seem appropriate then for others having additional capabilities to assist with that maintenance should the overwhelmed bank so desire. This assistance seems appropriate when overwhelmed, but would not seem prudent in routine maintenance, which should be within the capabilities and resources of each genebank.

Collaboration in distribution is possible in a number of areas. One important area is to work with quarantine officials to help better understand quarantine needs and to work toward reduction in restrictions where possible to promote the free flow and use of germplasm between nations. Joint distribution would not seem tenable because this does touch on areas most commonly used by genebanks to justify their existence, e.g. numbers of accessions distributed and the tracking of their use.

Cooperation in evaluation is possible. The banks could share the information they have accumulated. They could keep one another appraised of their evaluation priorities. In those areas of common interest, they could attempt to coordinate screening efforts to reduce duplication of effort. It may be as in collecting, that screening priorities could differ for each genebank based upon the specific needs of their user communities, but cooperation would be helpful when those goals overlap.

There are other areas where cooperation could occur such as in training of personnel. Each bank could assist in training personnel where that bank has unique expertise or experience. This could involve the training of technical staff or scientists. Assistance could be provided to new as well as to established genebanks.

Genebanks could cooperate by sharing in the maintenance of accessions restricted by quarantine officials in a particular country but not by those of another country, if a genebank in the

latter country would be willing to accept such materials. A particular need here would be to identify one or more genebanks willing to accept clonal collections from collecting expeditions which often face lengthy quarantine testing and consequently are subject to loss.

They could assist each other in safeguarding their collections and making sure they are available to other users in the event of natural or man-made disasters or political changes preventing free exchange of germplasm by placing an adequate sample of seed of each accession in one or more genebanks. This safeguard would be a simple but effective means of assuring that the majority of the germplasm maintained as true seed would be available to others regardless of the calamity or political changes that may occur in the future.

Now, where can the Inter-Regional Potato Introduction Project (IR-1) and its associated USDA, ARS research staff assist in this cooperation? IR-1 should be able to assist with collecting and classification of accessions, through the services of the USDA, ARS taxonomist. Future collecting has received high priority by the IR-1 Technical Committee. IR-1 is and has always been willing to share any of the accessions it has with other genebanks or with fellow scientists.

In maintenance, IR-1 could assist particularly in advising on the maintenance of true seed of wild species for which it has accumulated considerable experience. It could possibly assist with the maintenance of accessions for genebanks that are

overwhelmed. It does have considerable ability for expansion of its maintenance capability. It could assist in providing advise on clonal maintenance, but actual assistance could be more difficult because of present strigent quarantine regulations. Also, IR-1's *in vitro* maintenance is done under contract and not directly by IR-1 staff, so the amount of assistance in *in vitro* maintenance that could be offered is questionable.

IR-1 could distribute a sample of all of its true seed holdings to one or more genebanks for safekeeping. It would be generally understood that this seed would not be made available for routine distribution, but that a portion of it could be used by the recipient genebank for its own increase and distribution if it so chose.

It could also assist in the training of personnel at either the technical or professional level. This could be accomplished by actually working side-by-side with IR-1 personnel rather than in a more structured setting. IR-1 would welcome visiting technical and support staff.

IR-1 could make an effort to inform other banks of its priorities for evaluation and what areas it would actively be pursuing so that efforts could be coordinated and perhaps species/accessions of mutual interest could be identified and incorporated into the screening process where feasible. Minimally, IR-1 could share its screening results with other interested genebanks.

As has been pointed out, there are a number of areas of potential cooperation, that could improve the overall germplasm preservation effort in potatoes. Genebanks should be encouraged to cooperate as much as possible in these joint endeavors within the limits and constraints permitted by those they serve and by their funding. Minimally this could simply take the form of better communication and exchange of information among genebanks. It could also take the form of direct assistance with normal project activities when a genebank becomes overwhelmed, such as in the area of maintenance of seed or clonal accessions. Much of this cooperation would seem to be able to be done within existing resources. More extensive collaboration, such as maintenace of accessions, collection, etc. could require the transfer of funds to genebanks willing to cooperate, but with budget restrictions which prevent such cooperation from occurring. Better cooperation would seen to serve both national and international interests and should be fostered whenever and wherever possible.

INTER-GENE BANK COOPERATION - THE GERMAN-DUTCH CONNECTION

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1. Introduction

The 150 - 200 known wild and cultivated tuber bearing species of Solanum, many of which are inter-fertile, form a common gene pool of importance to the cultivated potato.
Considerable progress has been made in clarifying taxonomic relationships within the genus, notably through the work of Ochoa, Hawkes and others. Evaluation of genetic collections is progressing at various centres. The possibilities of gene transfer between species through conventional breeding and by utilizing recombinant DNA techniques are becoming better understood. All these developments highlight the need to conserve genetic variations available within the genus.

In the past few years genetic conservation has become an issue of intensive debate also in a wider context; this concern with genetic resources is perhaps unfortunately linked with propietary rights and base materials for plant breeding (FAO, 1983).

It is suggested that all countries where potato is grown as a major crop share a common responsibility to safeguard its genetic resources. This can only be achieved by international cooperation. The central role CIP should and is playing is clearly expressed in its PROFILE 1970 - 1995, which states that by 1990 it will concentrate on research to include maintenance and utilization of the world collection and the distribution of material resulting from research with the collection. Additional activities are necessary by genebanks in the various centres of diversity and genebanks in major production areas.
This paper attempts to define the possible role of the German-Dutch Potato Collection in the wider context of genetic conservation.

2. The present structure of the German-Dutch Potato Collection

The original objective of the German-Dutch cooperation was to establish a wide collection of tuber bearing species of Solanum for the purpose of plant breeding and research. Hence the objective was not genetic conservation per sé.

At present the collection consists of 2 537 accessions; 1 773 accessions of 90 wild species, 686 accessions of 6 primitive cultivated species and 78 accessions of hybrids and unclassified material.

The reason why species are included and the number of accessions per species is partly determined by their known or presumed interest to plant breeding. However, random factors have played a major role as well; regarding the material from the starting collections (EBS, WAC) and material obtained from other collections and expeditions.

For instance, the more common species such as S. acaule, S. chacoense, S. gourlayi, S. infundibuliforme, S. megistacrolobum, S. microdontum and S. spegazzinii are over-represented and on the other hand many Peruvian species and species from the Northern Centre of diversity (Central-Southern Mexico) are underrepresented.

3. Present constraints and future needs

The German-Dutch Potato Collection has reached a point where decisions have to be made on how to proceed. Obviously such decisions should be guided by the following factors:

1. Working collections needed for research and plant breeding.
2. The role assigned to the German-Dutch Potato Collection in overall genetic conservation and research.

A major constraint for the actual size of the collection is obviously maintenance. Regeneration and availability of the collection is complicated by quarantine requirements.
To realize a collection that satisfies these requirements is both time consuming and expensive. A second complicating factor is that for some species like S. oplocense, andigena, pinnatisectum the climatic conditions in Braunschweig appear not suitable for flowering and/or seed setting.

3.1. Collection needs of the German-Dutch Potato Collection for research and plant breeding

For research and plant breeding the German-Dutch Collection aims at assembling a "representative" sample of most if not all species of tuber bearing Solanums. Representative in this context means that accessions within species should represent broadly the total genetic variation present in the particular species. Ideally it should be based on knowledge of area of distribution and environmental characterization (a-biotic and biotic stress factors) of habitats in which the species are found. It presumes extensive eco-geographical data which often are not yet available. This covers the original objectives of the collection. In the present situation it would mean that a considerable number of new species should be added to the collection while from others the number of accessions could be reduced and in some cases other accessions added.

3.2. The role of the German-Dutch Potato Collection in genetic conservation

As stated before, countries where potato is a major crop should in one way or another contribute to the overall conservation of its genetic variation. Ex-situ conservation is primarily determined by the threat of genetic erosion and extinction. This means generally a much more extensive collection than is necessary for research or on-going breeding programmes. From the work of notably Ochoa the need for genetic conservation activities is beyond debate. However, the main question remains as to how this should be done and what role various participants should play.

This issue has been addressed in previous CIP planning conferences in 1976 and 1979. The 1976 conference led to a proposal for "A cooperative system for the rejuvenation and interchange of botanical seed of wild species and primitive cultivated forms of potato" (June 1977).

Organization and procedures were spelled out in some detail. Important roles were assigned to the U.S. Potato Collection at Sturgeon Bay and the German--Dutch Collection at Braunschweig base collection for responsibility of wild species from South America and respectively wild species from the Northern Centre of diversity (Mexico and Central America). However, for various reasons, neither genebank was in the position to accept such responsibilities at that time, and apparantly no specific action was taken.

For the German-Dutch Collection a major constraint is regeneration due to stringent quarantine regulations. This will continue to put a limit to the number of accessions that can be handled at a level well below the numbers required for overall conservation.
The necessary conclusion is, that collections of Potato germplasm for genetic conservation are better maintained in or near their centres of origin, in casu South- and Central America.

A possible role of the German-Dutch Collection could be to contribute to overall research, assist in strategically generating disease free stock of the various species, contribute to evaluation and documentation, and store duplicate collections.

4. Some views on the overall issue of Genetic Conservation

With some modifications the 1977 proposals (June 1977) would in our view still seem relevant today and might merit reconsideration. Since that period there is a growing realisation that genetic conservation is urgent and requires international cooperation. In a broader context the problems of genetic conservation are being addressed by the FAO (1983), in a continuous manner by the IBPGR and by a study recently started by the National Academy of Science in the USA (global management of genetic resources). In these discussions some general trends are becoming apparent:

- the important role of countries in centres of diversity (primarily in the tropics and the sub-tropics)
- the need to realize a form of international funding to enable selected gene banks (both national gene banks and gene banks of international institutes of the CGIAR) to develop and execute adequate programmes.

The FAO discussion has, perhaps unfortunately, infused a political element in the debate linked to proprietary rights and control over natural resources. However, this must now probably be taken into account further complicating an already complicated problem.

It might be worthwhile for the present planning conference to contribute so the overall discussion by analysing the situation in potato. Available options should be worked out.

This would entail:

- Defining the role of CIP providing overall leadership and its material contribution.
- Identifying national gene banks in various regions of diversity, their possible tasks and appropriate facilities and budgets requirements.
- Defining role and functions of gene banks outside regions of diversity (notably in Europe and North America).

While one cannot as yet be too optimistic about the realisation of such structural international financial arrangements, proposals could help in obtaining a clearer focus on inter-gene bank cooperation. Furthermore, it might provide specifically the FAO commission on Genetic Resources some clearer insight in workable arrangements.

5. Documentation

A matter requiring immediate attention would seem to improve the availability of information of existing collections. Some gene banks have published Index Semina, however others have not. The establishment of a World Potato Data Base (WPDB) would seem both possible and necessary - and could be considered by the Planning Conference. The WPDB should in first instance start with entering available passport data (Phase I) followed by available evaluation data (Phase II).
Various options would seem to be possible

a) An overall WPDB at CIP

To ease this enourmous task an alternative could be

b) - WPDB for primitive forms of potato at CIP
   - A division of labour for a WPDB for wild species between IR-1 programme at Sturgeon Bay and the German-Dutch Collection at Braunschweig

c) - A separate WPDB for potato varieties (S. tuberosum subsp. tuberosum) could be considered. Progress is being made with a European Data Base for Potato Varieties (Kehoe, 1986), probably at Braunschweig.

6. Evaluation

Potato has become a world wide crop grown in the tropics, sub-tropics and temperate zone. Each environment has to some extent specific requirements for evaluation. The German-Dutch Potato Collection cooperates in evaluation with specialised institutes in the Federal Republic of Germany and the Netherlands with an emphasis on diseases (e. g. nematodes, late blight, wart resistance etc.). Data obtained are published from time to time. However, the overall flow of information from evaluation programmes of the various gene banks could be much improved. The Planning Conference could consider ways of doing this. In some crops (i. e. tomato, rice) this is for instance done by the publication of Genetic Resources Newsletters.

As a follow up, evaluation data need to be entered in appropriate data base systems. CIP is well placed to take a lead in such activities which probably would need considerable strengthening of its Data-Information-Section.

Concluding remarks

An attempt is made in the present paper to review genetic resources work in potato from the point of view of the German-Dutch cooperation.
The views are based on internal discussions. In many instances it states what we consider desirable now and in the future rather than what can be achieved with the organisational resources presently available. Ultimately we share a common problem; how to make a meaningful contribution to the overall conservation and utilization of genetic resources of potato and how to secure the necessary funding. This undoubtedly will take time. However, a first step must be clear objectives and goals based on a realistic evaluation of what can be achieved.

SEED CONSERVATION OF POTATO GENETIC RESOURCES- IBPGR STANDARDS - THEORETICAL IDEALS AND PRACTICAL REALITY

Miguel Holle

Introduction

Sexual potato seed is classified as orthodox seed. This means that the seed moisture content can be reduced (2% in potato)(19), that seed can be stored under cold temperatures and that it will survive then for long periods. In countries of the temperate region, sexual potato seed will survive for 10-20 years under room laboratory conditions.

In the present paper, data on the process of the potato sexual seed from production to long term storage is examined. Three sources of data are used: one, the published literature; two, the answers provided by curators who handle sexual potato seed, to a questionnaire sent by the author; and three, the IBPGR Seed Advisory Committee Reports (5, 6, 7).

Fruit ripening and initial seed cleaning

Fruits (berries) are normally harvested going one time thru the field thus age of fruit is variable. The relation with seed age has not been carefully ascertained. Curators estimate age of ripe fruit between 6 to 9 weeks after pollination with a range of 4 to 12. Terms such as "botanically ripe" and "quite soft" are also used to define moment of harvest for obtaining botanical seed. Soft berries releasing strong aroma may be a good indication of ripening. In the case of seed for germplasm purposes, the practice of sib mating will tend to produce a fairly uniform age of fruit and seed in any one accession, since the pollinations per accessions will be done at one time. Post harvest fruit ripening is practiced by taking 2-10 week old berries and holding them for 1 to 8 weeks at 4 degrees centigrade.

An experiment where seed from green plus 4 weeks of after ripening and nearly-ripe fruit were germinated, showed:

Fruit stage at harvest	Time to full ripe (weeks)	% Germination
Green	4 weeks at 20°C	80-90
Nearly ripe	1 week	20-60

Thus, one has to avoid nearly ripe fruit (for example berries, 5 to 7 weeks after pollination) (22).

Over-ripe fruits are not too influential on seed viability. While fermentation of seed from mature berries for 96 hours at 30°C and a 10% clorox solution for 10 minutes gave seed of better germination rate than the nonfermented control. Fruits matured above 30°C produced seed with poor germination. Still precise information on the effect of duration and environmental conditions on fruit ripening to optimize seed storage is lacking (18).

Seed is extracted into water, some genebanks use a blender; others squeeze the seed into a sieve. The washing is repeated so that light seed and debris float and can be thrown out, while heavy seed stays on the bottom and is kept.

The majority of the genebanks **do not** treat the seed before storage. Only four special treatments are mentioned: 1. Using water at 38°C while extracting seed from berries; 2. Adding sodium hypoclorite to desinfect; 3. Treating with 10% hydrocloric acid for 15 minutes before washing; and 4. Immersing into a Germisan 2% water solution for 3 minutes .

Drying potato seed

Half of the genebanks reported to be drying under room conditions (5-25°C and 20-29 percent relative humidity). Special conditions that are used to obtain dry seed are: 23°C and 60% relative humidity for 4 to 10 weeks; air dried over potassium salt; or 20% relative humidity over silica gel for 10 days. These cases result in 5-6.9% moisture. For drying under room conditions resulting seed moisture contents were not reported.

Toasted polished rice with less than 1% moisture content has been tried as a dessicant for potato seed stored in closed jars. Using 30 grams of rice for 20 grams of potato seed, an initial moisture content of

11.5% was reduced to 4 to 5% (19). This is considered a safe level of storage. Germination was not affected when silica gel was used at 20°C for one day to attain 5% seed moisture; or for 7 days to attain 2.5% (1). A negative effect on viability is present at 14% moisture after 28 weeks, and at 7% or less no problem is encountered.

The general IBPGR preferred standard for seed moisture content is 3-7%. Initial seed moisture percentages are reported as 5, 7, 10, and 12%. This parameter is essential for the interpretation of seed viability in storage. It seems that the main drawback to determine percent moisture with the oven dry method (14 hrs. at 103°C) is that 4 grams of seeds are needed. An appropriate method non estimative or with much less seed wastage is worth defining for potato.

Conditions of the store rooms where botanical seed of potato is stored

Three types of storage enclosures are reported:

1) Open or air conditioned rooms are used in 6 cases. Temperatures range from 15 to 25°C, with one location reporting 8-10°C. Relative humidities fluctuate from 40 to 70%.

2) Cold rooms are common. Fourteen genebanks favor this type of storage. The temperature ranges from -0°C to 8°C while the relative humidity is between 10 and 45%.

3) Freezers are present in 6 genebanks. Three are major ones. Temperatures range from -10 to -21°C.

A su[illegible] of the ranges of the conditions reported is shown here compared w[illegible] IBPGR Advisory Seed Committe (7) suggestions:

Type of Room	Number	Temperature Range	Relative Humidity
Air conditioned	6	15 to 25°C	40 - 70%
Cold	14	-1 to 8°C	10 - 45%
Freezers	6	-10 to -21°C	-
IBPGR Normal	-	0°C or less	Hermetically sealed containers
IBPGR Preferred	--	-18°C or less	"

In general, there was no preference for seed packet material. When paper is used it is left open, while laminated foil packets are heat sealed. Several genebanks use laminated aluminum foil, metal, or glass containers. A few, simply use dessicators. Although moisture equilibrium of the seed with the storage environment is an important parameter, curators do not define them if packets are not hermetically closed. In a storage at 4°C and 30% relative humidity, sexual seed of potato will attain a hygroscopic equilibrium of 6.3% moisture content (1).

Experiences at the National Seed Storage Laboratory, Fort Collins, Colorado, USA showed that potato sexual seed can be preserved at -196°C for base collections when adequately dried. It will also survive liquid nitrogen exposure even after repeated freeze-thaw treatments (21).

Potato seed quality in storage

Botanical seed of potato shows different degrees of dormancy depending on factors such as species, location of production, age of fruit or seed. Soaking the seed for 24 hours in a 1500 ppm Giberellic acid (GA_3) solution has proven successful to overcome dormancy in many cases. Similarly, after ripening seed in storage for up to six months has also been reported to be effective.

In general, the percent germination for fresh seed of cultivated clones is 90-100%, while wild species range from 80-100%. Germination tests are done in petri dishes with seed on water soaked filter paper at 15°C - 20°C with counts at 10-14 days. Lower or higher temperatures inhibit germination. There are important species differences at 25°C and 30°C germination temperatures. Alternate temperatures leave no significant effect on germination (8, 9). Most curators show a drop of viability after 5 years. After 20 years, germination ranges from 50 to 90%. A couple of genebanks report 100% (See tables 1-2; Annex 2).

Flowering and Fruit Set (9, 13, 16, 22)

Flowering is one of the highly differential parameters in tuber-bearing Solanum spp. Obviuosly, this creates difficulties in sexual seed production. For germplasm and plant breeding purposes, treatments such as stolon pruning, grafting, cut stem technique, or the "brick" method can be used to improve flowering and fruit set. It should be appreciated that not all materials may produce seed in a given environment.

Pollen fertility is usually low, especially during early flowering. Viable pollen can be dried with silica gel and stored at -20°C to 5°C for

2 months, and even one year at the lower temperature. Pollen viability can be tested in the laboratory by using the aceto carmine pollen stainability test. Fruit set of 28 to 34% has been obtained with stored pollen. Recent research suggests more than only one pollination will produce heavier seed.

Variation of numbers of flowers per inflorescence in 38 cultivars was between 6 and 26; fruits can range from 4 to 9 and fruit set between 29 and 95%. Seed size ranges from 0.43 g. to 0.7 g. per 1000 seed. Plump seeds should be selected, discarding small and shriveled ones. The latter does not seem to influence germination, but affects seedling size at an early stage. Berry size does not seem to influence seed size as expected, thus selection for quality should involve physical seed parameters (16).

The knowledge of pollen quality, pollination, fertilization and fruit set under field conditions has to be analyzed and optimized. It is possible that more than one site has to be used to regenerate seed of large and variable germplasm collections.

Considerations for Regeneration of Seed Supply for an Accession

It is apparent from the data presented that storage of botanical seed with high germinability is a fairly easy process. This being the case, attention should be given to the genetic nature of the populations being stored.

Important considerations are:

-- What is the number of individuals used to produce the seed?

-- What is the number of fruits per individual that will provide the botanical seed for storage?

-- Ideally, 12000 seeds should make up a representative accession in a heterogeneous species like tuber bearing Solanum spp. (7).

Initial genetic variability of an accession is determined by the number of individuals that participate in the formation of sexual seed as ovule and pollen parents.

In the case of wild species, experienced collectors have given the following parameters:

Situation	No.of plants collected	No. of seeds per berry
Ideal	50	50
Usual	10	100-250
Not Uncommon	1-5	As many as possible

In the case of clones, twenty plants are bulk pollinated assuming that each plant has an equal chance to contribute genes to the next generation. Additionally, clones considered duplicates are put into seed by planting 20 seedlings and producing enough seed for storage in the cold room.

Research has been suggested on the effect of seed production and storage procedures on the genetic composition of the accession. An additional query is: does the progeny from seed of duplicate clones show the most important characteristics of the representative clones kept in the field collection? The reviewer found no published articles on this subject. Regeneration should proceed when the initial germination - which usually was 85 to 95% - drops 15% and thus would read 80 to 85%. Another criterion considers a reduction of 10% germination from the initial figure as the time to rejuvenate seed stocks.

Sib-mating seedlings from open pollinated accessions can result in greatly increased inbreeding, and desirable genotypes will "disappear" although the genes are still present. How important it is to maintain a definite genotype will influence the approach to the regeneration process. If necessary a genotype might have to be preserved from other tissues for this situation.

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ANNEX 1

Basic suggestions of the IBPGR Advisory Committee on Seed Storage (1982, 1984, 1985)

Size of individual accessions	Heterogeneous populations should ideally have 12,000 seeds or a minimum of 4,000. Homogeneous populations should ideally have 4,000 seeds or a minimum of 3,000.
Storage behavior	Orthodox as potato sexual seed; or of various recalcitrant types.
Seed treatments	Should be avoided whenever possible.
Seed drying	Seed moisture should be between 3 to 7% arrived at by drying at 15°C and 15% relative humidity. Measurement of seed moisture should follow ISTA methods, adapted to the usual small samples.
Containers for storage	Hermetically sealed containers made of glass, tin cans, or laminated foil.
Seed Storage Temperature	Acceptable at subzero; preferred at -18°C or less.
Seed viability monitoring	Initially with 400 seeds if available, followed by sequential testing at 5 - 10 years with 200 seeds minimum. Based on this, after 10 years or more, regeneration should proceed if germination is below 85% especially for cultivated varieties. Gibberellic acid (1500 ppm-GA3) application is recommended as dormancy is still present after prolonged storage.
Data management seed storage management	Acceptable: passport and seed management data. Preferred if genebanks hold or are able to locate characterization and evaluation data.

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ANNEX 2. GENEBANK MANAGERS OR LITERATURE REPORT:

Seed Viability under Medium Term Storage (*Solanum* spp. tuber bearing)

Case	Storage Temperature	Seed package	% Seed moisture	Length storage	Percent germination
4	- 18°C	Paper	ND	8-10	12 - 95
9	15 - 25°C	Paper	ND	7- 8	60 - 70
11	8 - 10°C	Paper	ND	5	60
12	20°C ± 5	Paper	ND	5	Variable
13	3 - 4°C	Paper	ND	5	80
21	5 - 45°C	ND	ND	10	70
23	-20 to 20°C	ND	Low	4	All similar
* 1	-10°C	Laminated	5% ± 1	5	75- 100
7	5°C	Paper	10%	5	70
14	5°C	Glass vial	14 - 17%	5	50 - 70

ANNEX 2(cont.). GENEBANK MANAGERS OR LITERATURE REPORT:

Seed Viability under Long Term Storage (*Solanum* spp. Tuber bearing)

Case	Storage temperature	Seed package	% Seed moisture	Length storage	Percent germination
* 2	4°C	Laminated**	ND	20	60
10	5°C	Laminated	ND	20	100
15/16	Room	-	ND	13	Adequate
18	5°C	Sealed tubes	ND	20	75
19	3-4°C	-	ND	20	75
* 5	3°C	Paper	6.9%	20	70-100
* 8	2°C	Laminated	5%	20-25	Good
6	3°C	Laminated	12%	20	50- 90
22	-10°C	-	5-6%	20-30	ND?

* Major genebanks

** Laminated foil, usually aluminum

References: (2, 3, 4, 14, 15, 17, 20, 23)

ANNEX 3.

Institution	Country	Age of fruit weeks	After ripening	Seed extrac.	Seed treat.	Seed drying °C	RH %	Duration days	% Seed Moist.	Seed Packet mater.	Open or closed	°C	RH %	Germi. Initial	Final	Maximum length reported years	Observ.
Grose Luse-witz	DDR	5- 7	No	Blender	No	20-22	40-75	2	ND	cano	sealed	-20	—	80%	80%	8	Open packet no surv
FAL	FRG	4-6	0-8 wks 4°C	Squeeze	No	23	60	4-10 wks	6.0	paper	open	3	30	—	700-100	20	in sml boxes cardb.
L FK	DNM	8-12	No	Blender Rept.wash	No	room	20-50	2- 3	ND	paper	open	15-25	40-50	50-90	60- 70	8	cv vari ation
VK	BULG	Botani-cally ripe		Blender	Yes[-1]	room	60-70	3- 5	ND	paper	open	16-20	60	70-90	ND	ND	
NBPGR	INDIA	9-10	1 wk.	Blender	Yes[-2]	25°C	-10	1- 2	ND	Al foil	ND	5	—	—	100	20	Pack in Dessic.
Hokkaido	Japan	Mature		Blender	No	10-20	ND	2- 3 wk	10	paper	ND	5	—	90	70	5	
TARI	ROC	6- 7	Till soft	Squeeze	Yes[-3]	20	ND	4 wks	ND	paper glass vial	ND	3- 4	55	85	80	5	Stored in case sil.gel
CIP(1) CIP(2)	PER PER	4 6-4	No No	Blender Squeeze	No Yes[-4]	Room 15	60-90 20	ND 10	8-9 5	paper Al foil	open sealed	4 -10	— —	90 80-100	60 70-100	20 5	Dry w/ sil.gel
INIAP	ECU	8	No	Squeeze	Yes[-5]	room	—	2- 3	ND	paper	open	8- 10	70	5	60	5	
ICA	COL	6	No	Blender	No	25	60	2-3 wks	12	Al foil	sealed	3	—	0	50- 90	20	
UACH	CHL	ND	NO	Squeeze	No	24	30	2-3	ND	paper	open	-18	—	3- 96	8 - 95	5	variab. /acces.
AIRCAN	CAN	12	Yet at	Blender	No	room	—	3-5	ND	paper	open	20±5°	—	normal	variab	5	Rm Str.
USDA	USA	4-8	No	Blender	No	airdry w/K+ salt	ND	—	5	Al foil	sealed	2	—	ND	good	20	sibmat.
NN 1(H)	—	Green soft	No	Squeeze	No	Room sil.gel	—	1- 2	ND	Al foil	seladed	—	—	—	—	—	
NN 2(C)	—	Phisio maturi ty	Yes[-6]	Blender	Yes[-7]	25-27	70-80	6-10	14-17	glass	sealed	6	80	30- 40	50- 70	5	

NOTES:

1. Germisan 2% water solution for 3 minutes on seed.
2. Seed treatment with 10% hydrocloric acid for 15 minutes.
3. For easy seed extraction 10% hydrocloric acid for 20-30 minutes.
4. Desinfect seed with sodium hypoclorite.
5. Seed extraction and washing in 38°C on blotter of paper towel.
6. Fruit for after ripening in plastic bags fro 2 weeks until soft.
7. Seed into sodium hypoclorite solution and 1 gram of Arnan in 1 liter of water and Volaton 2% to kill seed insects (moths).
8. Seed when extracted from fruit is left to float and sink. The floating seeds are thrown away.

ANNEX 4

Conditions of storage and sexual seed viability for potato variability in genebanks that are components of the IBPGR Network (1987)

Genebank	Directory Report	Survey Report	% Initial Germination	Years Time	% Indeterminate Germination	Years Time	% Final Germination	Years Time
CIP A	5-6% seed moisture laminated Al foil hermetically sealed ar 0°C and -10°C	5% ± moisture in laminated Al foil hermetically sealed at 0°C and 45% RH or -15°C frostless.	85-100	0	75-100	5	?	20
CIP B	—	Not determined in packages, same as above at 4°C	90	0	80	5	60	20
Long and Medium Term								
Bulgaria BGRO 1	Glass jars at 5-18°C	Not determined. 16-20°C Lab room and 60% RH in paper bags	70- 90	0.5	—	—	—	—
Long Term								
Chile CHLO3	Plastic bags at -18°C	Seed moisture not determined in open paper bags at -18°C	54- 96	3-4	8- 92	5-7	12- 95	8-10
Poland POLO2	Glass jars at -1°C	—	—	—	—	—	—	—
FAL/FDR GNPG	Sealed cans at -10°C	paper bags of seed with 6.9% moisture in cold room at 3°C and 30% RH	100	0	80-100	5	70-100	20
Colombia ICA COLO2	Laminated Al foil hermetically sealed at 3°c	12% seed moisture in laminated Al foil hermetically sealed at 3°C	0	0	100	5	50- 90	20
Japan JPN08	Plastic at 5°C	10% Seed moisture in paper bags plastic cans with silica gel	90+	0.5	70	5	?	-
Japan JPN1	Plastic at 0-5°C	—	—	—	—	—	—	—
USDA-Fort Collins		5% seed moisture in laminated foil hermetically used at 2°C		Not specified				20-35

REPORT ON RECENT POTATO COLLECTING EXPEDITIONS

Robert E. Hanneman

The Inter-Regional Potato Introduction Project's (IR-1) Technical Committee has given high priority to collecting wild potato species. Funding has been sought from the USDA, Agricultural Research Service to support IR-1 collecting activities. IR-1 has been fortunate to have been granted support for collecting expeditions to the southwestern part of the United States (Arizona and New Mexico) (1978), Argentina (1983), Boliva (1986, 1987), and Mexico (1982-84). Each of the foreign expeditions has been a joint effort supported in part by all parties. It is IR-1's belief that the sharing of effort in terms of paying salaries, travel, supplying vehicles, equipment, and personnel is crucial to the overall success of the mission. In this way, all parties have had a part in an important effort and can lay claim to and can be proud of what they have accomplished. Materials are shared equally among the participant's institutions, unless agreed to otherwise by all parties.

In August of 1978, Dr. D. A. Ugent of Southern Illinois University, Carbondale, Illinois, led an expedition to Arizona

and New Mexico to search for Solanum fendleri and S. jamesii. He was accompanied by Mr. R. W. Ruhde, USDA, Agricultural Research Service, Madison, Wisconsin. This expedition lasted approximately three weeks and resulted in 14 S. fendleri and six S. jamesii accessions being collected. They noted that they were unable to find species in several of the localities where 20 years earlier, Dr. J. G. Hawkes had reported finding them. The most significant threats noted to the species were over-grazing, encroachment by civilization, and deforestation. The most promising remaining area appeared to be the White Mountains of New Mexico.

Funding for collecting in Mexico was granted by the USDA, Agricultural Research Service in 1982 and again in 1983 and 1984. A joint Canada-Mexico-U.S. expedition began on September 13 and lasted until November 20, 1982. Participants were Dr. T. R. Tarn, Agriculture Canada, Fredricton, New Brunswick, Canada who served as the leader, Mr. A. Rivera-Pena who served as the Mexican representative, and Mr. R. W. Ross, the IR-1 representative. This expedition was intended to survey the country to lay the base for future collecting by identifying important new areas and by confirming the importance of areas identified by previous expeditions as worthy of further collection. In light of the survey nature of this expedition and the country-wide drought, only 52 accessions

were collected, but a wealth of information was garnered for planning future expeditions.

In 1983, another expedition to Mexico was undertaken - a joint Canada-Denmark-Mexico-U.S. expedition. This time, A. Rivera-Pena was replaced by J. Gomez as the Mexican representative, and Dr. J. P. Hjerting, a well known Solanum taxonomist from Copenhagen University, Copenhagen, Denmark, joined the expedition. The expedition began on August 15 and ended November 8, 1983. One hundred and forty-five collections were made in what proved to be a good season. Collections were made in the states of Hidalgo, Veracruz, Queretaro, Nuevo Leon, Jalisco, Durango, Guerrero, San Luis Potosi, Coahuila, Oaxaca, Puebla, Zacatecas, and Mexico. The most serious observed threats to wild species were over-grazing by goats and other domestic animals, deforestation, erosion, and expanding modern agriculture.

In 1984, a second Canada-Denmark-Mexico-U.S. expedition was fielded, lasting from September 11 through November 13. The same personnel participated as in 1983. One hundred and seven collections were made. Collecting concentrated again in less explored areas. The states collected in were Mexico, Aquascalientes, Zacatecas, San Luis Potosi, Jalisco, Coahuila, Hildalgo, Puebla, Tlaxcala, Nayarit, Oaxaca, Chiapas, Veracruz, and Queretaro. Again, the same threats due to over-grazing, deforestation and modern agriculture were noted.

In 1983, Dr. K. A. Okada, Instituto Nacional Technologia Agropecuaria (INTA), Balcarce, Argentina, agreed to lead an expedition to the mountainous areas of the provinces of Salta and Jujuy in northwest Argentina. Participants were Mr. O. A. Garcia, and Miss A. M. Clausen from INTA, Balcarce, Argentina and Miss S. M. Saufferer, USDA, Agricultural Research Service, Beltsville, Maryland. The expedition began January 15 and ended May 10, 1983 and resulted in the collection of 217 accessions. It was supported in part by the International Board for Plant Genetic Resources (IBPGR) as well as by the USDA, ARS and INTA. A severe drought occurred in a large portion of the area, making it difficult to collect berries. Again, threats to the species were noted from over-grazing and from a decline in agriculture which eventually would lead to a reduction in native varieties as well as wild species.

In 1986, funding was obtained for collecting in Bolivia. The intent of this joint Argentina-Bolivia-U.S. expedition, was to go to areas unexplored by previous expeditions or which had been noted by them to be worthy of more detailed exploration. This expedition was undertaken on February 22, and lasted until April 12, 1986. The participants were Dr. R. W. Hoopes, Cornell University, Ithaca, New York, who served as leader of the expedition, Dr. K. A. Okada, Instituto National Technologia Agropecuaria (INTA), Balcarce, Argentina, who served as the expedition taxonomist, and Mr. I. Aviles, who

served as the Bolivian representative. Collecting was done over a wide area of Bolivia, but access to many areas was limited due to unusually heavy rains making roads and travel difficult. Nevertheless, 116 accessions were collected. Mr. Ruben Trujillo served as the driver/mechanic whose services were crucial to the success of the expedition, and whose fluency in Quechua proved extremely valuable in communicating with local people in more remote regions.

Funding for 1987 has been approved by the USDA, Agricultural Research Service for collecting in Bolivia. The participants will be the same as in 1986 with the exception of a possible replacement for Mr. Aviles, who has taken a new job and may not be able to participate.

All of the expeditions have proven to be very valuable by adding new germplasm, by expanding the known range of several species, by rediscovery of several species only known by herbarium specimens or from the literature, and by the discovery of several potentially new species. To this end, we are grateful to those who so generously gave of their time and endured hardship to provide the potato community with additional accessions of a broader range of known and new species. Their dedication begins the lengthy work of putting these materials into a more useful form to serve agriculture around the world. It is to these collectors that we owe much. We thank the governments, institutions and personnel who have shared so generously and worked so cooperatively to further these efforts.

THE UTILIZATION OF WILD POTATO SPECIES IN BREEDING

Peter Schmiediche

1. Introduction

When we talk about the utilization of wild potato species in breeding today in 1987, we cannot help but think of the many contributions made by several generations of scientists in this field. Hawkes (1958) wrote a review paper about the whole issue of the utilization of wild potato species almost thirty years ago. In this paper he distinguished three phases in the utilization of wild species in the history of potato breeding. The first phase started in about 1824 and probably reached its peak by the middle of the last century, when as a result of the catastrophic late blight epidemics in Europe, particularly, however, in Ireland, the introduction of "new blood" into the then existing (and through the epidemics greatly narrowed down) genepool of cultivated potatoes was advocated by European and North American botanists. This "rejuvenation" of the cultivated germplasm was to be achieved through the utilization of newly discovered wild species from South America. Unfortunately, however, the species scientists tried to utilize then were mainly triploid such as Solanum **maglia** Schlechtd. from Chile or triploid cytotypes of the species S. **commersonii** Dun. from Brazil/Paraguay. At that time the very concept of ploidy had not yet been discovered, and it is obvious now why these particular attempts of utilization failed. Some experiments independently carried out by Klotzsch (1852) and Lindley (1848) at about the same time with S. **demissum** Lindl. were much more successful but of no real consequence then. S. **demissum** became important in potato breeding only much later in the first quarter of the 20th century.

The lack of taxonomic work in the Genus of tuber-bearing Solanum was another hindrance for the utilization of wild species. With the re-discovery of Mendel's work at the turn of the century and with the highly fruitful work of the taxonomist Georg Bitter (1912), the foundation for the scientific utilization of wild species in potato breeding had been laid. One of the first geneticists to make use of the newly discovered genetic principles in potato breeding was Salaman. Hawkes (1958) considers the work of Salaman along with the work of Bitter and a little later Müller in Germany and Reddick in the United States the beginning of the second phase of the utilization of wild species in potato breeding. The most successful accomplishment during that period was the extensive utilization of the Mexican hexaploid species S. **demissum** as a source of resistance to late blight.

The fact that S. demissum was later discovered to possess "only" major genes against the late blight fungus and that major genes have the tendency to be overcome by new races of the pathogen does in no way obscure or invalidate the tremendous contribution of these early breeders. In fact, let it be clearly stated, although minor gene resistance is considered preferable and superior today, we still rely largely on major gene resistance in practice, and, moreover, S. demissum is now thought to also have contributed a good measure of minor genes and thus horizontal resistance to potato breeding.

The third phase of the utilization of wild species began with the publication of the Russian discoveries of the polyploid series of cultivated potatoes as well as of a tremendously large and variable wild genepool from 1927 onwards. The scientific evaluation of the Russian and later also British, American and German collections revealed such characteristics as frost-resistance in S. acaule Bitt. (Bukasov, 1933), resistance to the potato cyst nematode in S. vernei Bitt. et Wittm. (Ellenby, 1948) or immunity to virus X in S. acaule (Ross, 1954) to mention just a few. All of these species were in one way or another utilized in potato breeding. By 1976, about 200 of the approximately 600 varieties listed in the "Index of European Potato Varieties" (Stegemann and Loeschke, 1976) had wild species in their pedigree. However only 6 of a total of about 200 wild species had been utilized in European breeding by that time.

With the foundation of CIP and a new interest in genetic resources in general and potato genetic resources in particular, the fourth phase in the utilization of wild species in potato breeding might be stated to have begun.

2. The utilization of wild Mexican potato species.

The Mexican hexaploid wild potato species S. demissum was the first wild potato species ever to be successfully utilized in modern potato breeding. More than 200 of the 600 European potato varieties registered in one or the other European country in 1976 had S. demissum in their ancestry (Stegemann and Loeschke, 1976). As has been mentioned before, S. demissum was chiefly used for its valuable late blight resistance, and there are indications (Schmiediche, unpublished) that European varieties with S. demissum in their pedigree might have a measure of other resistances (possibly to Pseudomonas solanacearum) as well.

2.1. Research on the utilization of S. bulbocastanum Dun.

The Mexican diploid species S. bulbocastanum was found to be strongly resistant to late blight more than 20 years ago,

and Hermsen (1966) started to work on the utilization of this species in the early sixties in order to make its late blight resistance available to breeding. Through the use of two bridging species and through persistantly high numbers of pollinations (in one case 20.000), germplasm of S. bulbocastanum was finally combined with that of tetraploid potato cultivars, and some of the hybrids retained the original resistance of S. bulbocastanum (Hermsen et al., 1973). This was achieved alone with conventional crossing and breeding methods and showed clearly that even the traditionally difficult to cross Mexican diploid wild potato species can be successfully utilized in modern potato breeding. These double-bridge hybrids have meanwhile been successfully utilized in CIP's breeding program for resistance to late blight.

Other Mexican species such as S. stoloniferum, S. verrucosum, or S. pinnatisectum have on various occasions been utilized for their resistances or as bridging species. S. stoloniferum has been extensively used in Europe for its PVY resistance, S. verrucosum has been a useful bridging species in crosses of S. etuberosum x S. pinnatisectum hybrids with cultivated potatoes (Hermsen et al., 1981; Chavez, 1984), and S. pinnatisectum has also been utilized as a bridging species in tapping the PLRV resistance of non tuber-bearing S. etuberosum (Hermsen and Taylor, 1977).

3. The utilization of wild South American potato species.

3.1. Work on wild ancestors of cultivated potatoes.

The whole concept of the utilization of wild genetic resources received a decisive boost in the mid-seventies when CIP sponsored three research projects in the form of Ph.D. theses in this area. These three research projects were essentially biosystematic in nature as each one elucidated the origin of one or two of the eight cultivated species. Three of the cultivated species, S. ajanhuiri (Huamán, 1975) and S. juzepczukii and S. curtilobum (Schmiediche, 1977) were confirmed or found to have at least one wild ancestor. Huamán and Schmiediche showed clearly that it was now possible to re-synthesize and to improve the existing varieties of these three cultivated species by going back to their wild and cultivated ancestors. The idea of breeding and evaluation for desired characteristics within the wild genepool before combining wild genotypes with cultivated ones was a clear departure from commonly accepted principles of the utilization of wild species. Traditionally, genotypes of wild species with the desired characteristics would be cros-

sed to the cultivated tetraploid cultivar which was lacking these characteristics, and in a series of seven to eight backcrosses, the original cultivar was recovered as nearly as was possible.

This research on the origin of four cultivated species - the fourth one was S. chaucha (Jackson, 1975) which has, however, no immediate wild ancestors - essentially confirmed what had been thought to be possible, on taxonomic grounds. The confirmation of the putative ancestors of several cultivated potato species made it now possible to utilize the wild progenitors of these particular cultivated species in a major way, should this become necessary.

3.2. Research on inter-specific crossability

Along with the bio-systematic work, which elucidated the origin of several cultivated potato species, large numbers of inter-specific and inter-series crosses were tried in the collection of wild species at CIP. The information that has accumulated about inter-species and inter-series crossability was slowly gathered over many years and is now available for the large-scale utilization of wild potato germplasm whenever necessary. It should be noted that in one way or another, in many cases, however, through the use of bridging species, every crossability barrier that was encountered was also, without exception, overcome.

3.3. Wide crosses

The utilization of the immediate wild ancestors of cultivated potatoes was shown to be relatively easy. However, wide crosses involving different taxonomic series or distantly related species within the same series are of a more complex nature. Direct hybridizations between many wild species or between wild species and cultivated tetraploid potatoes have, in many cases, turned out to be very difficult or impossible. Of all the taxonomic series of tuber-bearing Solanum, the series ETUBEROSA, is biosystematically farthest removed from cultivated and most wild potato species. Although this series has, on morphological grounds, been included with the tuber-bearing Solanum, it does not even contain any tuber-bearing species.

When many years ago the species S. etuberosa was discovered to be highly resistant or even immune to the Potato Leaf Roll Virus (PLRV), it used to be thought that only with the advance of the protoplast fusion technique would it ever be possible to utilize this species in resistance breeding (Hawkes, pers. comm.) as natural hybridization with cultivated potatoes appeared to be out of the question.

However, a long series of hybridization attempts, crossing S. etuberosa with a large number of other wild species showed that this Chilean species can be crossed with the Mexican species S. pinnatisectum at a very low rate of success (Hermsen and Taylor, 1977). The diploid F_1 moreover was completely sterile as there was no apparent homology between any of the chromosomes from the two species. Duplication of the chromosome number resulted in a highly fertile new and man-made allo-tetraploid potato species which became known as EP.

The discovery of the principles of the endosperm balance number (EBN) by Johnston et al.(1980) made it possible to determine that both species, S. etuberosa as well as S. pinnatisectum, had each only one EBN which was almost certainly responsible for the fact that the two species, which are completely isolated geographically, would have crossed. Although the EBN hypothesis has not been accepted by all researchers working with wild potato species, there is mounting evidence for its correctness.

The tetraploid EP was, in turn, successfully crossed with the Mexican species S. verrucosum (Hermsen et al., 1981) and the Peruvian species S. acaule (Chavez, 1984). The resulting hybrids became known as VEP and EPA respectively. VEP was triploid, and its chromosome number had to be doubled for further crosses, however, eventually both, EPA and VEP, were crossed with genotypes from the cultivated diploid species S. phureja. The hybrids of the latter set of crosses retained some of the original resistance to PLRV, and these S. phureja x EPA/VEP hybrids were further crossed with tetraploid cultivated potatoes.

Even if it had now become possible to obtain hybrids from very wide crosses indeed, it was not possible at each stage of crossing to grow the hybrid seeds conventionally. However, in vitro culture of hybrid embryos was successfully used to overcome strong germination problems as well as the destruction of embryos that was observed in some of the hybrid seeds if embryo and endosperm were not separated in time (Chavez, 1984; Spack et al., 1987).

This is the most successful example of the utilization of a wild species that is biosystematically far removed from the cultivated potato. In fact, until now no species that is farther removed from the cultivated potato has been utilized in breeding. The production of a potato-tomato hybrid by protoplast fusion (Melchers et al., 1978) has so far had no consequences for potato breeding. It is important to point out that the several crossability barriers that were overcome in the utilization of S. etuberosum were overcome with the use of traditional breeding techniques (bridging species) in combination with modern in vitro methods (embryo culture).

3.4. The utilization of wild species in breeding for resistance to **Pseudomonas solanacearum.**

Pseudomonas solanacearum exists in a variety of races and specific strains with varying degrees of pathogenicity. The system of classification itself is an expression on the complex nature of this pathogen. Whether a given strain of the pathogen will be more or less virulent depends largely on environmental conditions, mainly temperature and soil humidity or the degree of salinity of a given soil. In saline soils on the Peruvian coast, for instance, the bacterial wilt pathogen has never established itself. Interaction of Pseudomonas solanacearum with other pathogens, such as root-knot nematodes (**Meloidogyne** spp.) is well documented (Jatala et al., 1975; Jatala and Martin, 1977a; 1977b).

The extreme specificity of the pathogen-host-environment-interaction has so far prevented the breeding of genotypes with general resistance under all conditions. Even if inoculum samples were taken from infected plants in a specific country or region and brought to Peru in order to screen genetic material for those places at CIP, the problems caused by the specificity of the pathogen-host-environment-interactions could not be solved.

Preliminary evidence obtained in CIP's breeding program for resistance to bacterial wilt indicates that the genetic base for resistance is different in each species that has resistant genotypes. The resistance allels in one species are almost certainly different from those in another species although in each case these allels determine resistance to the same pathogen. Recent evidence obtained in work with wild species suggests that contrary to the major gene inheritance observed in S. phureja (although the exact number of genes involved is not known), the resistance found in wild species is probably polygenically determined.

Considering the complex variability and interactions of the bacterial wilt pathogen it was decided to respond with a breeding program that would develop maximum genetic variability for the trait "resistance to bacterial wilt" into one genetic population. This would equip the population with a type of broad spectrum resistance that would meet any serious challenge of infection in the field. This goal was to be achieved by combining various sources of specific resistance into one genetic population (Schmiediche and Martin, 1982). The idea of breeding a population with this type of broad spectrum resistance is conceptually akin to the principle of breeding genetic populations with field or

horizontal resistance to **Phytophthora infestans**. This type of broad-spectrum resistance was to be combined with earliness and adaptation to heat, as potato cultivation is expanding into hot and humid regions where the bacterial wilt problem is most serious.

3.5. Wild species sources of resistance to bacterial wilt.

In the diploid wild potato species **S. sparsipilum**, **S. chacoense**, and **S. microdontumn** bacterial wilt resistant accessions were identified at CIP about eight years ago. A population combining resistance from these three species as well as from the cultivated diploid species **S. phureja** was assembled and tested against four strains of races 1 and 3 in a two-year screening program. The original population consisted of 11,500 seedlings of which only 40 clones, representing eight families, survived the rigors of screening against four different strains of the wilt pathogen.

In the 1980/1981 growing season these 40 clones were grown in the Andes at an altitude of about 3,200 m, and they were intercrossed in 203 different combinations. More than 100,000 seeds were obtained, and almost 10,000 seeds from 43 families were sown and the seedlings tested against the particularly severe strain 013 of race 3. Of the 7468 seedlings tested, 2,410, or 32.69%, survived. The control DTO-33 was virtually wiped out demonstrating the effectiveness of the inoculum. When the original wild population had been tested against the same pathotype, it showed a similar percentage of survival (about 30%), and the increase on a population basis after one cycle of recurrent selection appeared rather small. However, the increase in individual families was quite dramatic as survival rates of up to 67% were recorded. In two parallel screening programs resistance to the root-knot nematode **Meloidogyne** spp. and against the potato tuber moth (**Phthorimaea operculella**) was also identified in this population.

3.6. A breeding strategy for the utilization of wild bacterial wilt resistant species.

Before starting to utilize the broad spectrum resistance to bacterial wilt present in the MBN population, a strategy had to be developed that would permit quick and efficient transfer of the resistance to the tetraploid level and that would allow quick and efficient combination with commercially acceptable agronomic and quality traits.

Mendiburu et al. (1974), Peloquin (1983), Leue (1983) and Peloquin et al. (1985) have clearly demonstrated that the most efficient way to use diploid wild germplasm is to cross

the selected diploid wild clones with **tuberosum** haploids. This appears to eliminate any wild characteristics in the diploid hybrid population obtained from the wild species x haploid cross.

Consequently, the breeding program would have to be carried out at the diploid level in an attempt to "nobilize" the wild germplasm (Hermsen, 1977) before going to the tetraploid level in a tetraploid x diploid (TD) cross. Such "nobilization" would considerably reduce the necessity for backcrosses to the tetraploid parent, and the probability of obtaining resistant genotypes with good agronomic characteristics in the F_1 following a TD-cross would theoretically be quite realistic.

The notoriously low male-, and often also female-, fertility of **tuberosum** haploids make the use of this material as crossing partner in the "nobilization" of wild germplasm, however, less attractive in practice than in theory. Therefore, cultivated diploid potatoe species were considered in order to achieve "nobilization". Cultivated diploid potatoes are abundantly fertile and easily cross, as a rule, with most wild species from the taxonomic series **Tuberosa** and the series closely related to that series. Moreover, a population of the diploid cultivated species S. **stenotomum**, S. **phureja** and S. **goniocalyx** had been selected for high yield, high dry matter content and good agronomic characteristics and performance in a breeding program at North Carolina State University (NCSU). This most advanced cultivated diploid germplasm was therefore chosen to be hybridized with the bacterial wilt resistant wild material. At this point, it should be pointed out that there is little difference genetically between a **tuberosum** haploid and highly developed cultivated diploid clones except that the haploid carries some of the sophisticated quality and agronomic traits customarily present in **tuberosum** varieties.

Before the NCSU cultivated diploid population was crossed with the bacterial wilt resistant wild population, it also was screened for resistance to bacterial wilt. Bacterial wilt resistance had been observed in that population under field conditions but it had never been systematically tested. Since S. **phureja** was part of that population, the presence of resistance to bacterial wilt was not unexpected. When this population was tested against strain 013 of race 3 some families had resistance levels of 25-30%.

From the resistant wild MBN population, 167 clones representing 42 families were planted and crossed with bulk pollen from the resistant members of the cultivated NCSU population. In early 1983, 10,000 seedlings of the MBN x NCSU hybrid progeny were planted and 500 clones were selected exclusively for agronomic characteristics and tuber shape.

The hybrids, which combined one genome from the wild population and one from the cultivated diploid population, had agronomic traits at least as good as those found in a common Andean cultivated diploid potato. This represented a considerable improvement over the almost pure wild population. This diploid hybrid population was screened against bacterial wilt strain 052 (Biovar III, Race 1) from Taiwan and against strain 204 (Biovar II, Race 3) from Peru. The survivors of this evaluation were crossed in tetraploid x diploid crosses with the most advanced tetraploid germplasm resistant to bacterial wilt. An abundant number of unreduced (2n) gametes were apparently present in the diploid population. The first TD progenies were planted in clean fields on the coast of Peru to get an impression of their agronomic and quality potential. A second set of the same progenies was planted in fields heavily infested with bacterial wilt in the Department of Cajamarca, Peru, where the first S. phureja derived BR clones had been tested 15 year earlier. Genetically this tetraploid population contained four specific sources of resistance, and of the four genomes three came each from a different species, either wild or cultivated. This combination assured a high degree of heterozygosity and a theoretical broad spectrum resistance.

The high degree of heterozygosity resulted in a high yielding, vigorous and most heterotic crop. Family averages of more than 2 kg/plant were the rule, and the highest yielding seedling in the population produced 7.6 kg of tubers. These results were obtained in clean fields on the Peruvian coast. The limitation of this material, its relative lateness, also became apparent in the first growing season. The crop was barely ready for harvest after 126 days which is too late for many tropical sites. The tuber shape of more than 90% of the individual clones was tuberosum-like with shallow eyes. In a field of 1200 seedlings, only one had distinctly wild characteristics.

However, the relative lateness of the material made at least one backcross to early and heat adapted material necessary. Such early and heat adapted populations were already available at CIP. It should be remembered that resistance to bacterial wilt was found to only express itself fully in material well adapted to heat. The excellent agronomic characteristics of the early heat adapted populations resulted in further agronomic improvement of the first backcross generation (bacterial wilt resistant tetraploid population x early heat adapted population). The progenies of this first backcross generation were tested in a large experiment under heat stress conditions in Peru, and about 200 highly promising selections were made. several sets of seed have in the meantime gone to Region VII for field testing under high infection pressure. Early results indicate that a good portion of genotypes of this population are indeed resistant under very adverse conditions (such as are found in Mindanao, Philippines).

As indicated before, one part of the first set of TD progenies was planted under conditions of heavy infection in Peru. In order to conduct a proper Randomized Complete Block experiment with three replications in two sites, the families to be tested were multiplied by cuttings, and the rooted cuttings were transplanted to the field. The two checks for this experiment were Molinera and BR-69-84, which are bacterial wilt resistant clones with resistance derived from S. phureja. Preliminary results are summarized in Table 1. The average yield per plant appears to be rather low, but top yielding clones with 1.8 kg/plant were also found in this population. The low average yield reflects the fact that all individual plants had been raised from stem cuttings. More important, however, was the heavy attack of the bacterial wilt pathogen on the resistant clones Molinera and BR-69-84. The latter clone was virtually eliminated and in Molinera more than half of the plants were lost. The highest loss in the new material (on a family basis) was 24.58%, and the lowest was 6.56%. These preliminary data suggest that development of broad spectrum resistance to bacterial wilt, appears to be a potentially important defensive strategy.

TABLE 1. Average yield and losses through wilting of material with broad spectrum resistance to Pseudomonas solanacearum.

FAMILIES	YIELD/PLANT (Grams)	WILTED PLANTS IN PERCENT
1. 44A (S-51.1 x bulk 052)	468	14.57
2. 15A (S-20.3 x bulk 052)	393	17.15
3. 21A (S-23.1 x bulk 052)	456	24.58
4. 4A (S- 9.2 x bulk 052)	413	10.00
5. 29A (S-26.5 x bulk 052)	378	10.09
6. 14B (S-21 x bulk 204)	416	11.46
7. 22B (S-26 x bulk 204	458	9.23
8. 35B (S-50 x bulk 204	543	12.22
9. 20B (S-25 x bulk 204	457	10.61
10. 10B (S-20 x bulk 204	431	6.56
11. Molinera (check 1)	475	56.99
12. Clone BR-69-84 (check 2)	345	77.2

Test sites: Heavily infested fields in the Department of Cajamarca, Peru, during the 1984-85 growing season.

Experimental design: Randomized Complete Block, two sites, three replications.

Principal collaborator: Ing. W. Martin Eslava L.

3.6. Conclusion

The results obtained so far with wild species suggest that the utilization of new sources of resistance to bacterial wilt to develop a type of broad spectrum resistance will be an important strategy in the genetic control of this disease. Despite the initial success reported with **S. sparsipilum**, **S. chacoense** and **S. microdontum** additional sources of resistance should be investigated. In the last two years, at least four more sources, all of them wild species and possibly more effective than the ones already in use, have been identified.

Combining all of these sources into one resistant super population is not envisaged as experience with the bacterial wilt pathogen have shown that it is wise to develop populations differing in sets and combinations of sources of resistance. Such populations can be used according to the specific needs of the various world regions where the bacterial wilt problem exists. At the moment it appears that breeding programs utilizing wild species or wild species derived parental material may be able to meet the challenge of is pathogen in combination with agronomic measures wherever potato growers are faced with it.

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Strategies for the Conservation of Potato Genetic Resources

A G E N D A

Monday, February 9

08:30 Welcome and opening remarks.
Dr. R.L. Sawyer, Director General.

08:45 Review of Recommendations of October 1979 Conference.
P. Gregory

STATUS OF POTATO GENETIC RESOURCES AVAILABLE IN GENE BANKS

Chairman: H. Mendoza

09:00 Status of the Collection at CIP.

Wild Species - C. Ochoa - P. Schmiediche

09:30 Native Andean cultivars - Z. Huaman

10:00 Coffee

10:30 Status of the IR-1 USDA Potato Collection.
R.E. Hanneman

11:00 Present State of Maintenance and Evaluation of the German-Netherlands Potato Collection.
R. Hoekstra

11:30 The role of international cooperation in the development of national potato collections in Latin America.

a. The case of Argentina - A. Okada

11:50 b. The case of Colombia - L. Lopez

12:30 Lunch

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11:50 b. The case of Colombia - L. Lopez

12:30 Lunch

Monday afternoon

IN VITRO MANAGEMENT OF SELECTED GENOTYPES OF TUBER-BEARING *SOLANUM* RESOURCES

Chairman: P. Schmiediche

14:00 Status of the *in vitro* potato collection at CIP and new approaches for long term conservation.
J.H. Dodds

14:30 Status of *in vitro* collections in other gene banks.

IR-1 USDA. - R.E. Hanneman

14:45 BGRC. - R. Hoekstra

15:00 Discussion - Seed regeneration of duplicate accessions not maintained clonally.

Tuesday, February 10

INTER-GENE BANK COOPERATION FOR POTATO GENETIC RESOURCES CONSERVATION

Chairman: M. Holle

08:15 CIP's Role in the development of an inter-gene bank cooperation for potato genetic resources conservation.
Z. Huaman

08:30 HOW EACH POTATO GENE BANK CAN COLLABORATE IN A JOINT EFFORT TO CONSERVE POTATO GENETIC RESOURCES

Suggestions from the USDA Inter-regional potato gene bank.
R.E. Hanneman

09:00 Inter-Gene Bank Cooperation - The German-Dutch Connection.
R. Hoekstra

09:30	Discussion
10:00	Coffee
10:30	Seed Conservation of Potato Genetic Resources: IBPGR Standards - Theoretical Ideals and Practical Reality. M. Holle
11:00	Discussion
11:30	Report on recent potato collecting expeditions by the IR-1 USDA gene bank. R.E. Hanneman
12:00	Discussion - Inter gene bank collaboration for future collecting expeditions.
12:30	Lunch

Tuesday afternoon

Chairman: R.E. Hanneman

14:00	The utilization of wild potato species in breeding. P. Schmiediche
14:30	Discussion - Determining interests and distributing responsibilities among gene banks for the exploitation of wild species with desirable attributes for breeding.
15:30	Discussion - Duplicating seed stocks for security.
16:00	Discussion - Distribution of genetic materials to breeders.
16:30	Naming a Chairperson to make recommendations.

Wednesday, February 11

07:45	Flight to Huancayo.
10:00	Tour to CIP facilities.
12:00	Lunch

Wednesday afternoon

01:30 Visit to the potato germ plasm collection.

Thursday, February 12

07:45 Flight to Lima.

10:00 Formulation of recommendations.

12:30 Lunch

02:00 Development of a joint proposal for an inter-gene bank cooperation for potato genetic resources conservation.
P. Gregory, Chairman

Friday, February 13

08:30 Summary of recommendations and establishment of research priorities for future activities at CIP.
P. Gregory, Chairman

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