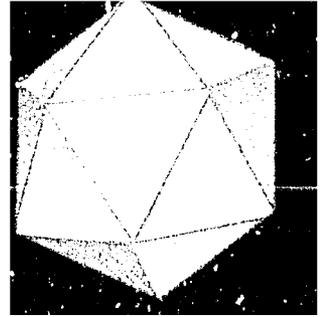


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**Control
of Virus and
Virus-like**

**Diseases of
Potato and
Sweet Potato**

**Report of the
III Planning Conference**

Held at the International Potato Center, November 20-22, 1989

PRESENTATION

At CIP, Thrust IV research is concerned with Control of Virus and Virus-like diseases. It plays a decisive role in the improvement of potato and sweet potato because its objective is to develop control measures for virus diseases. Because of CIP's goal to disseminate improved germplasm, there is a need to develop sensitive methods for detection and identification of previously known viruses as well as those found only in the centers of diversification of the potato and sweet potato. To develop these methods, however, a thorough knowledge of virus pathogenesis and structure is needed. On the other hand, the improvement of germplasm also requires input from virology, for instance, to search for and utilize virus resistance genes, or to free accessions infected with viruses and maintain them free. Due to the broad scope of our mission, virology must use other expertise available at CIP such as in breeding and genetics, physiology, bacteriology, nematology or entomology.

To accomplish our goals, however, we feel that the scientific community should also participate by periodically reviewing our objectives and procedures and by helping us to develop new ideas to improve our work. Therefore, this III Planning Conference had the objective of involving the scientific community to participate in our task with the assurance that the recommendations emerging from the conference will be followed as closely as possible.

It is our intention that these proceedings will serve as a permanent guide of our objectives and procedures until a new conference takes place. Our efforts to produce a document that expresses as much as possible the participants' point of views is a rewarding experience.

L. F. Salazar
Thrust IV Manager

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Objectives of the Planning Conference

P. Gregory¹

I would like to thank the visitors who have come to help us in this Planning Conference. Such conferences represent a very important strategy for timely review and planning of CIP's research activities. A very special aspect of the Conferences, as exemplified here today, is that there is strong participation by the best available experts from developing and developed countries. During this Planning Conference on "The Control of Virus and Virus-like Diseases of Potato and Sweet Potato" our objectives are as follows:

1. to review CIP's potato virology work since last Planning Conference (1980);
2. to review the relatively new activities that have taken place in sweet potato virology since the initial planning in the Sweet Potato Conference held in 1987 and;
3. to develop recommendations that will guide for the next 3-5 years "in house" and collaborative research with National Agriculture Research System (NARS) scientists and others.

In working towards these objectives we must not only think in terms of scientific quality and productivity, but also in terms of how well the work has contributed, or will contribute, to CIP's impact on NARS and the people they serve. In fact, any activity that is unlikely to strengthen CIP's practical, agricultural impact must take a low priority for access to our resources however scientifically interesting it may be. This raises the question of balance in our programs. For example, how much more sensitive do our potato virus and viroid detection methods need to be? As rapid progress is made in the development of more sophisticated molecular probes for detection should we, as a result, be researching more and more sensitive methods or would it be preferable to do other kinds of things in order to serve clients? Resolution of such issues can be greatly helped by the expertise of the people we have here today--visiting experts from developing and developed countries, and CIP staff and management representing our Headquarters' and Regional operations. In achieving our objectives, let us all be as constructively critical as possible. Do not hold back. In my opinion we have an excellent virology program here. It is well appreciated throughout the world for the scientific quality and quantity of its work and for the tremendous practical impact it has had on the NARS. But, even though CIP's virology program is so strong, I hope that all of you can challenge CIP to make it even better. Let's spend the next 3 days doing just that.

¹Director of Research, International Potato Center (CIP), Lima, Peru.

Recommendations of the III Planning Conference on the Control of Virus and Virus-Like Diseases of Potato and Sweet Potato

Germplasm Transfer and Virus Elimination

1. For potato, established mechanisms for potato germplasm transfer, techniques for virus eradication, documentation, and export policies are adequate and should be continued unchanged.
2. Sweet potato germplasm should be transferred (import/export) either as true seed or as in vitro cultures.
3. Sweet potato clonal materials will be exported only as pathogen tested in vitro shipments, except for use in germplasm conservation and for pathogen eradication, with appropriate approval from recipients.
4. Current research should emphasize and result in the identification, detection and control of sexually transmitted seed borne diseases. In the meantime, true sweet potato seeds (TSPS) will be exported only from crosses made from pathogen-tested plants grown under controlled conditions. However, as an exception TSPS could be exported for conservation purpose, with appropriate approval from recipients.
5. Based on research results in item 4 (above), TSPS from parents tested for freedom of pathogen known to be seed borne could be exported. Seed lots will be tested for seed-borne pathogens when reliable testing methods are developed.
6. The documentation for export should include the following phytosanitary options:
 - a. The mother plants tested and found negative to sweet potato feathery-mottle virus (SPFMV), sweet potato mild-mottle virus (SPMMV) and sweet potato latent virus (SPLV) (by serology), and to potato spindle tuber viroid (PSTVd) (by nucleic acid spot hybridization test-NASH). The mother plants also tested negative to the above viruses and other agents infecting the indicator hosts *Ipomoea nil* and *I. setosa*.
 - b. True seed harvested from pathogen-tested parental plants and grown under controlled conditions.
 - c. True seed harvested from mother plants tested and found free of sexually transmitted pathogens. A statistically valid sample of these seeds also tested and found free.

d. Other. (This option will be used to identify material of unknown health status).

Options (a) to (c) above will be redefined and described when the sexually transmitted pathogens are known and testing methods developed.

7. Research should emphasize methods to measure genetic variation in the process of virus eradication, in vitro conservation, and field propagation.
8. While the true benefits of thermotherapy remain questionable, CIP should continue to apply this virus eradication technique. Further research is needed on the improved sensitivity and scope of pathogen detection methods.

Breeding for Virus Resistance

General Recommendations

The development of priorities to introduce resistance to viruses into potato and sweet potato should be based on the overall mandate of CIP to improve the sustainability of potato and sweet potato.

Specific Recommendations

Potato

1. The important viruses/viroids of potato are PLRV, PVY, PVX, PVS, PVM, PVA, PVT, SB-22 and PSTVd.
2. The current program to incorporate resistance to PLRV in a background of PVY and PVX immunity should be continued.
3. The significance of PVS should be determined, particularly with regard to the breaking down of late blight resistance. Breeding efforts for resistance to PVS should be established, if the interaction is confirmed.
4. A policy statement should be developed to facilitate the development of resistance to viruses of local importance such as PVM.
5. PVT, SB-22 and PSTVd should be dealt with as quarantine problems, rather than as breeding problems.
6. Bioengineering will be of greatest benefit in introducing single-gene traits (X,Y) to widely adapted cultivars. The contribution of bioengineering will be to introduce traits to existing genotypes where conventional breeding would not maintain the desired qualities. Each stage in the construction of these varieties should be carried out by an institution with a comparative advantage.

7. Bioengineering techniques should be combined with conventional breeding for the development of PLRV resistance.
8. Testing and release of transgenic plants should follow the internationally accepted guidelines.
9. The early screening and evaluation of germplasm for resistance to viruses should be implemented in the regions.
10. Trials to assess the stability of resistance to viruses be extended to other regions.

Sweet Potato

1. Ongoing efforts to identify sources of resistance to SPFMV should be continued. Resistant genotypes will benefit from both breeding, and research on sweet potato viruses.
2. Resistance to other viruses and/or virus diseases should be undertaken only after:
 - a. Identification and characterization of sweet potato viruses that cause economically important diseases.
 - b. Thorough documentation of geographic distribution of these viruses.
3. We endorse the proposed project to determine if sweet potato viruses can be controlled by genetically engineered cross protection. CIP should attempt to maintain close cooperation, so as to obtain virus-resistant genotypes. These resistances will be retested at the greenhouse and in the field, following internationally accepted guidelines for testing of transgenic materials.
4. The potential of transformation with single gene traits is as important as that in potatoes. CIP scientists should be alert to any new development in this area.
5. CIP should assist NARS in developing testing procedures for the evaluation of advanced populations, lines, and the final release of new cultivars.

Virus and Viroid Detection

Recommendations

1. Identification and characterization of diseases

Identification and biological investigations of viruses and their diseases of potato and sweet potato should be continued at CIP, possibly with the help of external collaborative research initiated and coordinated by CIP. Where regional disease problems prevent germplasm movement, CIP should take full advantage of local and nearby expertise, e.g. in the cases of "wild potato viroid", PVT and novel sweet potato viruses should be investigated. The identification of new strains of known pathogens should be continued with all suitable technologies.

2. Techniques and reagents for detection

Detectability with the presently available techniques (ELISA, NCM-ELISA, NASH) is satisfactory under optimal conditions and with the best available reagents. However, reagents have to be diversified and improved. Areas of improvement include the development of strain specific antibodies, antisera or molecular probes for PVT, PVX, PVA, PVS and, especially, PLRV. New approaches include the use of the hybridoma technology, anti-idiotypic antibodies, and peptides derived from viral genome sequences obtained from other laboratories (if possible, as collaborative research). The improvement of virus purification techniques should also be continued. The feasibility of the polymerase chain reaction (PCR) for virus detection should be explored in collaborative research.

3. Appropriate technologies for CIP and NARS

Presently available techniques for virus and viroid detection are adequate for routine purposes. The capabilities of different national programs to use techniques such as NCM-ELISA, hybridoma technology, and virus purification methods should be updated and strengthened.

Collaborative help from other research institutes should be considered. The application of NASH within CIP's present concept is appropriate. Emphasis should be given to better sample preparation and possible adaptation for the use of the national programs.

Virus Control Approaches to Help NARS

Recommendations

1. We emphasize the importance of understanding the prevalent seed and propagation systems in developing countries. The knowledge of seed symptoms should include determination of incidence and economic importance of virus and viroid diseases, epidemiology, and currently practiced methods of crop production. We recommend that this information be used to evaluate the specific needs of each country, and recommend an integrated approach to improving potato and sweet potato production through control of virus diseases.
2. We agree with the current priorities of CIP, but emphasize that these need to be evaluated on the basis of information on the need of each country, as developed through recommendation No. 1.
3. The concept of NARS is not well understood and therefore NARS are less effective than they could be. CIP and NARS should develop strategies for awareness at the national and international level, of the identity and scope of the term NARS.
4. CIP, NARS, and each host country should interact to identify and transfer appropriate and sustainable technology for virus detection and control.

5. We recommend that CIP utilize scientist expertise that is currently available in developing countries/regions to help in transfer and application of technology.
6. Transfer is a dynamic process that needs to be paced with the capacity of each country to adapt technology. Mechanisms for transfer of new technologies should be developed along the model of antisera production.
7. We recommend to CIP use all available resources for training and technology transfer; for instance, CIP, CGIAR centers, NARS, the 5 intercountry networks, and other resources within each country, including the private sector.
8. NARS should be encouraged to identify areas that could best be served by the private sector.
9. We recommend CIP to identify areas for joint ventures in which CIP goals can be complemented by private sector, for example, utilizing expertise of private sector for the efficient production and distribution of user friendly kits with CIP participating in the area of antisera production.
10. We recommend exploring and improving all possible methods of increasing dissemination of information.

Overview of Thrust IV. Review of Previous Planning Conference Recommendations

L. F. Salazar¹

Planning Conferences have constituted excellent instruments for guiding CIP's research to achieve its goals in Third World countries. The virus Thrust has held two meetings previously. The first Planning Conference was held in 1977 and the second in 1980.

Before analyzing how the 1980 recommendations were implemented, it is important to examine the Thrust IV long-term planning.

The CIP profile was created to outline the long-term plan of the Center's goals as well as the strategies for reaching them (Sawyer, 1987). The Thrust IV outline now includes both potato and sweet potato activities (Fig. 1).

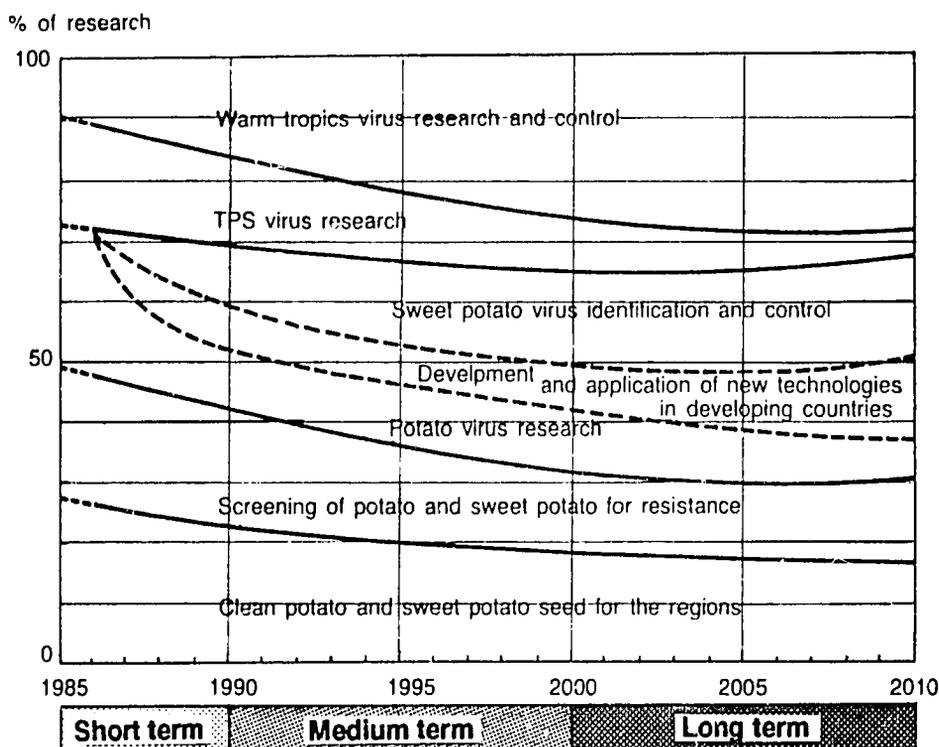


Figure 1. Thrust IV Outline.

¹Thrust Manager "Control of Viruses Diseases" International Potato Center, (CIP), Lima, Peru.

In terms of usefulness to NARS, one of the most relevant characteristics of the profile is the development and application of new technologies. Our strategy is to develop or adapt the appropriate technology for virus detection, identification and control in both crops by (a) using the same specialized personnel, thus allowing increased flexibility and efficiency in the program and, (b) allowing developing countries to employ advanced techniques as they are being perfected.

To achieve these goals, several approaches were used. It was first considered appropriate to develop serological kits at CIP and then transfer them to NARS. However, development of kits required an increased effort in labor and supplies. Our second approach was to transfer the technology available to NARS and guide them to the production of basic reagents. Examples of this approach will be presented by NARS visitors in this Planning Conference.

Our third approach is to link our efforts to the private sector as a way of transferring the technology and, at the same time, providing the reagents needed.

Recommendations of the 1980 Planning Conference

The recommendations that emerged from the Planning Conference in 1980 are the following:

Phytosanitation

1. Maintain priority on research for detection of novel viruses and other pathogens that may threaten the health status of exported germplasm.
2. Superior clones in the seed production program should be indexed on a wider host range to increase the chances of detecting novel virus or atypical strains of characterized viruses.
3. Upgrade laboratory facilities at CIP to increase efficiency of virus testing related to such procedures as automation of ELISA.
4. Efficiency of research leading to PSTVd detection should be increased by:
 - a. searching for more useful indicator hosts at CIP-Lima.
 - b. encouraging collaborative research in the development of detection techniques in appropriate laboratories and ensuring that CIP scientists are aware of new techniques.

Health Status of Exported Germplasm

A designation of quality is needed for labelling 3 categories of seed health and genetic constitution:

Tuber Seed and In Vitro Culture

1. superior high quality tuber seed originating from meristem culture designed for distribution.
2. same health quality as 1 but tuber families as segregating lines.
3. field-grown seed tubers that might include germplasm of wild species that have passed visual inspection.

True Potato Seed (TPS)

1. seed tubers from pathogen-tested parents should be distributed for on-site TPS production in the region.
2. TPS should be grown in CIP-Lima from parents known to be free from PSTVd.
3. Ten percent of TPS should be assayed for seed-borne viruses and PSTVd from parents of unknown status.
4. TPS in any quantity with no statement of freedom from seed-borne pathogens should be available for countries willing to accept responsibility.

For CIP receiving

Precautions should include:

1. post-entry testing of TPS and seed tubers for pathogens and pests.
2. accepting no material without a certificate from exporting countries.

All introductions should be addressed to the office of the Director General or his designate. Potato germplasm being introduced at CIP (Lima or regions) from the several sources of introduction should be tested for freedom from disease and potential danger to the CIP program. Priorities for such testing will be made on the basis of space, facilities, personnel available, as well as source.

It is recommended that regional multiplication and distribution centers be upgraded to:

1. increase the amount of potato materials available in the regions by increasing simple on-site virus testing.
2. increase the utilization of rapid multiplication methods in national programs.

Novel approaches

1. A study has been started on virus incidence, virus identification, and effects of yield with seedling plants grown from true potato seed (TPS) as a food source.
2. Within the near future, studies of strain variation within PLRV isolates obtained from wild potato collections will be completed. This will include a study of effectiveness of cross protection as a means of alleviating PLRV damage.
3. Present equipment does not permit a study of DNA sequences in PSTVd complementary to PSTVd-RNA.
4. Studies of *S. tuberosum* x *S. pinnatisecticum* are now under way in the PLRV resistance program.

General recommendations

1. Presently Virology has no contracts with other research programs. However, close ties with progressive research programs elsewhere are maintained and possibly may be more successful than contracts in servicing the needs of virology.
2. Greenhouse improvements are now on the drawing board and substantial improvement in quality of growing space is anticipated. However increasing demands for greenhouse space by other programs within CIP may limit the program in virology.
3. Limited work on vector research is now under way. Observations on PLRV resistance indicated that within certain accessions of *S. brevidens* some factor inhibits multiplication of the virus on the host. Further work on this is justified.
4. Recognition is given to the work of C. E. Fribourg and he has been named in a courtesy appointment.
5. Currently, there are 5 research scientists on the staff at CIP. However, two of these (Ing. C.E Fribourg and Dr. E. Fernandez-Northcote) have joint appointments in the Universidad Nacional Agraria.

Implementing the Recommendations

Staff

The number of virology staff members is approximately equivalent to four full-time research scientists. Thus, in order to meet the recommendations of the previous planning committee, another research scientist is needed.

Phytosanitation

All recommendations under this heading have been implemented throughout the years, excepting the search for useful indicator hosts for PSTVd. The reason for this omission was that efforts were being directed instead to the development of nucleic acid spot hybridization (NASH) tests. In connection with this work, the collaboration of the Microbiology and Plant Pathology Laboratory of the Beltsville Agricultural Research Center, USDA, made available by Drs. T. O. Diener and R. Owens, is greatly appreciated.

The detection of novel viruses was given lower priority than that recommended by the 1980 Planning Conference. The reason for this was that PSTVd appeared in some of our stocks, and efforts had to be directed toward developing technologies for viroid detection, eradication and control.

Health status of exported and imported germplasm

Our current procedures and strategies for exported germplasm will be reviewed by J. Bryan in his paper on transfer of CIP germplasm.

One of the aspects that deserves attention under this heading is the fact that after the 1980 planning conference, CIP developed strategies for distribution that, without being too conservative, were in general more severe than the recommendations emerging from the Planning Conference. For instance, CIP's policy prohibits distribution of TPS with no certification of freedom from seed-borne pathogens. The obvious reason is that CIP does not want to assume the responsibility of accepting risks to developing countries with limited knowledge of the importance and potential danger of potato viruses. The same rule applies to seed tubers that have passed only visual inspection.

On imported germplasm, CIP basically followed the recommendations from the Planning Conference, though some modifications had to be adopted to expedite the process.

Overview of Thrust IV

Thrust IV includes Headquarters and Regional Research Projects, Collaborative and Contracted Research, as well as student theses having Virology as the basic discipline.

Laboratory facilities for Thrust IV are inadequate to present needs but laboratories have recently been built.

A complete list of current activities has been published in CIP's list of research projects. Personnel involved in Thrust IV at headquarters is shown in Table 1.

Table 1. Personnel in Thrust IV (Headquarters, 1989).

	Type	No.
Project leaders	PhD	3
	Post-Doctorate	1
	M. Sc.	2
	Visiting Scientist	1 ^a
Research Assistants (B.S. or equiv.)		2
Technicians		4
Laborers		4
Students	PhD.	1
	M.Sc.	2
	B.S.	2

^aMr. M. Nakano, TARC, Japan. (Two years post.)

Some of the most relevant aspects in which the Thrust is involved can be summarized as follows:

1. Development and application of most adequate technologies for production of germplasm for export.

A process that might appear very simple becomes complex because we have to make sure that materials are exported free of the known viruses, and also to attempt to discover any unknown virus in the material. For this reason, we still use a set of indicator hosts to test any clone entering the "clean-up system" in addition to ELISA, NASH, etc. for known agents.

2. Development of techniques and basic reagents to help NARS, especially in seed production.

The development of serological kits, NASH kits, transfer of technologies for virus detection, consultancies, and training are some of the strategies used to accomplish this objective.

Collaborative and contracted research has played an important role in our attempts.

3. Search for sources of resistance to important viruses, and the development of resistant genotypes.

PLRV, PVX, and PVY have been studied more extensively. These aspects will be addressed by other members of the Thrust during this Conference. Immunity to PVX and PVY has been well developed until the present, whereas resistance to PLRV met some unexpected complications that are now being taken into consideration in the program.

4. Understanding the mechanisms of resistance to important viruses.

These studies became necessary as a consequence of our previous goal.

5. Identification and characterization of previously unknown viruses.

This was especially important for sweet potatoes though several "new" viruses are still found in potatoes.

On sweet potatoes, SPFMV is the most widespread virus, occurring as isolates or strains differing in virulence and other properties. Several other viruses are under study. For instance, one of them, coded C.2, has been found to be prevalent in our germplasm and was also previously reported in Japan.

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Main Virus Diseases of Potato

L. F. Salazar¹

Introduction

Leafrolling, crinkling and mosaic in the leaves were observed in potato following its introduction in Europe. Subsequently, studies showed that the cause of "degeneration" of potatoes was in part due to viruses introduced in the early history of the crop in Europe.

At present, close to 30 different viruses, excluding strains, have been found in potato. The viruses and related agents that affect potatoes can be divided into two groups well-defined by their association to the host (Table 1).

Table 1. Classification of potato virus by their association to the crop.

Virus dependant on potatoes for survival and spread	Virus not dependant on potatoes for survival and spread	Undetermined
Potato Virus X	Tobacco rattle virus	Potato black ringspot virus
Potato Virus Y	Tobacco mosaic virus	Potato spindle tuber viroid
Potato virus S	Tomato spotted wilt virus	Potato yellow vein virus ^a
Potato virus M	Cucumber mosaic virus	Potato deforming mosaic virus ^a
Potato mop-top virus	Tomato blackring virus	
Potato aucuba mosaic virus	Tobacco Ringspot virus	
Wild potato mosaic virus	Solanum apical leaf curling virus	
Potato virus T	Potato yellow dwarf virus	
Potato leafroll virus	Bet curly top virus	
Andean potato latent virus	Alfalfa mosaic virus	
Andean potato mottle virus	Tobacco necrosis virus	
Potato virus V	Tobacco streak virus	
Potato stunt virus		

^aTheir virus origin has not been yet determined.

Those that depend on the potato for survival and spread usually show a restricted or moderate host range, whereas those that do not depend on the potato for survival and spread,

¹Thrust IV Manager "Control of Virus Diseases," International Potato Center (CIP), Lima, Peru.

have a wide host range, and may cause economically important losses in other crops (Salazar, 1977). Some viruses are common in all areas where potatoes are grown whereas others are only prevalent in specific regions.

The largest variability in potato viruses is found in the Andean region, especially in those viruses that depend on potato for survival and dissemination. This is probably due to their long association with a variable host.

Even though only a few viruses are known to cause significant yield reduction, at CIP we are concerned with all of them. The main reason is our need of producing pathogen-tested materials for export.

Comparative importance of potato viruses

The potato is one of the plant species in which the largest number of viruses are found. As seen in Table 2, viruses belonging to different plant virus groups have been recorded in potato. On a worldwide basis, most economically important viruses are in the Luteo-, poty-, potex- and carlavirus group. However, other viruses or viroids might reach significant importance under certain conditions. This is the case of PSTVd, whose ease of mechanical and seed transmission makes it especially important in breeding programs and germplasm collections.

Table 2. Plant virus groups and members affecting potato.

Virus group	Typical member in potato	No. of viruses found in potato	Particle size (nm)	Type and No. of nucleic acid strands	Natural transmission
Alfalfa mosaic virus	AMV		58,52,42 x 18	RNA (3)	Aphids
Capilloviruses	PVT	1	640 x 10	RNA (1)	True Seed
Carlaviruses	PVS	2	640 x 11	RNA (1)	Mechanical Aphids
Camoviruses	APMV	1	28	RNA (2)	Beetles
Cucumoviruses	CMV	1	30	RNA (4)	Aphids
Furoviruses	PMTV	1	300 x 18	RNA	Fungi
Geminiviruses	BCTV	2	17 (pairs)	DNA	Leafhopper
	SALCV	1	17 (Triplets)	DNA	
Iilarviruses	TSV	1	28	RNA (4)	Thrips
Luteoviruses	PLRV	2	25	RNA (1)	Aphids
Necroviruses	TNV	1	26	RNA (1)	Fungi
Nepoviruses	PBRV	4	28	RNA (2)	Nematodes
Potexviruses	PVX	2	520 x 13	RNA (1)	Mechanical
Potyviruses	PVY	3	740 x 11	RNA (1)	Aphids
Rhabdoviruses	PYDV	1	380 x 75	RNA (1)	Leafhopper
Tobamoviruses	TMV-PMTV	1	300 x 18	RNA (1)	Mechanical fungi
Tobraviruses	TRV	1	190-45 x 22	RNA (2)	Nematodes
Tomato spotted wilt virus	TSWV	1	80	RNA (1)	Thrips
Tymoviruses	APLV	2	28	RNA (1)	Beetles
Viroids	PSTV	1	-	RNA (1)	Mechanical

Viruses in the Andean region such as APMV, APLV, PVT, and especially PMTV, become a serious limiting factor for seed production. Under some conditions, uncommon viruses may spread and cause severe yield losses. One example of this situation is TSWV in some regions of Brazil, or even SALCV in the area of San Ramon in Peru.

Specific virus strains might become a threat to potato production if allowed to spread from their localized areas. One of the oldest examples is PVY^N, which was introduced into Europe from South America (Smith and Dennis, 1940). Now PVY^N is one of the most important potato viruses in Europe. PVX^{HB} the strain that breaks the immunity to PVX available in *Solanum acaule*, still seems to be restricted to some areas of the Andean region. Therefore, at CIP the pathogen detection system aims to maintain this situation. But not all viruses or virus-like agents originated in the center of origin although this may appear to be the case.

Some agents appear to have been adapted to the potato outside its center of origin: PSTVd, for example, has never been found in the Andean Region in native cultivars and potato species. The high temperatures required by the viroid for multiplication are not prevalent in the Andes and, this seems to support our hypothesis that PSTVd became a pathogen of potato outside its center of origin. A similar situation seems to occur with PVM and even PVA, two viruses which are uncommon to Andean potatoes but prevalent in Europe and North America.

Effect of viruses on potatoes

Usually the first and most striking effect of virus diseases is the production of symptoms. As a general rule, the more severe the symptoms, the higher the yield reduction. However, there are exceptions to this rule because symptoms depend on the host genotype, the virus strain, and the environmental conditions under which the host is growing.

One of the classical examples of the above is that of PLRV. In the Andean region, most native cultivars belong to species of *S. andigena*, *S. stenotomum* and others, whereas in Europe and North America most cultivars are derived from *S. tuberosum*. PLRV in the Andes shows quite different symptomatology than it does in the European cultivars. This originally led us to believe that symptoms of stunting, deep chlorosis, and plant erectness were caused by an unknown virus; this disease was even given a name: enanismo amarillo. Rodriguez and Jones (1975) identified the cause of these symptoms as PLRV.

The variability of the effect (symptoms and yield reduction) has been documented for a number of viruses. The differing symptoms caused by PVY^N and PVY^O, or by strains of PVX, are well known.

The environmental conditions have a decisive effect on symptoms and on the effect of viruses. It is well known that potatoes grown at high elevations in the Andes show symptoms that are minimal compared with those in the coastal areas of Peru. This effect has other implications and will be addressed by L. Bertschinger in this Planning Conference.

In summary, viruses are known to have a tremendous impact on potato production, causing qualitative or quantitative losses.

The control of virus diseases

Prevention is the key factor in controlling virus diseases. Preventing infection can be accomplished with a short- or long-term approach. A short-term approach is the production of healthy seed, whereas the long-term approach could be the development of resistant genotypes. In both cases, the common denominator to achieving these goals is the development of expeditive, sensitive and reliable virus detection technology. It is with these objectives and prerequisites that CIP's virus program is operating.

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Viruses and Virus-like Diseases of Sweet Potato

J. Moyer and L. F. Salazar¹

The renewed interest of the international agricultural community in the sweet potato *Ipomoea batatas* (Lam.) L. has resulted in a dramatic rise in the need to exchange primitive and improved sweet potato germplasm. Sound horticultural practices and national quarantine regulations require that sweet potato germplasm intended for international distribution be free of known viruses. Sweet potato germplasm free of known viruses is also needed for commercial production and research purposes. Unfortunately, our ability to certify plants free of viruses and of agents responsible for recognized diseases of unknown etiology has not kept pace with this burgeoning need.

Field observations and assays have revealed virus symptoms and the presence of one or more viruses in virtually all sweet potato grown from materials that have not been virus-tested. In many instances the endemic nature of these viruses has facilitated the natural incorporation of high levels of tolerance to local viruses, via selection and propagation of asymptomatic plants. Although tolerance to viruses has improved production of sweet potato, it has made diagnosis difficult and, in some areas, has resulted in a general complacency about the importance of virus diseases in sweet potatoes. There is, however, justified concern that a virus isolate which is mild or latent in one location on one group of cultivars, may have considerably greater effects, either by itself or in combination with other viruses, when introduced into a new geographic location where local cultivars have a different genetic background. Thus, the necessary precautions must be taken to prevent the inadvertent distribution of viruses with germplasm.

A concerted effort is being made in several laboratories to discover the etiology of those diseases with symptoms frequently associated with virus infections. Recently a group of sweet potato virologists developed a list of 14 different viruses or virus-like agents that infect sweet potato (Table 1). A summary of the best characterized viruses is given below.

Status of Known Viruses and Virus Diseases

Sweet Potato Feathery Mottle Virus

There are many strains of sweet potato feathery mottle virus (SPFMV), which are found nearly everywhere sweet potato is grown. Some of the synonyms used for SPFMV isolates include

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Table 1. A list of recognized viruses known to infect sweet potato.

Virus	Vector	Distribution	Assay hosts ^a
CIP-Isolation (2-C6)	?	unknown	<i>I. setosa</i>
Cucumber mosaic virus (CMV)	aphid	widespread Cucumis sativus	<i>N. glutinosa</i>
Reo-like	?	Asia	<i>I. setosa</i>
Sweet potato caulimovirus (SPCLV)	?	widespread <i>N. megalosiphon</i>	<i>I. setosa</i>
Sweet potato feathery mottle virus (SPFMV)	aphid	worldwide	<i>I. setosa</i> ^b
Sweet potato latent virus (SPLV)	unknown	Asia	<i>I. setosa</i>
Sweet potato leaf curl virus (SPLCV)	<i>Bemisia tabaci</i>	Taiwan, Japan, Nigeria	<i>I. setosa</i>
Sweet potato mild mottle virus (SPMMV)	<i>Bemisia tabaci</i>	East Africa <i>N. tabacum</i> <i>N. glutinosa</i> <i>N. benthamiana</i>	<i>I. setosa</i>
Sweet potato mosaic virus (SPMV)		Taiwan	<i>I. setosa</i>
Sweet potato ring-spot virus (SPRSV)	unknown	Papua New Guinea	<i>I. setosa</i>
Sweet potato vein mosaic virus (SPVMV)	aphid	Argentina	<i>I. setosa</i>
Sweet potato yellow dwarf virus (SPYDV)	<i>Bemisia tabaci</i>	Taiwan	<i>I. setosa</i>
Unknown virus	?	Puerto Rico	<i>I. setosa</i>
Whitefly-transmitted component of sweet potato virus disease (SPVD)	<i>Bemisia tabaci</i>	Africa, Taiwan	TIB 8 sweet potato infected with SPFMV <i>I. setosa</i> ^c

Source: This is a list originally prepared by FAO/BPGR that has been modified with newly available information.

^aFrom FAO/IBPGR technical guidelines for the Safe Movement of Sweet Potato Germplasm.

^b*Ipomoea setosa* is frequently difficult to inoculate by mechanical transmission; graft transmission from sweet potato is the most reliable means of transmission.

^cSymptom expression is highly variable in *I. setosa*. This host should only be used after its reliability has been established in environment where test is conducted.

russet crack virus, sweet potato virus A, sweet potato ringspot virus, sweet potato leafspot virus, and probably internal cork virus (Cadena-Hinojosa and Campbell, 1981; Cali and Moyer, 1981; Campbell et al., 1974; Loebenstein and Harpaz, 1960; Moyer and Cali, 1985; Sheffield, 1957; Yang, 1972). Co-infection by SPFMV with an unknown virus is frequently a problem in determining the etiology of disease complexes.

The range of symptom types associated with SPFMV infection are as much a function of the host genotype and environment as they are of the virus strain or isolate (Alconero, 1972; Cali and Moyer, 1981; Campbell et al., 1974; Moyer, 1986; Moyer and Cali, 1985; Moyer and Kennedy, 1978). Symptoms on sweet potato leaves may consist of the classic irregular chlorotic patterns (feathering) associated with the leaf midrib, as well as faint or distinct chlorotic spots which in some genotypes have purple pigmented borders. These symptoms are observed predominantly on the older leaves. Vein-clearing, vein-banding and chlorotic spots are the predominant symptoms observed in the indicator host *Ipomoea setosa* (Kerr). However, symptoms may be mild, and leaves produced after the initial flush may be symptomless. Some strains of SPFMV cause necrotic lesions on the exterior of the roots (russet crack disease) while other strains induce symptoms on the interior of the root (internal cork disease).

SPFMV is the most thoroughly characterized (Campbell et al., 1974; Moyer, 1986; Moyer and Kennedy, 1978) sweet potato virus and serological procedures have been developed to detect it (Cadena-Hinojosa and Campbell, 1981; Esbenschade, and Moyer, 1982). SPFMV has many biological characteristics and cytopathic effects that support its classification as a potyvirus (Cali and Moyer, 1981; Campbell et al., 1974), even though its biochemical properties such as capsid protein CMr 38,000 dalton, RNA C 3.65 X 10⁶ daltons (Moyer and Kennedy, 1978) and virion length (850 nm) (Cali and Moyer 1981; Nome, et al., 1974) make it an atypically large potyvirus.

Sweet Potato Vein Mosaic Virus

Sweet potato vein mosaic virus (SPVMV) has been reported only in Argentina (Nome, 1973). Direct comparison of the particle morphologies of SPFMV and SPVMV indicated that SPVMV has a modal length of 761 nm, significantly shorter than SPFMV. Sweet potato plants infected with this virus are severely stunted and produce fewer new roots. SPVMV is also transmitted occasionally by aphids (Nome et al., 1974). Antiserum is not yet available to compare this virus to other known potyviruses or to assay sweet potatoes from other countries.

Sweet Potato Latent Virus

Sweet potato latent virus (SPLV), formerly designated Sweet Potato Virus N, has only been reported in Taiwan (Chung et al., 1986). As the name suggests, infection of many sweet potato cultivars by SPLV does not result in obvious foliar symptoms. The host range of SPLV includes many *Convolvulus* species, *Chenopodium* species and some *Nicotiana* species such as *N. benthamiana* (Domin). Although it induces mild symptoms in *I. setosa*, it can be easily detected in this host by serological procedures.

SPLV also has many characteristics of a potyvirus including production of characteristic cytoplasmic inclusions. However, all attempts at aphid and white-fly transmission have been unsuccessful. Thus, definitive classification of this virus awaits further characterization.

Sweet Potato Mild Mottle Virus

Sweet potato mild mottle virus (SPMMV) was isolated in East Africa from sweet potatoes exhibiting leaf mottling, veinal chlorosis, dwarfing and poor growth (Hollings et. al., 1976). SPMMV-infected *I. setosa* exhibit a bright yellow veinal chlorosis on as many as four leaves following inoculation. Subsequent leaves are symptomless. This virus was referred to as SPV-T in preliminary reports and may be the same as virus B (Sheffield, 1957). Virus B was also isolated from sweet potatoes in East Africa (Sheffield, 1957).

Although the morphology of SPMMV and its cytoplasmic inclusions are similar to that of other potyviruses, its biological characteristics differ greatly from the type member. Most notable among the divergent characteristics is the host range of SPMMV, which includes 45 species in 14 plant families (Hollings et. al., 1976). Additionally, SPMMV is vectored by the whitefly, *Bemisia tabaci* (Genn), and its virions are relatively unstable using purification procedures for other potyviruses (Moyer, 1986; J. W. Moyer, unpublished). Further it does not react to the universal monoclonal antibody for potyviruses (J. Hammond, unpublished data).

Sweet Potato Yellow Dwarf Virus

Sweet potato yellow dwarf virus (SPYDV), which frequently occurs with SPMMV, was described recently in Taiwan (Chung et al., 1986). The virion morphology and vector of SPYDV are similar to those of SPMMV. Neither virus is adequately characterized and a direct comparison has not yet been made to determine the extent of biochemical relationships, but sufficient differences have been reported to justify continuing the designation of SPYDV as a separate virus.

A Caulimo-like Virus

A virus with some properties similar to the caulimoviruses was isolated from sweet potato by grafting and has been provisionally designated as sweet potato caulimo-like virus (SPCLV). It was first isolated in Puerto Rico and has since been isolated from sweet potatoes grown in Madeira, New Zealand, Papua-New Guinea, and the Solomon Islands (Atkey and Brunt, 1987).

Early symptoms on *I. setosa* include chlorotic flecks along the minor veins with interveinal chlorotic spots. These symptoms may develop into a general chlorosis resulting in wilting and premature death of the leaves. Virions associated with SPCLV were typical of caulimoviruses, but some of the inclusions were similar to the fibrillar ring inclusions induced by geminiviruses.

Other Whitefly-Transmitted Agents

Other whitefly-transmitted agents isolated from sweet potato in Nigeria, Israel, Taiwan, and the United States (Chung et al., 1985; Girardeau, 1958; Hildebrand, 1958; Loebenstein and Harpaz,

1960; Schaefers and Terry, 1976) are also considered as separate agents, but a comparison of these agents has not yet been made nor have they been definitively characterized. They have properties different from SPMMV in that they are not mechanically transmitted, they have a narrow host range, and no virions have been identified for these agents. The sweet potato virus disease (SPVD) described in Nigeria is one of the most thoroughly investigated (Hahn, 1979; Hahn et al., 1981; Schaefers and Terry, 1976). This disease is due to the synergistic interaction of a strain of SPFMV and a whitefly transmitted agent. Diseases similar to SPVD, designated as Georgia mosaic and yellow dwarf, have been reported in the United States (Girardeau, 1958; Hildebrand, 1958). The sweet potato vein-clearing virus reported in Israel also induces symptoms similar to SPVD (Loebestein and Harpaz, 1960). Sweet potato leaf curl disease (SPLC) is another disease whose causal agent has been reported as being transmitted by *B. tabaci* (Chung et al., 1985; Yamashita et al., 1984).

Guidelines for Virus-testing of Sweet Potato

It is recommended that all sweet potato clones be placed in in vitro culture by meristem-tip culture accompanied by heat or chemotherapy as necessary for obtaining plantlets free of pests as determined by subsequent pathogen testing. All clones should, whenever possible, be stored in in vitro culture for multiplication and distribution to minimize opportunities for reinfection during maintenance. Each in vitro plantlet should be subcultured for pathogen testing. The youngest portion of the plantlet (apical 2 or 3 nodes) should be used to propagate plantlets as in vitro reference cultures; the remaining stem and roots can be used to propagate the plant in a screened greenhouse for pathogen testing. This strategy favors propagating for maintenance that portion of the plant having the least probability of containing viruses (the youngest), and propagating for virus-testing that portion of the plant that has the most probability of containing viruses.

Plantlets may be assayed at the time of subculturing by biochemical assays as a preliminary step in virus testing. It must be recognized, however, that a virus may only be detected in clones supporting high virus titers and that virus may not be detectable in all tissues. It is recommended that plants for virus testing should be grown in the greenhouse to produce stems with at least 10 to 15 nodes. These plants should then be assayed by making grafts to two separate *I. setosa* plants and to the sweet potato clone TIB 8, which is infected with a mild strain of SPFMV. The TIB 8 clone is used to detect the whitefly component of the SPVD complex. Nearly all known viruses infecting sweet potato also infect *I. setosa*. Although *I. setosa* is susceptible to many viruses that infect sweet potato and although it is a good assay host, the symptoms are not of diagnostic value. However, some viruses such as CMV and TSV, may not be reliably detected by these methods. Thus, mechanical assays directly from sweet potato to other virus indicators such as *N. benthamiana*, *N. clevelandii* Gray, and *Chenopodium quinoa* Wild are strongly suggested. In addition, as for other vegetatively propagated crops, it is recommended that each sweet potato plant be tested several times.

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The Importance of Potato Virus Diseases in Developing Countries

L. F. Salazar and P. Accatino¹

Introduction

There is no doubt that virus diseases of potato and sweet potato are important in developing countries. Qualitative losses such as reduction in marketable value of the crop are not yet important. However, quantitative losses such as yield reduction of vegetative reproduction material like seed or cuttings are commonly recognized. Viruses that depend for their survival and distribution on potato and sweet potato are widespread, but in many regions, viruses that do not depend on these two species for survival and spread are also prevalent. The abundant vegetation and high activity of virus vectors in the tropics almost all year round, create an excellent ecological environment for the spread of viruses. (Salazar, 1985).

In general, the tremendous importance of diseases is not recognized in most developing countries. Many diseases perhaps of equal or even more importance than those caused by viruses are not being given attention. Also virus diseases are overlooked because their symptoms are often less dramatic than those of bacterial diseases. This negligence is the result of the lack of knowledge of virus symptoms and detection methods. It is also caused by incomplete surveys and economic assessments of potato and sweet potato diseases.

Over the years, CIP has developed strategies that have permitted the effective transfer of the knowledge of potato virus diseases, and noticeable improvements have been made in several countries. It is expected that in the coming years, most developing countries will have obtained the conditions to utilize this knowledge.

Most of the achievements in this area were the results of the collaborative approach of CIP scientists with scientists of the National Agricultural Research Services (NARS) of developing countries. Of the many potato viruses recorded, most developing countries list, in descending order of importance, PLRV, PVY, PVX, and PVS, though in some regions specific strains can be found. For instance, the most widespread strain of PVX in Central America (PVX-GUA)

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appears to be more aggressive than other strains of the virus and, serologically, is distantly related to PVX^{HB}.

The knowledge of virus diseases that attack sweet potatoes is practically non-existent in most developing countries; but some countries have already shown great interest in initiating appropriate surveys. In China, for instance, a combination of survey and training courses has begun to yield results in controlling viruses. Sweet potato feathery mottle virus (SPFMV) was the one most commonly found in the samples examined. However, other unknown viruses also seem to be of importance (Table 1).

Table 1. Presence of virus determined by NCM-ELISA in samples collected in four provinces of China.

Virus (es)	Province ^a				Total (125)
	Sichuan (26)	Jiangsu (45)	Shan Dong (26)	Beijing (28)	
SPFMV ^b	5	4	2	0	11
SPLV ^c	1	4	4	4	13
SPFMV and SPLV	6	18	11	4	39
Other virus(es)	7	3	0	7	17

^aNumber of samples tested, appear in parentheses.

^bSPFMV = Sweet Potato Feathery Mottle Virus.

^cSPLV = Sweet Potato Latent Virus.

Adoption of technologies for the control of viruses

Developing countries are usually eager to receive and adopt new technologies. However, it is necessary to make a careful analysis of the technologies in relation to the facilities of the various countries and to the trained personnel available.

The adoption of foreign technologies without a careful analysis of their applicability, or the setting of standards too high to be realistic, has resulted in inadequate virus control. This was apparent in some new seed production programs where the limits for virus diseases were set at only 2-3% for certified seed. To achieve these levels, the organization of the several factors involved and considerable amounts of funds are required. These levels are unrealistic when compared with the potential yield reduction known to be caused by viruses (Fig. 1). At the beginning of each seed program, the seed purity and physiological conditions should be considered. Diseases and pests become important in a second stage, but the setting of standards for virus diseases should be continuously reviewed according to the program development. Good examples of this strategy are in the Chilean and Brazilian seed programs and the Tunisian multiplication program.

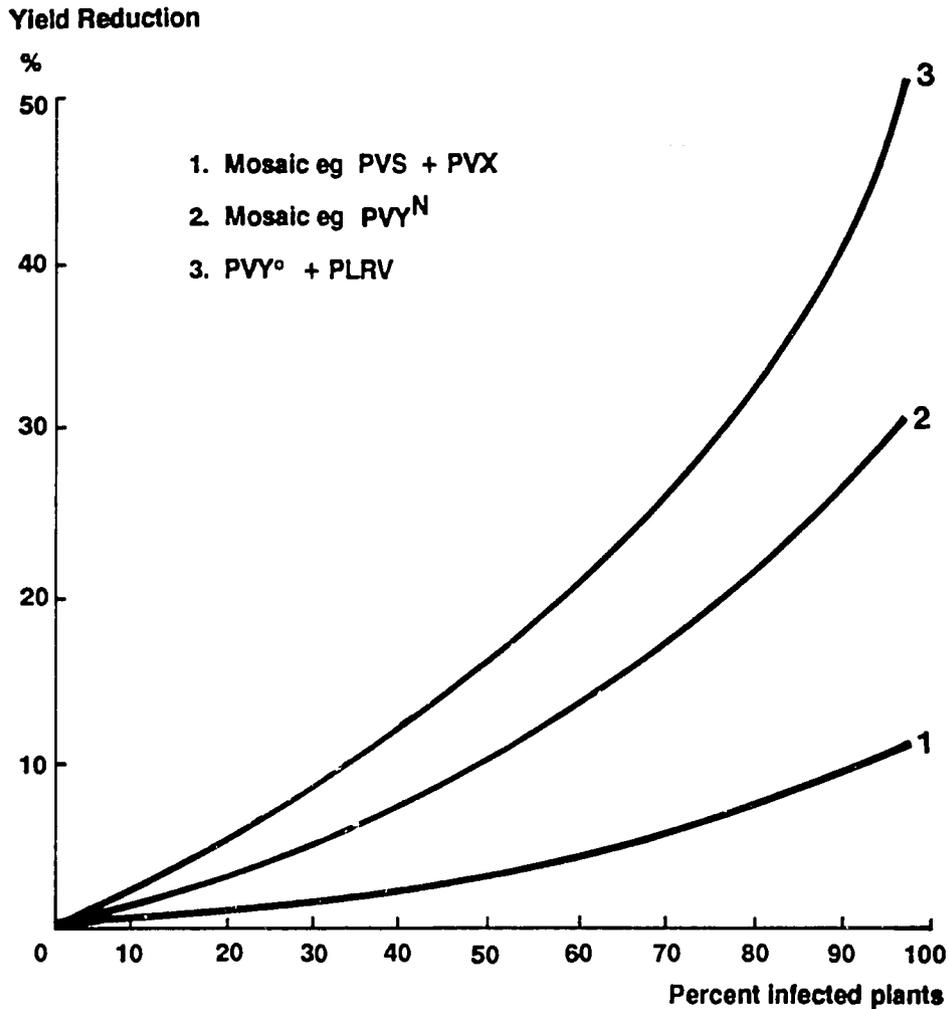


Figure 1. Yield reduction by virus infection in potatoes in relation to percentage of infected plants (From: Beukema and Vander Zaag, 1979).

It is common among NARS to make efforts for the control of virus diseases without obtaining appropriate support from other disciplines. For instance, the control of virus diseases by avoiding the vectors requires previous knowledge of the proper identification of the vectors and the dynamics of their biological cycles. An entomological input is certainly needed. The most common practice against vectors is the indiscriminate application of insecticides, with the well-known consequences to the ecological equilibrium and the unnecessary increase in the production costs.

The application of techniques for virus detection in NARS not backed up by in-depth training on general aspects of virus diseases can lead to erroneous attitudes. Some NARS workers who received training only in modern virus detection techniques forget the value of visual inspections. Omitting such inspections leads to the build up of other viruses among the materials, which may have devastating effects.

Approaches to virus control

From the beginning of its work with potatoes, CIP realized the importance of virus diseases, and therefore, several approaches were used to help NARS in transferring virus-free germplasm, developing and transferring of techniques for virus detection, training in those techniques, breeding for resistance, and improving seed production technologies. Our aim has been to apply the results of our research and to develop technologies for sustainable agricultural systems and we would like this Planning Conference to keep this aim in mind in its discussions and recommendations. Let us, then, review the approaches in some detail.

Viruses and the transfer of germplasm to developing countries

In order for CIP to achieve its goal of helping NARS to increase production of potato and sweet potato, it is important to identify the appropriate channels for transferring improved germplasm. CIP places great emphasis on avoiding the dissemination of diseases, especially viruses, by means of its pathogen-testing program, and the value of its program has been recognized (Thresh, 1980). NARS, on the receiving end of this program, has its own quarantine units, which usually are involved only in administrative matters, having little to do with the achievement of proper quarantine techniques. Developing countries rely on CIP's thorough testing for pathogens (especially viruses) in the germplasm distributed to them and, therefore, it is important to maintain our standards. For sweet potatoes we are following the same procedures used on potatoes. At CIP we are adamant about continuing to use NARS quarantine requirements because they permit CIP to maintain strict control over pathogen testing. Recently, the Director of Research of the CNPH-EMBRAPA of Brazil stated that they will accept sweet potato cultivars only from CIP because CIP has a trustworthy procedure for pathogen testing.

Development and transfer of techniques for virus detection

When the program was initiated in 1972, virus detection relied only on indicator hosts, visual observation of symptoms, and microprecipitation or chloroplast agglutination tests. Because of the unreliability of the serological tests available, CIP virologists developed the Latex test, which has increased sensitivity and is easy to apply.

By 1977, the double-antibody sandwich form of ELISA (DAS-ELISA) was introduced into our testing system. From then on, several improvements were made in ELISA, and the range of viruses tested was expanded.

The Latex test was widely distributed by NARS to test for PVX, PVY, PVS, APLV, and APMV. CIP later distributed DAS-ELISA to NARS in the form of a kit to test for the aforementioned viruses and for PLRV.

At present, the ELISA technique on nitrocellulose membranes (NCM-ELISA), which has higher sensitivity and is easier to apply than DAS-ELISA, is being tested at several NARS as an eventual replacement for DAS-ELISA.

The nucleic acid spot hybridization (NASH) test had been introduced by 1983 and since then, CIP has been using this technique to help NARS in detecting the Potato Spindle Tuber Viroid (PSTVd). Details of these techniques and new approaches will be presented at this conference.

The demand for serological kits from NARS (Fig. 2) far exceeded the capabilities of CIP's virology laboratory and, therefore, we have used other approaches to help NARS procure reagents.

The most successful approach to transferring the capabilities for serological detection was to guide selected NARS by means of collaborative projects which had two phases:

Phase I. CIP distributed crude antisera for different viruses to the selected countries for them to sensitize for latex and conjugate for ELISA. Personnel from selected programs were brought to CIP for in-depth training on sensitization with Latex or conjugation with enzymes. During this phase, which lasted from 1 to 2 years, the preparation began for Phase II.

Phase II. During this phase, the country produced its own antisera under our supervision and quality control system. Training on virus purification and antisera production was given on an individual "on-the-job" training basis. The antisera produced can be used to help other countries in the region.

Countries such as Argentina, Brazil, Tunisia, Korea, and China have followed this approach with excellent results.

In order to develop their own antisera supply, some programs, such as the Peruvian potato program and the PRECODEPA country research network, (composed of 10 Central American and Caribbean countries), used CIP laboratories, personnel and expertise for a number of years. At present, the Peruvian program is producing new antiserum batches at CIP laboratories. Our new approach also considers the private sector for production of reagents and services to NARS. CIP's impact on this new approach will depend largely on its strategy in reaching NARS and the private sector, and assisting in the interaction between them. CIP can act as a catalyst in the system and, in this way, improve the development of appropriate technologies.

As the previous examples show, when methodologies were developed, they were immediately transferred to NARS for evaluation and possible adoption. As new technologies are developed, they will continue to be passed on to the national programs. But we have a major

concern with this strategy and a couple of important questions should be considered. Is it a priority for CIP to find newer more sensitive methods of detection? Are NARS capable of absorbing the rapidly changing technologies developed at CIP? We think these are important subjects that should be addressed and discussed during this planning conference.

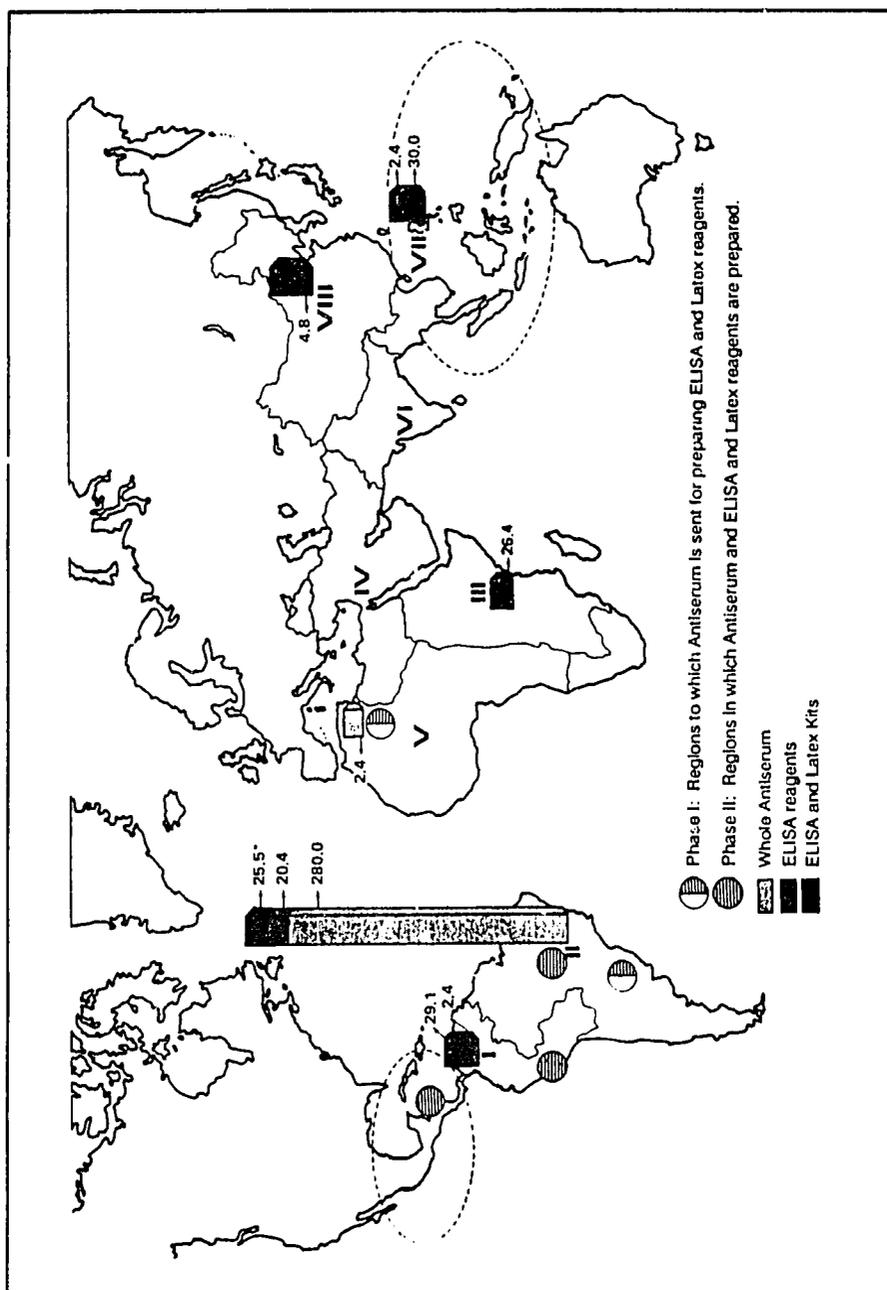


Figure 2. Distribution and production of immunological reagents in the CIP regions (1983-1988).

Training on virus diseases and their control

Specific details in training activities will be discussed in another paper during this conference. All developments on the control of virus diseases at CIP relied especially on techniques for virus detection and identification. However, training has been one of the most important factors in achieving results.

Individual training in virology was time-consuming for the Thrust and often conflicted with the research. Therefore, group training either at headquarters or in developing countries became an alternative so as to reduce individual training at headquarters. At present, individual training is only given as part of collaborative projects and on specific techniques.

In its formal training, CIP offers two virology courses each year: one on general virological aspects and application of techniques for virus detection, and the other on specific techniques.

A new approach to training has been used in China, whereby a survey on sweet potato virus diseases is combined with training on application of detection techniques. For a country as large as China, this is a very interesting approach, since trainees are requested to collect and send a number of diseased plants in their areas of work and send them to a central place before the course starts. During the course, trainees use the samples collected in different provinces to learn the techniques. In this way, the results collected through a number of courses will serve to build a thorough knowledge of the disease situation. We anticipate that the demand for these types of courses will increase in several countries and for other disciplines as well.

The true potato seed (TPS) program

The TPS program at CIP was aimed at providing the farmer with rapidly developed virus-free material. This was possible because only a few viruses can be transmitted through the sexual seed of the potato. Therefore, if parental materials are free of PSTVd and PVT, the TPS produced will constitute an excellent virus-free basic material.

Additionally, hygiene precautions should also permit seedling tubers produced from TPS to be used as a source of planting materials with low virus content.

Development of seed production programs

Seed production projects have been used by CIP as the basic approach for the development of National Potato Production Programs in developing countries. The main objective of the seed programs is to produce pathogen-tested planting materials. Virus control approaches, especially virus detection methodology, have been instrumental in these developments.

Virus resistance program

CIP considered that genetic resistance to viruses in developing countries was one of the best alternatives to the control of virus diseases. Research in Thrust IV, therefore, dedicated much of

its efforts to searching for virus-resistance sources, and to developing resistant genotypes. The efforts have been successful. At present, immunity to PVX and PVY is available in many genotypes, and good levels of resistance to PLRV have been identified and are being used in the many breeding programs of developed and developing countries.

Resistance studies on sweet potato viruses are now concerned with searching for sources of resistance for the most important viruses, i.e. SPFMV and SPMMV.

Concluding remarks

Directly or indirectly, virus diseases have a decisive impact on potato and sweet potato production in developing countries. Even technologies that at first glance appeared exempt from the virus influence had to be re-examined in detail. For instance, rustic and diffused light stores were found to be sources of virus dissemination, if precautions were not taken in their development and handling.

We are making rapid progress at CIP on approaches to virus control in sweet potatoes, and the initial efforts for the identification and characterization of the main viruses should lead to the development of appropriate control measures in the near future.

Finally, we want to acknowledge the dedicated and high-quality work on control of virus diseases and on detection methods that NARS of more than 50 developing countries have carried out successfully, permitting the improvement of potato and sweet potato research and development in their countries. We look forward to this Planning Conference discussion and to the recommendations that will permit us to continue meeting the challenges of the changing needs of our clients, the NARS.

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Advanced Immunological Techniques for Virus Detection

P. Gugerli¹

Plant virus serology has made remarkable progress over the last decade and rapid development through inventive assay design can be predicted in the future. The modern immunoassays include latex-based agglutination techniques, immuno-electron microscopy (IEM) and a wide range of enzyme immunoassays (EIA). Since the introduction of the very versatile enzyme-linked immunosorbent assay (ELISA) for the detection of plant viruses (Clark and Adams, 1977), EIA techniques have rapidly replaced biological and more traditional immunoassays. Several modifications of the ELISA technique have been examined in view of a simplification or an increased detectability. The use of fluorogenic substrates (Torrance and Jones, 1982), modified antibodies (F(ab₁)₂-fragments) (Adams and Barbara, 1982), enzyme amplification (Johannson et al., 1985, Torrance, 1987) or time-resolved fluorometry (Siitari and Kurppa, 1987) are some of the major modifications. The originally described ELISA procedure has, however, survived well. Indirect (Hawkes et al., 1982) and direct antigen-binding, dot-enzyme immunoassays (Dot-EIA) on nitrocellulose or nylon membranes, instead of the polystyrene solid phase of ELISA, are further useful variants of the solid phase EIA. The combination of electrophoresis, electrophoretic blotting of the separated antigens, and their enzyme-immuno detection (Blot-EIA) (Rybicki and Von Wechmar, 1982) is another powerful technique for plant virus analysis.

Ten criteria for an immunoassay

At least ten criteria can be considered for the characterization of immunoassays:

1. Detectability (detection limit)
2. Specificity (reaction with the desired antigen)
3. Sensitivity (signal/reactant ratio)
4. Accuracy (conformity to reference)
5. Precision (reciprocity of standard signal deviation)
6. Simplicity (easy handling and few steps)
7. Rapidity (rapid procedure)
8. Stability of the reagents
9. Aptitude to automation
10. Low costs

Table 1 shows a tentative quantitated characterization of the currently available techniques. None of the techniques is perfect. Therefore, the next generation of immunoassays should

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simultaneously satisfy more criteria, especially those of rapidity, simplicity and detectability, e.g. the simplicity of the latex test should be combined with the high detectability of the best enzyme immunoassay. A one-step (homogeneous), noncompetitive immunoassay based on the proximal linkage of two enzyme systems (Tijssen, 1985) might serve this purpose. Such a procedure requires neither a solid phase nor a separation step of free and bound label. The goal is to bring closely together on a target antigen, two different enzyme systems, using two different non-competing enzyme-labeled antibodies, where one enzyme produces the substrate for the other (e.g. glucose oxidase/peroxidase).

Table 1. Characterization of immunoassays.

Criteria	Latex	iSEM	ELISA	DOT-EI	BLOT-IA
Detectability	-	+	+	+	+
Specificity	+	+	+	+	+
Sensitivity	-	+	+	-	+
Accuracy	-	-	+	-	-
Precision	-	-	+	-	-
Simplicity	+	-	-	+	-
Rapidity	+	-	-	-	-
Stability	+	-	+	+	+
Automation	-	-	+	-	-
Costs (low)	+	-	-	-	-
	5	3	7	4	4

+ High performance, - Low performance

The reagents of the next generation of immunoassays are carefully selected antibodies. The primary requirement for the development of the next generation of immunoassays is the production of high affinity antibodies of adequate specificity which are also necessary in order to make available immunoassays more reliable, and applicable under variable conditions. In fact, the potential detectability of the best immunoassays now available is satisfactory. Emphasis has therefore to be put on the development of the required antibodies, on standardizing them, and on training people in the optimal application of these techniques.

The hybridoma technology is best suited for use in producing the required antibodies for the improvement of the detectability, specificity, accuracy, and precision of available immunoassays, as well as for the development of the next generation of immunoassays such as the homogeneous proximal linkage procedure described above. The hybridoma technology generates large numbers of in vitro cell lines, which can be clonally expanded, each producing a unique, genetically-defined antibody (monoclonal antibody). In contrast with polyclonal antisera, which consist of a pool of

different specific and non-specific antibodies, monoclonal antibodies possess a unique affinity towards a given epitope on an antigen.

The selection of the monoclonal antibody that best satisfies a required specificity is the most critical and cumbersome step in using the hybridoma technology. An appropriate screening, immediately after the primary activity test of the original cultures obtained from a fusion experiment, consists in the analysis of a large number of virus specific antibodies with a limited number of well-selected virus isolates belonging to different strain groups (e.g. PVY-N, PVY-O and PVY-C) as well as to different viruses (e.g. PVY, PVV and PVA). After subcloning the retained original cultures and further verifying their immunological activity, the next step in the selection procedure consists of analyzing a limited number of monoclonal antibodies with a large number of virus isolates covering, if possible, the spectrum of a worldwide collection. CIP can make a significant contribution in this respect. The listed selection procedure yields suitable antibodies for the immunoassays now available. However, the development of the next generation of immunoassays, such as the proximal linkage-system, requires a selection of at least two antibodies that can bind on a given antigen without much mutual interference. ELISA competition assays, where enzyme-conjugated and unlabeled antibodies (competing antibodies) are simultaneously reacted with a selected panel of viral antigens, can be used for this secondary selection.

An even more precise selection of the desired antibodies can be obtained by the reaction of the monoclonal antibodies with synthetic peptides derived from the amino acid sequence of the viral protein-coat. Since more and more viral genomes are sequenced, their primary coat protein structure can be obtained and exploited for the synthesis of a range of small overlapping peptides. This approach allows the mapping of some of the antibody binding sites along the linearized protein coat molecule. It also permits the identification of unique linear epitopes. In the case of the potyviruses, the protein-coat amino acid sequences of several key isolates are now available for comparative analysis. However, in order to recognize potential epitopes on a well-conserved common amino acid sequence, peptides might be used for the development of antibodies for diagnostic purposes.

The production of antisera specific to viruses that are difficult to purify (e.g. potato leafroll virus) is still not easily achieved, and prevents the use of modern immunoassays for routine phytodiagnostic purposes in some countries. Published data of protein-coat amino acid sequences can be analyzed (hydrophilicity profiles) and the structure of those peptides which can be derived, most likely represents antibody binding sites on the viral capsid. As a first step, a specialized laboratory should investigate which peptide is the most promising in producing useful antibodies. After this step, it would be less expensive to buy the selected peptide from commercial companies, for the easily repeatable "in situ" production of antisera, than to repeat the whole procedure dependent on the cumbersome virus purification method. The proposed approach could yield antibodies that might be cheaper than the currently available monoclonal antibodies.

Conclusions

The optimal application of modern immunoassays is satisfactory for the detection of the important potato viruses. The methods—none of which is perfect—cover the requirements for multiple applications in research and in routine diagnostic work in the laboratory and in the field. Emphasis has to be put on the development of more reliable reagents. The new reagents will not only improve the use of the currently available virus detection techniques, but they will also serve for the development of the future generation of more rapid and simple tests with a detectability equivalent to the most advanced existing procedures. The standardization of the reagents, the distribution of reagents, the mechanization of procedures for routine purposes, and training in the procedures involved, need to be continued.

Results on monoclonal antibodies used as examples in this paper were extracted from published data (Gugerli and Fries, 1983) and from the author's unpublished data. Unpublished PVY protein-coat amino acid sequence data are from P. Malnoe (RAC, Nyon). Unpublished results on peptide/antibody interactions are from collaborative research work by M. Vuonti (University of Helsinki) and the author.

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Hybridization Techniques for Viroid and Virus Detection. Recent Refinements

R. A. Owens¹

More than 8 years have elapsed since the initial report of practical diagnostic procedure for PSTVd based upon nucleic acid hybridization (Owens and Diener, 1981). During this time, several modifications of the original methodology have been reported. Perhaps the most important modification has been the substitution of single-stranded complementary RNA probes for double-stranded cDNA probes labeled by nick-translation. When RNA probes are used, non-specific binding of the probe to the nitrocellulose membrane can be virtually eliminated by a simple post-hybridization incubation with pancreatic RNase. Despite the well-known disadvantages of radioactive probes, most groups continue to label their RNA (or DNA) probes with [³²P] and visualize the resulting hybrids by autoradiography. Thus far, diagnostic protocols using biotin-labeled probes, require that samples be purified by phenol extraction before analysis.

Diagnosis of viroid disease by nucleic acid spot hybridization (NASH) was developed in our laboratory as a practical "spin-off" of our continuing investigations of the molecular mechanisms of viroid replication and pathogenesis, and NASH continues to be an integral part of these studies. Certain limitations, related primarily to a lack of sensitivity, have gradually become apparent. A detection limit of 1 pg PSTVd (approx.) may appear adequate, but a simple calculation involving the molecular weight of PSTVd (1.25×10^5) and Avogadro's number (6.023×10^{23}) reveals that there are actually 48,000 molecules in that 1 pg of PSTVd. Although CIP's experience with NASH suggests that this level of sensitivity is adequate for the reliable diagnosis of PSTVd, I would like to cite two other examples that illustrate a need for hybridization assays with increased sensitivity.

Symons and coworkers (1981, 1981a) have reported that the concentration of ASBVd may vary 1,000-fold within an individual infected avocado tree and 10,000-fold among different trees. Therefore, accurate ASBVd diagnosis requires the use of purified (and concentrated) nucleic acid samples isolated from pooled tissue samples. In asymptomatic AIDS infections as few as 1 in 10^5 peripheral blood mononuclear cells may be virus-infected. Because each infected cell contains approximately 6,000 molecules of retroviral mRNA, the overall level of virus-specific RNA available for assay is less than 1 molecule per 15 cells. Fortunately, several strategies to increase the sensitivity of conventional nucleic acid hybridization assays are now available. One

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group of strategies [e.g., the Polymerase Chain Reaction (or PCR)] seeks to specifically amplify the sequence of interest **before** analysis. Alternatively, amplification can be achieved **after** hybridization through the use of a replicable hybridization probe. In the paragraphs below, I have attempted to indicate briefly how these two different strategies may prove useful for the detection of virus and virus-like diseases of plants.

As discussed by Ehrlich (1989), the PCR is a method for the *in vitro* enzymatic synthesis of specific DNA sequences that requires two short oligonucleotide primers hybridizing to opposite strands of a DNA duplex and flanking the region of interest. Twenty to 40 repetitive cycles of template denaturation, primer annealing, and extension of the annealed primers by a thermostable DNA polymerase result in the exponential accumulation of a specific double-stranded DNA fragment whose termini are defined by the 5' ends of the primers. RNA can also be used as a template for amplification following the synthesis of complementary cDNA using reverse transcriptase. Studies with HIV (discussed in chapter 19 of Ehrlich, 1989) have shown that thirty cycles of PCR are sufficient to detect the presence of 1-10 molecules of proviral DNA in the presence of nucleic acid extracted from the equivalent of one million host cells. Although HIV-specific RNA does not appear to be amplified as efficiently as proviral DNA, many more copies are potentially available for amplification.

Figure 1 illustrates how the PCR can be used to amplify a specific portion of a member of the PSTVd viroid group. Sequence conservation within the central conserved region allows the same primers (e.g., HAD3 and RAO2) to be used with several different viroids. Although we have used low molecular weight RNA from infected plants (10-20 ug) as the source of viroid RNA in our experiments, total RNA preparations should also be satisfactory. Because this 10 ug of low molecular weight RNA will often contain as much as 10,000 pg (i.e., $\geq 0.1\%$ wt/wt) viroid RNA, our data do not yet allow any firm conclusions about the relative sensitivities of PCR and NASH for viroid detection. However amplification of viroid-specific cDNAs by the PCR has already greatly simplified the task of determining the nucleotide sequence of newly discovered viroids.

A second strategy to increase the sensitivity of nucleic acid hybridization involves the use of Amplifiable probes (i.e., molecules in which the probe sequence is embedded within the sequence of a replicatable RNA). In the protocol presented in Figure 2 the replicatable RNA is MDV-1, a small variant RNA molecule that is capable of autocatalytic (exponential) replication by QB replicase. This hybridization protocol differs from conventional NASH in several significant respects.

In contrast to conventional NASH, all hybridization reactions are carried out in solution rather than with a "target" RNA attached to a solid support. Furthermore, the single pathogen-specific probe has been replaced by a combination of "reporter" and "capture" probes. As shown in Figure 2b, both the reporter and capture probes contain sequences complementary to the target. The poly(dA) tail attached to the 3'-terminus of the capture probe allows the convenient

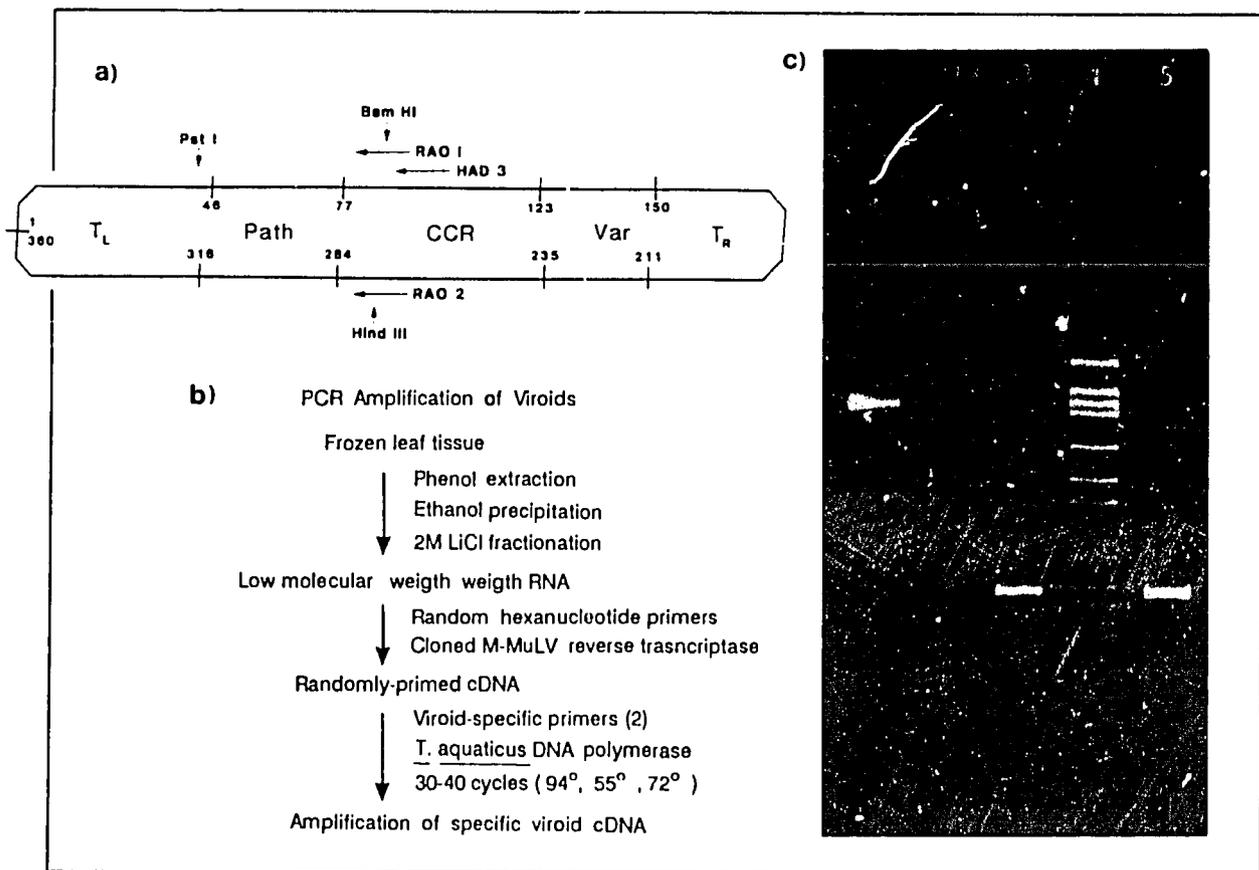
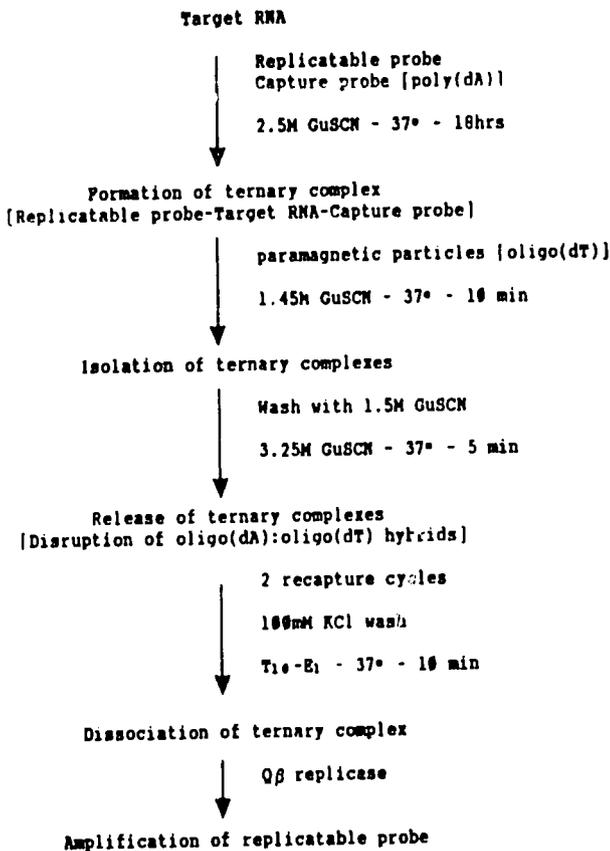


Figure 1. Amplification of viroid cDNA by the polymerase chain reaction (PCR). (a) Structural organization of TASV-Ivory Coast. Boundaries of the left (T_L) and right (T_R) terminal loop, pathogenicity (PATH), central conserved (CCR), and variable (VAR) domains within the rod-like native structure are indicated. *Bam*HI and *Hind*III are restriction sites present in various PSTVd-group viroid cDNAs. HAD3 (5'-CTCCAGGTTTCCCCGGG-3'), RAO-2 (5'-GCGGATCCGGTGGAAACAACACTGAAGC-3') and RAO1 (5'-CCCGGGGATCCCTGAAGG-3') are oligonucleotide primers used for amplification of viroid cDNA (HAD3 and RAO-2) and nucleotide sequence analysis. (b) Simplified protocol for synthesis and PCR amplification of viroid cDNA. (c) Visualization of a 217 bp cDNA representing the left side of a CEV-TASV chimera. Lane 1, 500 bp fragment derived from a bacteriophage lambda control DNA template provided by Perkin-Elmer Cetus; lane 2, no template; lane 3, randomly-primed CEV-TASV cDNA template; lane 4, *Hae*III digest of pBR322 recombinant pVB-6 (CRESS et al., 1983); and lane 5, TASV cDNA template. Positions of reference fragments containing 234 and 213 bp are indicated by dots in lane 4.

a)



b)

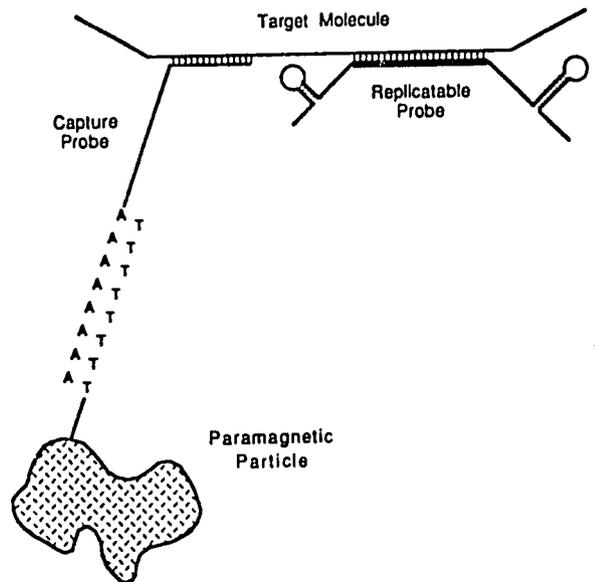


Figure 2. A reversible target captures hybridization strategy utilizing a replicable RNA probe. (a) Generalized protocol for the formation and purification of a ternary complex containing a "target" RNA that has been allowed to hybridize with an oligo(dT)-containing "capture" probe plus a "reporter" probe that is able to undergo autocatalytic replication by Q β replicase. (b) Schematic representation of the complex formed when a capture probe links a reporter probe-target RNA hybrid to an oligo(dT) group on the surface of a paramagnetic particle.

isolation and purification of the ternary reporter probe-target-capture probe complex via oligo(dT)-coated paramagnetic particles. Thompson et al. (1989) used a multiple capture-release strategy to dramatically reduce non-specific binding of ³²P-labeled reporter probe to the solid support (i.e. the paramagnetic beads). By using an amplifiable reporter probe that can be replicated autocatalytically by QB replicase, Lomeli et al. (1989) have been able to detect as few as 10,000 target molecules. This is an almost 50-fold increase in sensitivity compared to that of conventional NASH.

Neither the PCR nor the solution hybridization protocols described above may ever be suitable replacements for conventional NASH in the day-to-day operation of CIP. However certain features of each technique may prove quite useful to CIP's fundamental research program. Because the PCR requires only very small amounts of pathogen nucleic acid that need not be highly purified, it is ideally suited for the rapid characterization and sequence analysis of viroids and viruses. In several current solution hybridization protocols, the hybridization reaction is conducted in the same guanidinium isothiocyanate solution used to disrupt the tissue samples to be tested. Because no further sample processing is required, sample preparation is greatly simplified. It remains to be seen how successful this approach will be with plant tissue, however. Finally, there is no apparent reason why amplification before hybridization via the PCR could not be combined with amplification after hybridization via an amplifiable probe when maximum sensitivity is required.

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CIP Efforts at Virus Detection by Serological Means

J. Castillo¹

Efforts at CIP to detect viruses by means of serology are within the framework of the objectives of the Serology Project. These objectives are:

1. to produce antisera against common potato viruses as well as against little known and new viruses.
2. to evaluate serological techniques and their adaptation to CIP's needs.
3. to transfer this technology to the CIP Regions and to National Potato Programs by:
 - a. training for the production of antisera and the efficient use of serological tools;
 - b. producing standardized sets of serological reagents, to be sent to CIP's Regions, National Programs, and research institutions; and
 - c. encouraging the production of antisera and/or their processing by countries within the CIP Regions.

Antiserum Production and Distribution

The production of rabbit polyclonal antisera was started in 1974. By 1976, antisera to Potato Virus X (PVX), Potato Virus Y (PVY), Potato Virus S (PVS), and to the Andean Potato Latent Virus (APLV) were already available as crude antisera for worldwide distribution. Processed polyclonal antisera in sensitized Latex, or as immunoglobulins and enzyme conjugates packed in kits with the required chemicals for on-site testing, have been distributed since 1978 (Fig. 1).

Polyclonal antiserum is still the main source of antibodies. These are produced in rabbits which are easier to maintain and handle than goats, guinea pigs or chickens. The main drawback of polyclonals is their broad specificity. To purify these antisera and decrease unspecific reactions, several approaches have been tried with variable success.

Monoclonal antibodies have been produced since 1988. Hybridomas are known to produce single antibody molecules and therefore are of great specificity. They can be grown under laboratory conditions, producing any amount of antisera, and they can also be stored indefinitely in liquid nitrogen. After a period of adaptation, especially to water conditions, several clones have

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been produced to Potato Leaf Roll Virus (PLRV). These clones are under study to see how they can best be utilized.

Anti-idiotypic antibodies (A-I Ab) have been widely used in medicine, and they constitute one of CIP's approaches for producing low cost specific antisera under all laboratory conditions.

Number of samples (x1000)

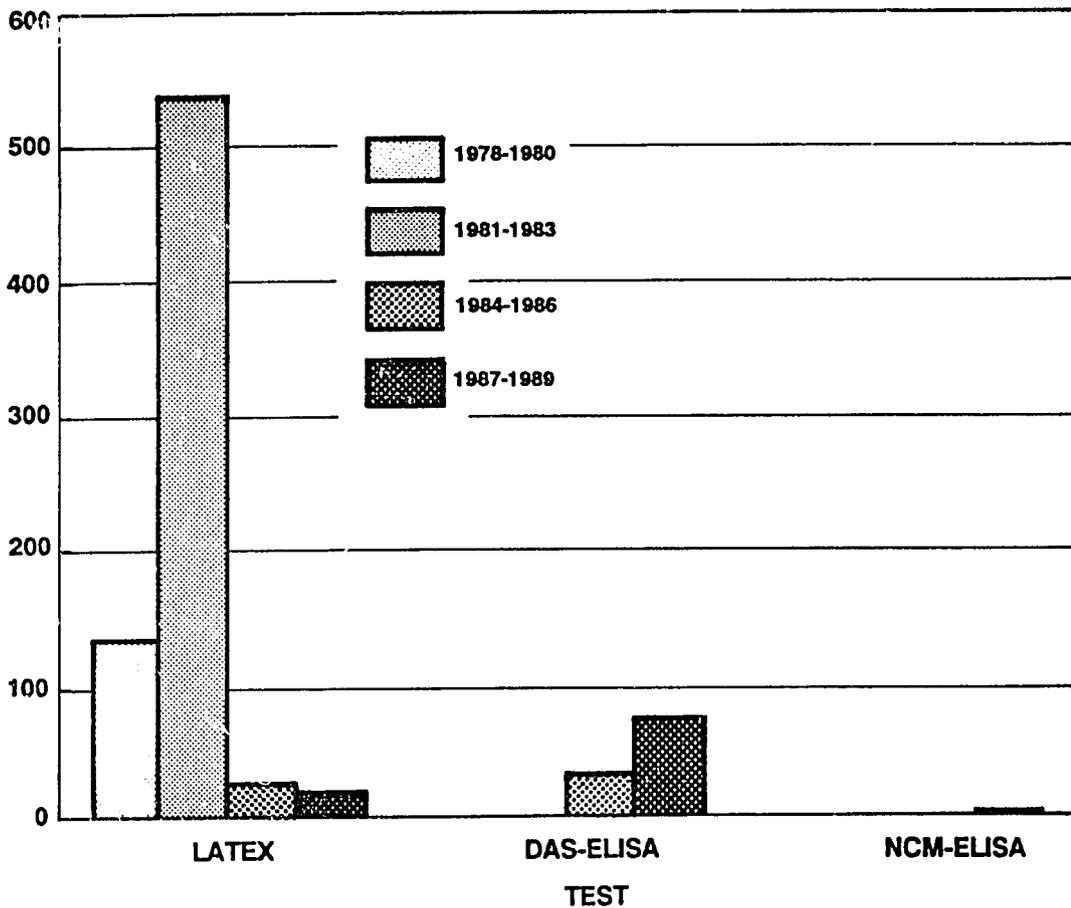


Figure 1. Distribution of standardized sets of serological reagents.

A-I Ab's are based on the antigenic properties of antibodies themselves. In this technique, an animal is injected with immunoglobulin G (Ab-1), generating Ab-2. A second animal is inoculated with the Ab-2, producing Ab-3, which has characteristics similar to those of Ab-1. Therefore $Ab-3 \simeq Ab-1$. This offers the appealing possibility of producing Ab-2's of selected monoclonal antibodies at CIP headquarters to be sent to the regions, where they could be injected into animals to obtain good antisera on-site.

Evaluation and adaptation of current and new serological techniques

Before being adopted, serological techniques are subjected to careful evaluation and adaptation to CIP's needs, in the following aspects:

1. Sensitivity: to detect viruses at low concentrations, i.e. PLRV or Potato Virus A (PVA).
2. Specificity: to detect viruses in any virus-host combination.
3. Adaptability: to allow for a change of chemicals and/or procedures.
4. Capacity: to handle samples on a large-scale.
5. Efficiency: in the use of antisera.
6. Clarity of reaction: "Yes" or "No" answers and possibility of the quantification of reactions.
7. Possibility of standardization to make results reproducible; and
8. Reasonably long shelf life for chemical and serological reagents.

Serological techniques currently being applied at CIP

Gel diffusion for the detection of Tobacco Ringspot Virus (TRSV), APLV and the Andean Potato Mottle Virus (APMV), and microprecipitation for PVX, PVY, and PVS were the first serological tests used, in 1973 and 1974.

In 1975-76, Fribourg and Nakashima reviewed and adapted the Antibody Sensitized Latex (ASL), described by Abu Salih, Murant and Daft in 1968. By 1977, ASL to PVX, PVY, PVS, APLV, and APMV were widely utilized at CIP's headquarters and in the Regions. Kits were also developed, even for polyvalent tests.

Shortly after the enzyme-linked immunosorbent assay (ELISA) was described by Clark and Adams in 1977, it was adapted for use with potato viruses (Salazar, 1979). In 1980, ELISA was a routine check for germplasm and breeding projects. Kits for DAS-ELISA were developed the same year. Direct and indirect methods have also been compared and antibody-labelling enzymes other than alkaline phosphatase were assayed (Salazar and Nakashima, 1983).

ELISA on nitrocellulose membrane (NCM-ELISA) is in the process of evaluation and adaptation. It offers some advantages over DAS-ELISA because it requires less antisera and sap. Good results have been obtained when specific antisera are used. NCM-ELISA kits have already been developed and sent to be tested in the Regions.

Transfer of Serological Technology

Serological technology is being transferred to CIP Regions and to national programs. Eventually other research institutions around the world will become beneficiaries, in the following ways:

1. *Training.* Preparation of antisera and procedures to conduct serological tests are precise operations requiring qualified skill and, therefore, training has become crucial. It is also essential for the regional staff to know how to use the serological tools that are being sent. Training is offered to two groups:

Technicians. Trainees are taught to process antisera for Latex and ELISA. They also learn other serological techniques.

Scientists. Includes advanced training in virus purification, antiserum production and processing.

2. *Production of standardized sets of serological reagents.* Careful and detailed analysis of each serological methodology and of the range of conditions under which these techniques can be carried out, have preceded the development of kits. Studies were also made to reduce costs by the re-use of some materials. Each of the kits produced contains enough serological reagents to assay about 600-800 samples. Chemicals are diluted and packed to be readily usable without the need for exact measuring.

3. *Encouragement of antisera production at CIP Regions, in connection with National Programs.* To carry it out, 2 phases were planned:

Phase I: For this phase, the following countries were chosen in the Regions to utilize the antisera produced at CIP headquarters in serological assays:

Region	I:	Colombia
Region	II:	Brazil
Region	V:	Tunisia
Region	VIII:	China

Phase II: Antisera production in countries that have adequate facilities for this approach. To implement it, scientists from INIPA (Peru), ICA (Colombia), CNPH (Brazil) and PRECODEPA (Central America) have received advanced training. CIP has provided guidance, supervision and quality control.

The anti-idiotypic approach should allow us to extend this type of work to virtually every developing country.

Development of Molecular Probes for Use in NARS

M. Querci¹

Profile

Diseases caused by viruses are particularly important in developing countries and need appropriate control. More than 25 different viruses are known to infect potato in the world and to cause significant yield reduction (Salazar, 1989). In addition, the Potato Spindle Tuber Viroid (PSTVd) has been recognized for many years as one of the most important pathogenic agents. Crop losses caused by the viroid may reach 64% (Singh et al., 1971).

CIP Research in molecular virology, initiated only a few years ago, was primarily devoted to the development of a rapid and sensitive method for viroid detection.

Because viroids do not contain a protein coat, they cannot be detected by immunological techniques. The previous methods utilized, such as inoculation onto tomato plants, which show clear symptoms after 2-3 weeks, or electrophoretic separation of the PSTVd RNA on acrylamide gels, were demonstrated to be inadequate when large numbers of samples had to be analyzed and when a high sensitivity of detection was required (Salazar, 1988). Following the recommendations of the II Planning Conference in Virology, CIP initiated efforts to search for modern techniques for PSTVd detection. Accepting the offer of Drs. T. O. Diener and R. A. Owens from USDA, we began collaboration using recombinant DNA technology. The recommendation to search for new indicator plant species for PSTVd detection was never implemented at CIP because we preferred instead to explore new possibilities.

Recombinant DNA technology allowed the detection of the nucleic acids of the infecting agents immobilized onto a nitrocellulose membrane by hybridization with labelled probes specific to the pathogen.

After the development of the nucleic acid spot hybridization for detecting PSTVd (by Owens and Diener), efforts were made to develop the technology at CIP. Dr. Owens' visit to CIP and CIP personnel visits to USDA were instrumental in the development.

Over the years, improvements and modifications of the technique increased sensitivity and specificity and opened up new, broader applications for this research area.

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The nucleic acid spot hybridization method is now routinely applied for the detection of both viroids and viruses.

PSTVd Testing

The importance of PSTVd in potato is due to its easy and rapid spread in the field, to the difficulty in identifying the pathogen in the infected plants, and to its recognized transmissibility through tubers and seeds (Fernow, 1970), interfering with germplasm movement from one country to another. The most effective measure to control PSTVd on potatoes is the production and distribution of seed material free from the viroid (Diener, 1979).

The development of different techniques resulted in a more sensitive detection of the pathogen and a significant increase in the number of samples which can be analyzed every year (Fig. 1).

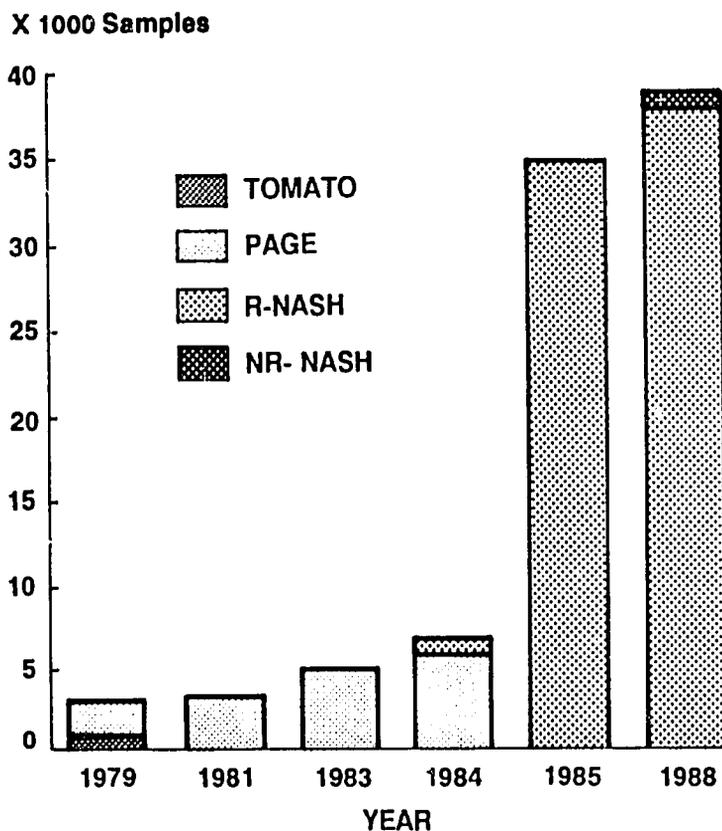


Figure 1. Evolution of testing methods for PSTVd detection permitted a significant increase in the number of samples tested/year.

At the moment, around 68% of the effort in PSTVd detection is devoted to the testing of CIP germplasm at Headquarters. In addition, the development of a testing kit permitted the detection of PSTVd in regions where no laboratory facilities are available.

Since 1984, approximately 42 countries have received CIP's support for PSTVd testing (Fig. 2). Samples are prepared and directly spotted onto the membranes included in the kit, which are then returned to CIP for processing.

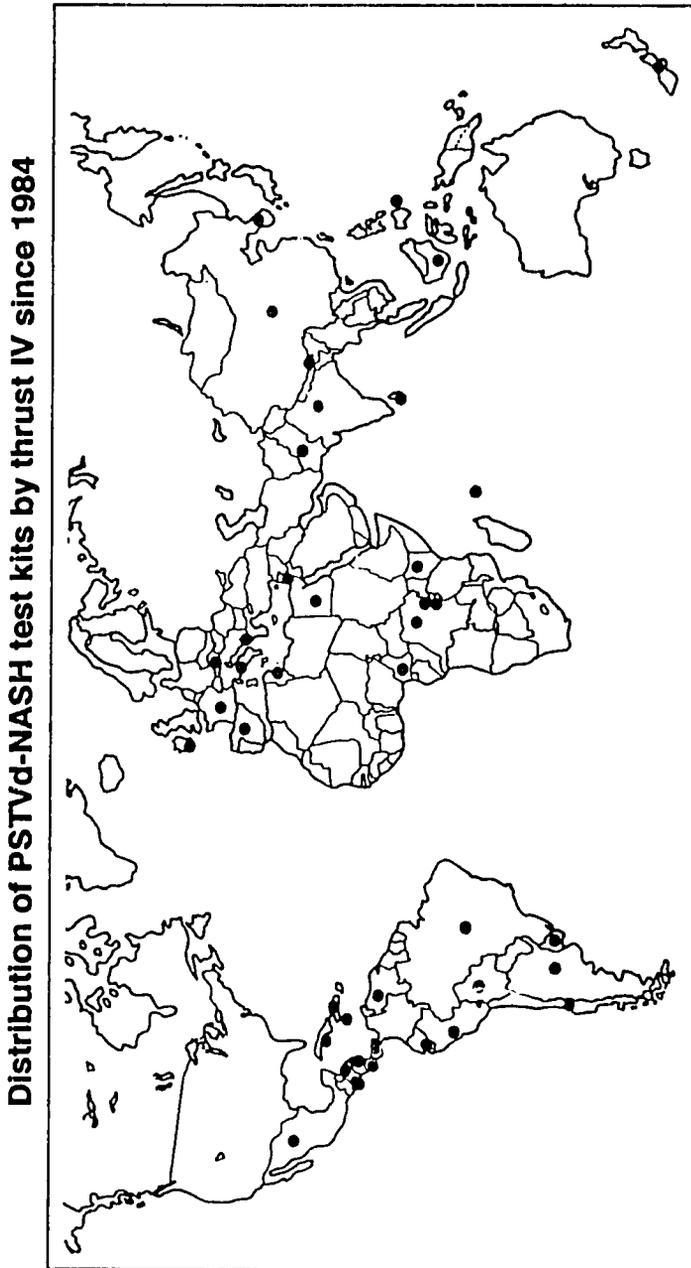


Figure 2. Distribution of PSTVd-NASH test kits by Thrust IV since 1984.

The growing interest in the development of techniques for the detection of pathogens by non-radioactive means led to the formulation of a new kit in which biotinylated probes are used. The technique is slightly less sensitive than ^{32}P labelled probes, but the results can be rapidly obtained since the test can be developed "in situ."

The non-radioactive kit, already delivered to some regions for testing, is still being improved to develop a simpler procedure and, if possible, to increase sensitivity.

Cooperative approaches

Regional and international cooperation is one of the ways of promoting technology transfer in developing countries. In the case of Peru, CIP offers information and facilities to help local institutions whenever possible. One example of this type of collaboration is the identification of the Avocado Sunblotch Viroid (ASBVd).

Since 1986, some avocado plants cultivated in Ica, Huaral and the Rimac valleys, have been exhibiting size reduction and fruit damage. Electrophoretic analysis and hybridization tests performed in the virology laboratory identified the ASBVd as the causative agent of the disease.

Maximum sensitivity of ASBVd is required for the indexing of avocados in view of the long-term implications of ASBVd infection of parent trees used as a source of propagation material, either seeds or graftwood (Barker, 1985).

Nucleic acid spot hybridization (NASH) testing of leaf samples using an RNA-probe is sensitive enough to detect less than 1 pg of purified viroid and a maximum dilution of infected leaf extract of almost 1/1000.

As shown in Table 1, different probes for the detection of several viroids have been prepared and are now available at CIP, offering the possibility of testing different crops for the presence of viroid-like diseases, so common in tropical and subtropical climates.

In spite of its small genome size, PSTVd is known to infect many plant species, causing symptoms in some and remaining symptomless in others (Singh, 1973). Sweet potato was not recognized as a host for PSTVd, but inoculation experiments at CIP demonstrated that approximately 30% of *I. batatas* cv Paramonguino plants were infected. PSTVd infection was demonstrated by NASH test and its presence confirmed by electrophoresis on acrylamide gel. Until now, no other *I. batatas* variety has been found on which PSTVd can reproduce. The discovery of the infection of *I. batatas* by PSTVd becomes important for CIP programs in disease control, especially because symptomless infection of sweet potato does seem to occur.

Table 1. Molecular probes available at CIP to detect viruses and viroids.

Viroids	RNA probe	DNA probe
Potato Spindle Tuber (PSTVd)	+	+
Citrus Exocortis (CEVd)	+	+
Avocado Sunblotch (ASBVd)	+	+
Tomato Planta Macho (TPMVd)	+	+
Tomato Apical Stunt (TASVd)	-	+
TASVd-PSTVd	-	+
Hop Stunt (HSVd)	+	+
Chrysanthemum Stunt (CSVd)	+	+
Viruses		
Potato Virus X	+	+
Potato Virus Y	+	+
Potato Leaf Roll Virus	+	+
Andean Potato Latent Virus	+	+
Sweet Potato Feathery Mottle Virus	+	+

NASH for virus detection

In order to achieve its objectives, CIP needs to assure the use and distribution of pathogen-free genetic material. For this reason, CIP has been emphasizing studies to detect lower virus concentrations. Molecular cloning experiments, developed with the support of the Plant Protection Institute in Beltsville, Md, produced cDNA libraries for most of the economically important potato viruses.

The procedure has been used for PVX, PVY, APLV, PLRV, and SPFMV; some of the clones obtained have already been analyzed and used as probes for both virus detection and studies on the mechanism of infection and strain relationships. In fact, molecular cloning and hybridization procedures are also applied for basic investigation of viral genome structure and resistance mechanisms.

Several PVX isolates have been studied and the specificity of hybridization confirmed differences between serotypes PVXO and PVXA.

The division reflects dissimilarities in the coat protein structure and, consequently, in the genome.

Our interest, is in the understanding of the molecular mechanism involved in the process in which the isolate HB is able to break immunity to PVX in potato.

Recent approaches also led to the detection of low concentrations of sweet potato viruses by NASH and the progress of virus infection in *Ipomoea* plants inoculated with SPFMV.

Detection using molecular probes starts 6 days after inoculation; first symptoms appear 10-11 days later. The degree of hybridization obtained, reflecting the concentration of virus present in the sap, indicates a maximum of virus concentration between 13 and 20 days after inoculation and a higher accumulation in the central part of the plant. The probe was sensitive enough to detect less than 0.2 ug of purified virus, i.e. approximately 9 pg of viral RNA.

Molecular probes for virus detection, until now used mainly for investigation or as support for the immunological procedures, have been shown to have more potential uses.

Immunological methods for virus detection are already well established, and because of the sensitivity of the ELISA procedure, most plant-virus diagnostic work makes use of this technique. There is great potential for the use of molecular hybridization analysis for the diagnosis of plant virus infection in cases where the virus concentration in the infected tissue is lower than the limits of serological detection. They will also be useful in cases where it is difficult to purify enough virus to obtain high quality antisera.

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Virus and Viroid Detection Techniques: Status, Constraints and Needs in NARS of Latin America

O. A. Hidalgo¹

Introduction

In Latin America, diseases induced by viruses and viroids are among the most important diseases, because they are transmitted through tuber seed as well as true seed. These tuber- or seed-borne diseases then occur with more or less intensity in the following season, reducing yields in different degrees, depending on the pathogen or combination of pathogens. In the majority of Latin America countries, most of the virus diseases in potatoes have been recognized, and they are being controlled by standard procedures.

The detection and control of virus and viroid diseases of potato in Latin America are more advanced than those of sweet potato. Significant progress has been made in Latin America in the characterization of potato pathogens, antisera production, and in the use of serological or other detection techniques in routine work for seed production and for research purposes.

Almost all countries in Latin America are now capable of employing serological techniques at their own facilities. Specialized training activities in these areas have covered the needs of the NARS in Latin America.

This document summarizes the current situation, constraints and needs of the Latin America National Potato Programs in the area of virus and viroid diseases of potatoes, emphasizing aspects of the methodologies for their detection. Brief considerations on the actual situation in the identification of sweet potato viruses are also included.

Current Situation of the Identification of Virus and Viroid Diseases of Potato

The most important and predominant virus agents have been recognized in the potato production areas of Latin America, either by specific studies or through the routine serological work performed for seed production purposes.

Among the predominant virus diseases recognized in Latin America are those induced by PLRV, PVX, and PVY (Salazar, 1980; Apablaza, 1980; Hinojosa, 1973; Santillan et al., 1980; Corzo, 1989). In addition, two Andean viruses, APMV and APLV, have also been recognized in

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Peru (Fribourg et al., 1978), Colombia (Corzo, 1989; C. S. de Luque Personal Comm., 1989), Chile (Apablaza, 1980; 1987) and Bolivia (Fribourg et al., 1978; Jones and Fribourg, 1978); in addition, APMV, has also been reported in Brazil (Avila et al., 1984; Barron and Salazar, 1984). Other viruses of varying economic importance have been found in CIP's germplasm collection or in native cultivars. These include: PVT (Abad and Fribourg, 1980; Salazar and Harrison, 1978), TSV (Abad, et al., 1982), AMV (Abad and Salazar, 1984), SB-22 (Fuentes and Jayasinghe, 1987), SALCV (Hooker et al., 1985), mot-top (Salazar and Jones, 1975), PVM (Motta, 1986), PVA (Nakashima et al., 1987) CALICO-TRSV (Fribourg, 1975), etc. In addition, there are three viruses that have been found in some Latin American countries (Table 1).

Table 1. Viruses found in some Latin American countries

Virus Disease	Importance	Countries
Tomato spotted wilt virus (TSWV)	Low	Brazil, Argentina, Uruguay
Virus (?) Mosaico Deformante ("Deformante" Mosaic Virus (?))	Low	Argentina, Uruguay, (?)
Potato yellow vein virus (?)	High	Colombia, Ecuador, Venezuela

There is no known report that yellow vein (amarillamiento de venas) is a disease induced by a virus, but all reports on its transmission suggest it is virus. Symptoms of disease have been successfully reproduced in potatoes by grafting and by using white flies (*Trialeurodes vaporarorum*) as vectors. Yellow vein is acquiring greater importance in Colombia, Ecuador and Venezuela because of its rapid dissemination (Saldarriaga, ICA/CIP Project Reports, 1988; 1989).

The viroid disease induced by PSTVd has been found in some CIP germplasm (Salazar et al., 1983). In most of the Latin American countries PSTVd has been tested by NASH; membranes have been sampled in the countries and processed at CIP with negative results. Extensive surveys have been conducted in Chile, Argentina, Brazil, and Venezuela, also with negative results (Salazar, personal communication, 1989).

Current Situation of the Identification of Sweet Potato (SP) Virus Diseases

Few SP viruses have been detected in Latin America (Salazar, 1987). The SP Vein Mosaic (SPVMV) was identified in Argentina but only some of its characteristics have been determined (Nome, 1973). This virus has not been reported in other parts of Latin America. The SP Feathery Mottle Virus (SPFMV) has been detected in Argentina (Nome et al., 1980), Venezuela (Olivero et al., 1984) and in Peru (Lopez and Salazar, 1987; Salazar, 1987).

Symptoms that may resemble SPFMV have been noted in several Latin American countries, but no identification studies have been carried out. In Brazil a mosaic on SP was reported in a region of the state of Rio de Janeiro. Virus particles (15 x 800 nm) were observed in plants with mosaic symptoms and chlorotic local lesions on *C. quinoa* were obtained by mechanical transmission. Symptoms and partial results were associated with a virus of the PVY group (Kitayama et al., 1975). Greater efforts are needed to characterize SP viruses in Latin America.

Current Situation of Constraints and Needs of Serological Techniques and Antisera Production for Potato Viruses

The use of the serological techniques Latex and ELISA for tuber-seed production as well as for research purposes is now routine in almost all Latin American countries (Hidalgo, 1989; Hidalgo and Rincon, 1989). Both serological techniques became popular in these countries because of the initial distribution of experimental serological kits and because of the extensive in-service training in virology and seed production offered for Latin American scientists (Salazar, 1989; Hidalgo, 1989).

In the early 1970's antisera for PLRV and other antisera needed for research at a few selected NARS were imported from the U.S.A. and some European countries. After 1975, when CIP started producing sensitized Latex, interest in serological techniques increased substantially. In 1979, interest again increased when the ELISA experimental kits were distributed and training was intensified (Salazar, 1989). Since that time, local production of antisera was recognized as an important component for the increase of local seed production. To meet local demand for antisera, a two-phase plan was initiated in 1983 with EMBRAPA-Brazil and supported by CIP (Avila et al., 1989; Avila and Dusi, 1989; Salazar, 1989). During Phase I, EMBRAPA prepared and distributed sensitized Latex kits using antisera produced at CIP, but processed in Brazil. ELISA kits were also produced under the same scheme and included antisera for PVX, PVY, PVS, and PLRV. Phase II, initiated simultaneously with Phase I, was completed in 1987 with the entire local production of antisera for the previously mentioned viruses plus APMV. CIP offered EMBRAPA *in situ* assistance, specialized training and technical support in antiserum production. Transfer of this technology to the private sector was initially attempted by EMBRAPA, but this sector did not show much interest at that time. At the present conference, a Brazilian scientist from EMBRAPA will illustrate the details of this process as well as the progress made recently in this area. A similar approach was followed by Argentina: in 1987 INTA-Cordoba presented serological kits for Latex and ELISA as part of the Phase I activities (Salazar, 1989). Phase II is continuing but once again no continuous supply of antisera for the local seed industry has been obtained yet.

The supply of antisera for PRECODEPA, the network that serves Mexico, Central America, and the Caribbean, was provided by CIP. CIP produced antisera for them as part of its responsibility as a network member. A similar agreement was made with the Peruvian

National Program to meet the needs of their Prebasic Seed Production Project (Salazar, 1989; Hidalgo, 1989).

The other Andean Potato Programs (Bolivia, Ecuador, Colombia, and Venezuela) received antisera from CIP for their small prebasic seed projects and for research. Venezuela and Bolivia, however, increased their operations and had to import from Europe antisera for PLRV detection. At present, a two-phase project, similar to those of Brazil and Argentina, has been initiated with ICA-Colombia, as part of CIP support to PRACIPA, the potato network of the Andean Countries.

At present, no major constraints to the use of serological techniques in the Latin American NARS are envisioned. Scientists have been sufficiently exposed to the serological techniques and they are now being practiced routinely. However, the National Program scientists must receive further training in the new serological techniques being developed, and hopefully will simplify them. Significant progress in the production of antisera has been made with official institutions of Brazil and Argentina, but strategies for large quantity production and continuous supplies of antisera, preferably with the participation of the private sector, must be developed. Antisera needs for the South Cone countries were calculated at the end of 1987 during a Seed Planning Conference of PROCIPA, with representatives of public and private companies of these countries (Hidalgo and Rincon, 1989). It was estimated that the South Cone countries required 977,000 tests per virus, per year (Table 2). Notice also in Table 2 that the private sector, composed of companies that produce prebasic and basic seed, and a company dedicated to potato virus diagnosis, reported that it would require almost 90% of the total estimated demand. Value of serological tests (Table 2) was estimated at approximately US\$60,000/year/virus.

Table 2. Estimated annual antisera needs per virus in the South Cone countries of South America^a.

Country	Number of Tests (x1000) per virus		Total
	Private Sector	Public Sector	
Argentina	491	10	501
Brazil	309	22	331
Chile	75	50	125
Paraguay	-	-5	5
Uruguay	--	15	15
Totals	875 (90%)	102 (10%)	977

^aSource: Hidalgo y Rincón (edits.), 1989.

Similar numbers of tests for the rest of Latin America (the Andean countries plus Mexico, Central America, and the Caribbean) might be required when maximum needs are taken into consideration. Considering the detection of five potato viruses in the seed production process, Latin America's antisera requirements might create a half-million-dollar industry antisera production.

Current Situation, Constraints to and Needs in PSTVd Detection

CIP offered assistance to all Latin American countries to conduct tests for PSTVd detection. NASH kits were developed and distributed to countries requesting them (Salazar, 1989). For Brazil and Argentina, a special service for PSTVd detection using NASH is being offered by CIP to private companies, at minimum cost. This assistance will be offered only until a private company is capable of offering the service. (Hidalgo and Rincon, 1989).

New non-radioactive techniques such as those already developed by CIP need to be refined and simplified if possible in order to use them under local conditions of NARS.

The demand for PSTVd tests is not too high, but the tests are required because of the intensive manipulations done with rapid multiplication techniques that increase the chances of PSTVd spread. Tests conducted so far in Latin America have shown negative results for PSTVd.

Virus and Viroid Detection Related to Tuber-Seed and TPS Production

Progress on seed production has been made in Latin America and is attributed to government decisions to support seed programs, active participation of the private sector, use of new technologies, a sustained training program, and technical assistance on demand. Progress has been estimated by comparing the situation of the existing seed programs in 1975 vs the situation in 1987 (Hidalgo, 1989). By 1987, prebasic and basic seed production programs existed in almost all Latin American countries. These in turn led to more formal seed certification programs. Progress has been more noteworthy in the South Cone countries because of the greater participation of the private sector (Hidalgo and Rincon, 1989). The rapid advancements can also be explained by the extensive use of the new technologies such as *in vitro* tissue culture, rapid multiplication techniques, and serological techniques. The rapid multiplication and serological techniques have played an important role because they made possible the rapid multiplication of materials, at the same time assuring the appropriate sanitary quality. Without this assurance, advancements might not have been possible. The intensive training program, carried out by National Programs and CIP in all Latin American countries during the last decade, positively influenced these developments.

In Chile, CIP has been carrying out the large-scale detection of PSTVd for TPS production since 1983. In a collaborative project carried out in southern Chile, the local research institute (INIA) and CIP produced large amounts of seeds for distribution by CIP. A large number of samples were taken from tuber sprouts before planting time, from leaves

during the crop-time and from seeds (TPS) at harvest, were placed in membranes in Chile which later on were processed at CIP using the NASH technique (Rojas and Hidalgo, 1987). Negative results were obtained, permitting CIP to export TPS to many countries in the developing world.

New, more sensitive detection techniques and more simplified ones are expected to be released in the near future as products of basic and collaborative research. National Programs and CIP scientists need to be continually informed on the new knowledge being generated to improve the efficiency of the seed programs. This is a continuous process which so far has been very successful in Latin America.

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Needs, Status and Constraints in NARS North Africa and Middle East

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Specificities of the potato industry in the Region

The potato industry in the Middle East and North Africa is, in the majority of cases, based on seed imports (generally from Europe and in a few cases from North America).

All countries have a "multiple-cropping" pattern. Several crops are grown per year, one of them utilizing the imported seed; the others are planted with local seed generated from the imported lots.

Several countries are linked to the European potato markets through:

1. the export of "new potatoes" in the early spring, and
2. the import of ware potatoes during the periods when the supply of local consumption potatoes is low.

The local seed programs

Over the past 20 years, several countries of the Region have initiated local seed multiplication/ production/ improvement programs. These programs aim either to produce good quality seed for those crops where no European seed is available or to produce local seed to substitute for a portion of the imported seed.

More recently, some countries have started programs to locally produce basic seed, which could subsequently be multiplied within the framework of pre-existing programs.

Virus testing and seed control laws were introduced in several of these programs.

Needs and constraints with reference to potato virology

1. *Genetic resistance.* Because of their links to European markets, the majority of the countries are bound to grow "European-like" varieties. This means that the introduction of CIP-

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generated resistant varieties has limited prospects unless the resistance is coupled with the required agronomic traits (earliness, shape, color, cooking quality, etc.).

2. *Virus detection.* ELISA testing is well known and applied throughout most of the countries of the region at research level. Nevertheless, its widespread use in the seed programs is limited by its high cost.

The plates, sera, buffers, etc. are imported, and the availability of foreign currency to the seed programs is limited.

For those countries (e.g. Tunisia and Morocco) which have started antisera production, the cost of the enzyme and plates remains prohibitive. Generally, the national seed programs pay local taxes on these imported supplies and this fact should be taken into consideration when calculating the cost-per-test and comparing it with the cost paid by CIP.

3. *Norms and procedures for seed control.* In general, one could say that the existing seed programs have a tendency to "over-test" for virus diseases. Field control and visual identification are not always properly called upon.

Few countries have started research to establish their local norms and procedures for seed control. Generally, European norms are adopted without the proper adaptation.

4. *The PSTVd dilemma.* Several countries could (and would) import seed from North America, especially in the years European seed is scarce and prohibitively expensive.

The existing European quarantine rules make it risky for these countries to open up to the North American market. They might see their exports of "new potatoes" to Europe limited or even prohibited, since these "new potatoes" have been hypothetically exposed to the risk of PSTVd infestation while grown in areas planted with North American seed. For this reason, several countries have expressed the need to acquire the necessary facilities and methods for PSTVd testing in order to protect their exports. Unfortunately, the available NASH technique is costly and not easily transferable.

Implications for the International Community (including CIP)

The needs and constraints outlined above could be better addressed by:

1. the availability of virus resistance coupled with adaptation and acceptable agronomic traits;
2. the search for cheaper detection methods even if they are somewhat less sensitive, starting with local antisera production;
3. enhanced cooperation in "seed production research" in order to establish locally acceptable production patterns with locally generated seed control norms and procedures;
4. the search for a "simpler" PSTVd detection technique which these countries could apply with limited investment in equipment and reagents.

Detection of Potato Viruses and Viroid in India

SM P. Khurana¹

The potato is one of the most important vegetables in India. Today it occupies about 0.84 million ha., but yields are low, averaging 15 t/ha, despite potato's high yield potential. By the turn of the century, not only will the area, planted with potato be doubled, but production will be improved by doubling the average yield. When seed imports were banned in the 1940s, potato seed production started as an ad hoc scheme of the ICAR at Kufri, following the guidelines of seed production in the UK (Pal., 1943).

History and Economic Importance

Initially, the prevalence and distribution of potato virus and virus-like diseases were investigated in relation to production (Vasudeva and Azad, 1952), but in the 1960s, the focus shifted to the spread, diagnosis and detection of viruses for their control (Nirula, 1962; Nagaich et al., 1969). Prior to caring for seed health, the virus incidence in seed crops was rather high in both the low lands (70-95%) and the highlands (20-90%). This was mainly due to rapid degeneration of seed stocks and poor crop management (Vasudeva and Azad, 1952). The situation, however, has changed in the last two decades because of the use of elite seed from hills alone and the development of the "Seed Plot Technique" for seed production in lowland areas. (Nagaich et al., 1969). Common mild/latent viruses, such as PVX and PVM are the most important viruses in Indian potatoes having an incidence of up to 70-80% (Khurana, 1985). Among aphid-borne viruses, PVY and PLRV are common, but their incidence is usually low (below 5%), particularly when crops are raised from healthy seed and prevention measures are adopted. Both *Myzus persicae* and *Aphis gossypii* are important aphid vectors; the former has greater significance due to its early appearance in lowland crops (Singh et al., 1982a). PVA, PAMV and PVM are of little importance due to very low/stray incidence (Khurana, 1985; Khurana and Singh, 1986). Similarly, the incidence of PVY, TNV, TMV, and TBSV is negligible (Khurana and Singh, 1983). TSMV has been rampant (up to 90%) in early-planted fields (Khurana et al. 1989), causing extensive stem/foiar necrosis in the central plains. In addition a mild strain of PSTVd was observed in cv. Kufri Chandramukhi and recently, a severe strain, causing unmottled curly dwarf, was found in cv. Kufri Jyoti. Yield losses of up to 20% due to PVX and PVS are common (Khurana and Singh, 1986). Higher yield depressions (40-60%) were observed in the spring crop than in the autumn crop (7-20%) due to PVY and PLRV (Singh et al., 1982b; Khurana and Singh, 1986). Degeneration of common Indian potato cultivars is rather slow in the NW/NE plains due to

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adoption of the seed plot technique (Singh et al., 1981; Vashisth et al., 1982) but it is rapid in the spring crop of tropical areas (Singh et al., 1984; Garg, 1985).

Detection

Virus

For production of nucleus/breeders' seed, a large number of visually healthy plants of desired varieties are selected at CPRS, Fagu (Shimla hills) at 10 m.a.s.l. on the basis of their being true-to-type in appearance, purity, and high yields. Four tubers from each such plant were grouped as one "clone" and indexed by grow-out tests of their single eyes. The eyes, scooped from tubers selected in hills, were indexed in the plains in winter and in the highlands in summer since no controlled-environment facilities exist. The plantlets were closely observed for 6-8 weeks and then subjected to the following tests: (i) chloroplast agglutination for PVX/S(M); (ii) biological for PVY/A on "A6" leaflets; (iii) earlier, phloroglucinol-HCl was also employed for histochemically detecting PLRV, but later Rhodamine-B was used (Lal and Khurana, 1983; S. Kumar et al, 1988).

With the imported kits, use of the ELISA technique was started in 1984 for indexing PVY and PLRV in seed at CPRI, Shimla. It was also tried for PVX/S(M) to test the reliability of the chloroplast agglutination test for PVX/S using indigenous antisera.

Latex kits from CIP for PVY, PVS, PVM, and APLV/ Δ PMV were also randomly used both for the routine indexing and quarantine purpose.

ISEM was also standardized for PVX, PVY and PVM in 1984. It was found to be a very effective detection technique. A simpler technique for ISEM was also standardized for detecting the virus in vector aphids and tuber sprouts (Garg et al., 1988b). For TSWV, ISEM was effective only when the samples were prefixed with glutaraldehyde, but for other (flexuous) viruses, the fixation affected particle trapping.

Since 1985, efforts have been made to standardize DAS-ELISA using indigenous antisera and enzymes. Initially, we successfully used horseradish peroxidase and locally produced antisera for PVX/S and Y for detection of the homologous antigens. Recently we adopted penicillinase-based ELISA for detection of PVX, where the whole antibody was used for trapping and conjugated F(ab)₁² for detection (Singh et al., 1989). Both of the indigenous enzyme-tagged ELISA systems were as effective as the ELISA kits (alkaline phosphatase) in detecting the common potato virus(es).

Currently, we are involved in developing quicker and more sensitive variants of ELISA. A simpler form of dot-ELISA (Bantari and Goodwing, 1985) has been standardized where we can analyze all samples using single (common) extraction buffer and avoiding the heat-treatment of sap for PLRV. In this case, use of a "template" for spotting samples increased the test's sensitivity.

Similarly, a simpler version of dot-ELISA was developed for detection of PLRV in single *M. persicae*. Also a very quick and reliable cocktail-magnetic ELISA (using plastic-coated magnetic beads) for both PVX and PLRV has been made in cooperation with Prof. Bantari, St. Paul, USA for detecting more than one virus in a sample at one time. Use of mixtures of polyclonal antisera for simultaneous detection of any one of the three or four viruses has been tried. At times, the backgrounds are high enough to give pseudo-positive results. It may be worthwhile to develop artificial polyvalent antisera from a mixture of different monoclonal antisera to overcome this problem.

With the help of ISEM sero-surveys and by ELISA, it was possible to pinpoint a number of common weeds in potato fields carrying potato viruses X, S, and Y. An association of a nuclear *rhabdo* with phloem-limited yet true seed-borne virus has been discovered from potatoes showing "leaflet stunt" and "leaf roll" symptoms, respectively.

Viroid

The mild isolates of PSTVd were readily detected on both potato plants and true seeds, and biologically on test plants of tomato cv. Rutgers developing stunting/albinism and vernal necrosis. However, these symptoms did not appear on *Scopolia sinensis* due to lack of production of the local lesions. Viroid association was also confirmed on the basis of absence of association with any virus(es); raised "eyebrows" russetting/roughening and discoloration of tuber skin; true-seed transmission; and ultra-structural variations in apical leaves.

PSTVd-infected plants had subcellular aberrations, viz. cell walls of young apical leaves were undulated and irregular in thickness, and contained large numbers of paramural bodies (Garg et al, 1988b). Cells from chlorotic areas had disorganized chloroplasts and a large number of electron-dense osmiophilic globules. The necrosed cells, however, contained condensed lamellar aggregates. Similarly, the viroid in wild tuber-bearing *Solanum* spp. has been detected on the basis of the above factors. The 'WPV' needs to be confirmed on the basis of mol. wt., nucleotide sequencing, etc. and for distinctness despite its greater virulence, wide host-range, ability to cause systemic necrosis/ash grey mottle in *Gomphrena globosa*, etc.

Conclusions

For the goal of enhancing production of virus-free seed in India, it is important to continue intensified research on detection and spread of potato viruses and viroids, including the development of cDNA and cRNA probes for both viroids and viruses. There is a lack of trained manpower, regional facilities for antiserum production and for application of latest immunodiagnostics for guaranteed supply of healthy (virus-tested) nucleus seed stocks. A global approach should be adopted for their management not only within the region country but also internationally to prevent the spread of viruses to newly-developed regions. CPRI and CIP can collaborate in this regard and help developing countries to achieve their aim of enhancing potato yields and production.

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Virus and Virus-Like Diseases of Potato and Sweet Potato in South East Asia: Status, Constraints and Needs

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Introduction

In South East Asia, average yields for potatoes and sweet potatoes remain far below their potential. Although several countries now have average potato yields of around 20 t/ha, there is still room for further improvement. Potential yields are calculated to be from 40 to 70 t/ha, depending on the environment. For sweet potatoes, only South Korea, Taiwan and some areas in Viet Nam and the Philippines have yields of 18 to 20 t/ha. Production levels in most of S. E. Asia are much lower, averaging 10-12 t/ha.

The role of viruses and virus-like diseases becomes increasingly important as one comes closer to the yield potential for the crop in a specific environment. This paper is a personal assessment of existing production levels, and the importance of viruses in limiting yields in S. E. Asia for both potatoes and sweet potatoes. Many of the official yield statistics are not correct. I have taken the liberty here to give my personal estimates and also to assess the future role of viruses as we strive to come closer to the yield potential for these two crops. Population pressure is forcing more intensive agriculture leading to higher yields.

Present Status and Constraints

Potato

At present viruses do not significantly limit yields in S.E. Asia (Table 1). Even though total improved quality seed represents only a small percentage of the total requirements, the general health standard of most crops in S. E. Asia is good. S. Korea was successful in overcoming a serious virus problem in the 1960s and 1970s with a combination of tissue culture, virus eradication and an excellent basic seed program (Kim et al., 1986). That program has resulted in a doubling of yield in the past 10 years, to 20 t/ha. A large unofficial seed program parallel to the national program has permitted most farmers to plant virus-free or slightly infected seed on most of the national area devoted to potatoes. The yield gap in S. Korea is primarily attributed to premature harvesting of the potato crop to plant rice. PLRV as well as PVY are the two major

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viruses present in S. Korea; they continue to reduce yield, particularly at low-elevations, where farmers replant small tubers from their previous crop.

Viet Nam is a country with relatively high aphid populations during the January-March season in the Red River Delta. The potato variety they predominantly utilize (Ackersegen) has excellent resistance to PVY. PLRV is not a problem as the 9-month storage period in diffuse light storage (DLS) seems to eliminate this virus if it is present. Although PVX and PVS are present, they may reduce yields by 10% when combined with mild levels of PVY. In the S. Viet Nam highlands farmers have effectively used tissue culture to renew their seed stocks after 3 generations (Uyen and Vander Zaag, 1985). In Viet Nam, the major component contributing to the yield gap is physiologically old seed tubers (after 9 months of storage in DLS) as well as a lack of inputs.

Taiwan has developed an excellent small scale seed program that has helped to completely overcome the virus problem (Ho et al., 1990). The highland areas of Indonesia, Papua New Guinea, the Philippines and Malaysia have naturally low levels of virus infection (humid conditions reducing aphid populations). Burma is a unique example of a country with 50 years of potato production using the cultivar Up-to-Date, without a seed program nor imported seed and still having only very slight virus-infection levels (Table 2). The seed source had no influence when yields were less than 20 t/ha (data not presented). With favorable inputs during the irrigated spring crop, yields came closer to the potential yield and the imported seed source gave higher yields (Table 3). We are now re-evaluating the need for a national seed program in Burma. The S. Pacific Islands (New Caledonia, Vanuatu, Fiji, Tonga, and French Polynesia) import most of their planting material from Australia. This seed is very healthy and there has been no reduction in yields due to viruses.

Most countries in S. E. Asia have trained virologists who use antisera and latex in detecting viruses in their seed production programs (Table 4). S. Korea and the Philippines have the largest laboratories for testing large numbers of samples. With CIP technical support, S. Korea and Thailand have developed their capabilities for producing antisera. PVX and PVS antisera production has been relatively easy and antisera for PVY are now being developed. Antisera for PLRV appear to be more complex to produce. PSTVd is not of any practical importance in S.E. Asia. CIP Region VII routinely evaluates its germplasm to assure that it is PSTVd free.

Sweet Potatoes

Little is known about viruses infecting sweet potatoes, although the relative area of sweet potato is large, with Indonesia, Viet Nam and the Philippines being among the top 7 nations in production (Table 5). Viruses are present in most countries of the region, but their influence in reducing yield is believed to be relatively small. However, this subject needs careful study to determine which viruses are present and how important viruses are in reducing yields at present. Sweet potato yields are relatively low as most subsistence farmers provide no inputs for growing the crop. With such a large gap between actual and potential yields, it is certain that viruses are

Table 1. Status of potato production and seed tuber sources and use in S. E. Asia as of 1989 (estimated).

Country	Area '000ha	Tissue culture	Certified Seed		Percent of total needs	Estimated average yield ---- (t/ha) ----	National potential yield	Yield gap due to viruses (%)	Major factor to large yield gap
			Annual Local prodn ---- (t)	Imported ----- (%)					
Burma	15	0	30	0	< 1	10	20	0	inputs
Indonesia	35	xx	500	1000	2	15	30	5	late blight
Malaysia	< 1	x	0	0	0	20	35	5	late blight
S. Korea	30	xxx	5000	0	20	20	35	15	premature harv.
Papua New Guinea	2	x	80	0	25	12	25	0	inputs
Philippines	5	x	200	50	2	20	40	5	late blight
Taiwan	3	xxx	1600	0	50	20	40	0	premature harv.
Thailand	1	xx	20	200	25	20	40	5	seed quality
Vietnam	40	xx	1000	100	3	10	25	10	physiological age
S. Pacific	1	0	0	1000	80	17	35	0	inputs

o, not used for potato; x, germplasm multiplication on a small scale; xx, moderate scale multiplication; xxx, large scale use.

Table 2. Virus levels tested by ELISA in 1989 of Up-to-Date harvested in August 1988 and April 1989 from introduced foundation seed as well as for local Up-to-Date and from the Rangoon market.

Seed source	Year	Virus			
		PVS	PVS	PVY	PLRV
Ireland	1987	*	-	-	-
Scotland	1986	-	-	-	-
Australia	1984	-	-	-	-
Australia	1983	-	-	-	-
Local (+e selection)		-	-	-	-
Local (seed farm mult.)		-	-	-	-
Local (market)		+(25%)	+(12%)	+(12%)	+(63%)

* -, means nones; +, mild infection; %, values are percent of samples infected.

Table 3. Effect of sources of Up-to-Date on growth and yield of potatoes (April, 1989) under high input farming using irrigation, fertilizer and pest control.

Seed source	Year received	Stems/ plant (#)	Canopy cover			Plant harvested (%)	Yield (t/ha)
			45 DAP	60	75		
Local		2.7	13	48	68	80	20.2
Local (+ selection)		3.3	18	60	78	74	29.2
Scotland	1986	2.5	15	63	90	72	33.6
Ireland	1987	2.3	17	63	90	67	34.6
Grand mean		2.7	15	58	81	73	29.4
CV (%)		27.2	14	19	4	16	11.1
LSD (0.05)		ns	ns	ns	9	ns	ns

Table 4. Status of virology work on potatoes in S. E. Asia.

Country	Virologist	Antisera utilization	Antisera production
Burma	no	no	
Indonesia	yes	yes	
Malaysia	yes	no	
S. Korea	yes	yes	PVX, PVS, PVY
S. Pacific	no	no	
Papua New Guinea	no	no	
Philippines	yes	yes	
Taiwan	yes	yes	
Thailand	yes	yes	PVX, PVX, PVY ^a
Viet Nam	yes	no	

^aTechnical support provided by CIP and some financial support from CIP for S. Korea and SAPPAD for Thailand. Both countries are to distribute antisera to other countries with CIP coordination.

Table 5. Area, yield and factors limiting yield of sweet potatoes in S.E. Asia as of 1989 (estimated).

Country	Area '000ha	Yield		Major factor limiting yield
		Subsistence ----- (t/ha)	Commercial -----	
Burma	4.7	5.2	10	inputs
Indonesia	210	8.7	12	inputs
S. Korea	25	-	21	temp.
Philippines	200	5	17	inputs/drought
Thailand	15	11	-	inputs/drought
Viet Nam	350	11	18	inputs/drought

not important. Commercial production is gaining importance, particularly near urban centers and in the more developed countries (S. Korea and Taiwan). The gap between actual and potential yield is decreasing (Table 5). Thus, viruses may become an important factor in the future.

In the South Pacific, sweet potato little leaf disease or witches' broom (mycoplasma-like organism) is currently a serious problem. It is spread by the black spotted hopper (*Orosius lotophagorum rikyensis*). It is a serious disease in some varieties grown in the S. Pacific during the dry season (SPC, 1984). Yields can be reduced up to 50% and 25% reductions are common (SPC, 1984). The black spotted hopper can transmit the disease to and from the morning glory making it difficult to control. Resistant germplasm is needed for this disease. Local cultivars appear to have some level of resistance while most introduced cultivars appear to be susceptible (personal observations).

Virology research and antisera production have not yet started in any country of S.E. Asia. Only at the Plant Research Institute in Victoria, Australia, in collaboration with Tonga and the Solomons, has some limited virus research been done.

Needs

Potatoes

1. Antisera production for PLRV is not yet possible in S. E. Asia. Should we continue developing this capability or we encourage imports from developed countries? Possibly, alternative, simpler methods could be developed.
2. Germplasm for S. E. Asia should have PVY immunity and also PVX and PVS immunity if possible.
3. Easier testing for PSTVd would be desirable.

Sweet Potatoes

1. Determine the major and minor viruses in the region.
2. Develop antisera or other simple methods for detecting the major sweet potato viruses.
3. Determine the relative importance of viruses in reducing yields.

Summary and Conclusions

Potato viruses are well documented in S. E. Asia. Their relative role in reducing yields at present yield levels is minor. S. Korea and Viet Nam have the greatest problem of aphid-transmitted viruses. Humid highland growing areas do not have a major problem with viruses. Future needs

should focus on developing simpler methods for virus detection, especially for PLRV and PSTVd. All germplasm from CIP should have PVY immunity and, if possible, PVX and PVS immunity.

Sweet potato viruses are not identified in all of S.E. Asia. Their importance in reducing yields needs to be determined. If this is significant, methods of virus detection using antisera and other simple methods must be developed.

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The Advances of Virus Testing in China

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Introduction

The most important diseases reducing potato yield in China are late blight, ring rot, black leg, bacterial wilt and some virus diseases caused by potato virus X, potato virus Y, and potato leafroll virus. During the 1970s, late-blight resistant varieties were widely adopted. Since then, virus diseases became a major problem in potato production.

Potato degeneration and potato viruses have been studied in China since the 1950s (Tian, 1956), but the studies on identification of potato viruses and diagnostic methods began in 1975 to meet the certification requirements of seed potatoes for virus-free seed potato production. During the last decade, some progress has been made in the isolation of viruses, development of diagnostic methods, antiserum production, and certification of seed potatoes.

After the establishment of CIP Region VIII Office in Beijing, a new project, "Development and utilization of potato virus detection techniques" was started in cooperation with CIP scientists. The highly sensitive, rapid and specific virus and viroid testing techniques have been studied and some of them are being used and introduced into the national program.

Isolation and identification of potato viruses and viroids

In 1979, a new virus strain of alfalfa mosaic virus was isolated and identified in addition to PVX, PVY, and PLRV. Potato calico virus is 18-74 nm in length and causes bright yellow blotches on potato leaves of different varieties like "Liwaihuang" (Zhang et al., 1983). Tests showed that this virus was different from potato tuber necrosis strain from California (Oswald, 1950) and tobacco ringspot virus-Andean potato calico strain in Peruvian potato cultivars from South America (Fribourg, 1977). Potato calico disease occurs often in potato crops planted in ploughed lucerne fields or next to lucerne fields.

During 1978-1980, potato virus Y, a tobacco vein necrosis strain of PVY from cultivar "Liwaihuang", and potato virus (PVS) from varieties "361", "278", and "Liwaihuang", were isolated and identified. The antisera against these viruses were also made (Zhang et al., 1983; Zhang et al., 1984). Further investigation showed that PVS is widespread in most potato cultivars in China.

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In 1983, PLRV was isolated and purified by filtration through Sephadex G200 column followed by sucrose density gradient centrifugation from potato B76-16 x 292-20, and Mira. The uniform isometric particles with average diameters of 23.93 ± 1.2 nm were observed under electron microscope H-600 (Zhaug et al., 1988). The purification method of PLRV has been studied. Meanwhile a yellowing agent-Beet western yellow virus was identified by inoculation of *Capsella bursa-pastoris* and *Nicandra physaloides* and re-inoculation of virus-free potato with *Myzus Persicae*. In ultra-thin sections of infected potato tissue, isometric virus particles 22-26 nm in diameter were found in cytoplasm of infected cells (Guo and Zhang, 1986). In 1987, the tobacco necrosis virus (TNV) was isolated from and identified in advanced line Dongnong 85-3334 (Wang Renyuan et al., 1988). In 1989, PVM and PVA were identified by ELISA. The potato aucuba mosaic virus (PAMV) and tobacco rattle virus (TRV) have been preliminarily identified in some varieties used for breeding by indicator plants at Keshan Potato Research Institute, but these viruses are transmitted only in limited incidence.

Potato spindle tuber viroid (PSTVd) was identified on some cultivars: Schwalbe, Mira, Kunnae, Iceland No. 3, Kexin No. 1, Kexin No. 2, Kexin No. 3, Kexin No. 4, "Tiger Head" and Tongshu No. 8, by polyacrylamide gel electrophoresis and by two dimensional electrophoresis (Schumacher et al., 1983; Tian et al., 1982; Zhang et al., 1982; Ma et al., 1987).

Although more than 30 viruses, viroids and MLOs infecting potato have been reported in the world, in China only PVX, PVY, PVS, PLRV and PSTVd are economically important. Some viruses such as PAMV, TRV, AMV, TNV, PVA, and PVM were found infecting potato, but were not widely distributed and caused little damage. Potato viruses reported from South America such as Andean potato latent virus (Gibbs, 1966), Andean potato mottle virus (Fribourg et al., 1977), Potato virus T (Salazar et al., 1978), Tobacco ringspot virus-Andean calico strain (Fribourg, 1977), *Solanum* apical leaf curling virus (Hooker and Salazar, 1983) and potato yellow vein virus, PYDV, and PMTV have never been found in China. Therefore, specific attention should be paid to avoiding infection of the above-mentioned viruses when introducing potato germplasm from South America and other regions to China.

Potato virus detection techniques

A rapid, sensitive and highly specific potato virus detection technique is very important for certification of seed potato as well as for selection of virus-resistant materials and evaluation of germplasm.

MAT, MPT, BFT and Latex test

In China, before 1974 only test plants and the slide agglutination test were used in detecting potato viruses X and Y. During 1975-76, the microprecipitin test (MPT), microagglutination test (MAT), and bentonite flocculation test (BFT) were studied and applied in certification of seed potatoes (Zhang et al., 1977). Later, the latex agglutination test was tested for detection of PVX

and PVY. The minimum amounts of PVX and PVY detected were 20 ng/ml and 2 ng/ml, respectively (Zhang et al., 1979).

Microslide gel double diffusion test

It was reported that the PLRV in extracts of infected potato could not be detected. However, it was found that the microslide gel double diffusion test is sufficiently sensitive for the detection of PLRV in infected extracts. The maximum dilution of stem extract which gives a clear precipitin line in diffusion was 1:4 and the minimum concentration of purified PLRV detected was 1.5 ng/ml (Hou and Zhang, 1988). Results showed that microslide gel double diffusion test is useful for detection of PLRV. Moreover, the microslide double gel diffusion test can be more readily used for determination of the titer of antiserum against PLRV than the precipitin ring test and double-agar diffusion test used by Murayama and Kojima (1974).

ELISA

During 1980-1982, ELISA was used for detection of PVY and PVY^N. The antibodies were labelled with horseradish peroxidase (HRP). The minimum amount of PVY and PVY^N detection were 40 ng/ml and 10 ng/ml, respectively, using the double-antibody sandwich method. The maximum dilution of both virus-infected potato leaf extracts giving positive reactions was 1/128 (Guo et al., 1983). In 1984, the antisera against potato leafroll virus were prepared in China (Zhang et al., 1987). The purified IgG to PLRV were labelled with HRP. The PLRV in leaf, stem and root extracts from infected potato and *Physalis floridana* were readily detected by ELISA. The minimum amount of purified PLRV in vascular tissues in rose ends of infected potato tuber after breaking dormancy could be also detected (Zhang et al., 1987).

DAS-ELISA

In order to further improve the sensitivity of ELISA for detection of PLRV, a new magnification system of biological reaction, the Biotin-Avidin-System, was used in ELISA in 1987. The rabbit IgG to PLRV was labelled with N-hydroxysuccinimide-6-biotinylamide caproate (BCNHS) and the PLRV was detected by Avidin-Biotin-Horse-radish peroxidase-complex (ABC-ELISA) and streptavidin-alkaline phosphatase (SA-AP ELISA). Tests showed that the minimal concentration of purified PLRV detected by SA-AP ELISA and ABC ELISA were 2.5 ng/ml and 1.25 ng/ml, respectively. The highest dilutions of PLRV-infected potato stem extract detected were 1:128 and 1:1256, respectively. Results showed that ABC-ELISA is about 20 times more sensitive than standard double antibody, sandwich method of ELISA (Hou and Zhang, 1988).

Dot ELISA

An enzyme-linked immunosorbent assay was tested on nitrocellulose membranes (NCM) used as solid phase carriers instead of polystyrene microtiter plates for detection of PLRV (Haskes et al., 1982). The rabbit antibody against virus was used as the first antibody and the sheep antibody to rabbit IgG, labelled with alkaline-phosphatase was used as a second antibody. The NBT and BCIP were used as substrate for visualization. Tests showed that the minimum amount of purified PLRV detected was a 1.5 ng/test spot and the maximum dilution of PLRV-infected

extract which gave positive reaction was 1:160. Dot ELISA is more sensitive than standard ELISA for detection of PLRV (Chen and Zhang, 1988).

ISEM

Another rapid, sensitive and specific detection technique for testing of PLRV in potato extracts is immunosorbent electronmicroscopy. It has been tested in China according to Roberts and Harrison (1979). The number of PLRV particles per standard area (SA=1000 μm^2) of grid in potato stems, leaves, sprouts and dormant tubers was 71364, 55909, 2273 and 909 per SA, respectively. The maximum dilution of ISEM was found to be four hundred times higher than that of common electron microscopy (Zhang et al., 1987).

Preparation of McAb

In order to improve the specificity of serological reactions the monoclonal antibodies to PVY, PVX, and PLRV were prepared by hybridoma techniques in 1985 and 1989 (Yan Kangsheng et al., 1985; Xiao Xiao Wen et al., 1989; Zhou Lan et al., 1989).

Page and return gel electrophoresis

Before 1980, in China, PSTVd in seed potato was detected by inoculation of Rutgers' tomato. Since then, polyacrylamide gel electrophoresis has been used for detection of PSTVd (Tian et al., 1982; Zhang et al., 1982). Later the more method, two dimensional gel electrophoresis method (Schumacher et al., 1983) has been used for detection of circular PSTVd-RNA under denatured conditions. By using this method the PSTVd infection in a widely growing potato cultivar, "Tiger Head" in Hebei province was verified and a certification program was established (Ma Xiufen et al., 1987). In 1987, the distribution of PSTVd in potato tubers was further studied by return electrophoresis with silver staining. It was found that PSTVd was easily detected in the rose end, heel end and pith of dormant tubers. After breaking dormancy, the concentration of PSTVd was obviously increased, especially in the rose end. PSTVd can be detected in as little as 2.5 mg of tuber tissue sample by this method (Ma et al., 1987).

Nonradioactive cDNA probe

Recently, the study on molecular hybridization of biotin-labelled cDNA probe for detection of PSTVd has been carried out and funded by the International Potato Center (CIP). The PSTVd cDNA cloned in pUC9 and pSTB5 plasmids were labelled with biotin-II dUTP by nick translation. The biotinylated PSTVd cDNA probe has been used for detection of purified PSTVd-RNA and PSTVd-infected potato extracts by NASH. The resulting hybrid was detected by using a streptavidin-alkaline phosphatase conjugate reacted with a substrate dye solution, containing nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP). Tests showed that the minimum amount of purified PSTVd-RNA detected was a 8 pg/test spot. The maximum dilution of PSTVd infected potato extract which give visible signals was 1/125 with pUC9 plasmid. The appropriate concentration of biotin-cDNA probe in hybridization solution, extraction buffer solutions and other test conditions was studied (Zhang et al., 1987). In view of the fact that the biotinylated cDNA probes have a long shelf life, can be stored at -20°C for at

least one year, do not need facilities specific for isotope work and are almost as sensitive as ³²P-cDNA probe, biotinylated probes should be easy to extend to national programs.

Preparation of molecular cloned cDNA probe of potato viruses: Recently in China the DNA copies of PLRV and PVY-RNA were prepared by reverse transcription of viral RNA and by nick translation of DNA-RNA hybrid, then cloned into plasmid pUC12 in JM109. The cloned PLRV-cDNA pLR6 and PVY-cDNA pPVY15 were hybridized to purified viral RNA and virus-infected extract by NASH. The minimum amount of PLRV-RNA detected was 100pg, and 156pg of PVY RNA by NASH. (Shi Yixing et al., 1989). The cloned PLRV-cDNA and PVY-cDNA probes are being prepared (Shi Yixing et al., 1989).

Currently, in China some serological virus detection techniques are being used as routine methods in seed potato inspection. Usually different potato viruses are detected by different methods; PVX, PVY, and PVS are detected mainly by ELISA. The PLRV in stems, leaves, and tubers is tested by ELISA, ISEM or microslide double diffusion test. Potato spindle tuber viroid in potato plants and tubers is detected by return gel electrophoresis and nucleic acid spot hybridization (NASH) with biotin-labelled cDNA and ³²P-cDNA probe.

Utilization and transfer of virus detection techniques

Before 1986, PSTVd was detected by test plants, Rutgers tomato, and PSTVd infection incidence was unknown. Now PSTVd can be effectively detected by NASH with Biotin cDNA probe or ³²P-cDNA probe and return gel electrophoresis. During 1988-1989, 1003 samples of 36 principal varieties of TPS producers from 5 potato production provinces were tested by NASH and return gel electrophoresis.

Now, incidence of PSTVd infection has been investigated throughout the country, from northeast to southwest China. Preliminary results revealed that besides two varieties, "Tiger Head" and "Lushu No. 1", which are severely infected, incidence of PSTVd infection in most principal cultivars, TPS procedures and selected lines is limited.

Recently efforts have been made to eliminate PSTVd from TPS, basic seeds and breeding parents.

In order to extend the rapid and specific virus detection techniques to national programs, ELISA has been simplified and transferred to potato research, extension and production institutions as kits. In 1989, 32 ELISA and 11 Dot ELISA Kits were prepared. 24 ELISA and 5 Dot ELISA kits were distributed.

The ELISA Kit has been applied in surveys of potato viruses in principal varieties, resulting in the improvement of the quality of seed potatoes, germplasm evaluation, selection, breeding and TPS production.

During the last 3 years, 1729 samples of 72 varieties and advanced lines from the main potato growing provinces have been tested by ELISA for detection of 8 potato viruses, PVX, PVY, PVY^N, PVS, PVM, PLRV, TRV and PVA.

Antiserum production

Before 1975, the antisera against potato viruses X and Y were prepared only in small quantities in individual research institutes for laboratory research use. In order to meet the increasing needs of large scale virus testing in certification of seed potatoes, an antiserum production program has been established. During 1975-1976, antisera against PVX and PVY with the titer of 1/2560-5120 in microprecipitin tests were prepared in freeze-dried form (Zhang et al., 1978; 1981). During 1978-1982, antisera against PLRV with the titer of 1/4096 in counterimmuno-electrophoresis were successfully prepared (Zhang et al., 1987).

During 1987-1989, 445 ml antisera, 110 ml Latex to PVX, PVS, 28 mg IgG to PVY and PLRV, 26 ml HRP-IgG to PVY and PLRV, 7 ml Ap-Sheep-anti-rabbit IgG have been prepared and made available.

Training

Since 1987, two training courses on utilization and extension of potato virus and PSTVd detection techniques have been held at Inner Mongolia University. Twenty-six young Chinese virologists and breeders from 10 provinces took part in these training courses. Some new virus and viroid testing techniques, such as ELISA, ISEM, return gel electrophoresis and NASH with biotinylated cDNA probe have been produced and extended.

In order to transfer the ELISA techniques, a special "on-the-job-" training course was held in 1989 for utilization of ELISA and Dot ELISA Kits. In the training course, the ELISA Kits and Dot ELISA Kits were applied and distributed.

Summary

During the last decades, some progress has been achieved in potato virus identification, detection techniques and antiserum preparation. In addition to PVX and PVY, the potato calico virus (AMV), PVY^N, PVS, PLRV, Beet western yellows virus, TNV, PAMV, TRV and PSTVd have been isolated and identified. Some recent virus detection techniques, ELISA, DAS-ELISA, Dot ELISA, ISEM, microslide double diffusion test, return gel electrophoresis, and NASH with Biotin-cDNA and ³²P-cDNA probe, were studied and developed. The McAb to PVY, PVX, and PLRV have been prepared. The molecular cloned cDNA probes to PVY and PLRV are also being prepared.

The ELISA, return gel electrophoresis, and NASH techniques have been utilized in surveys of viruses, gemplasm evaluation, certification of seed potatoes, TPS production, breeding, and selection for detection of potato viruses and PSTVd. The ELISA technique simplified as ELISA

Kits and return gel electrophoresis have been transferred to the National Potato Research and Production Institutions.

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Diagnósticos Vegetales S.R.L. -Success in Private-Public Interaction

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Introduction

Diagnósticos Vegetales S.R.L. (DV) is a private service company which performs post-control diagnosis of virus diseases and nematodes on seed potatoes. It works in support of the seed potato certification program of Argentina and of growers of non-certified seed potato. The company was founded in 1983 and is located in Mar del Plata, Argentina, 400 km south of Buenos Aires.

In Argentina, the annual potato production area is 120,000 ha. The certified seed area is about 4,000 ha, which covers 50 percent of the seed market; the other 50 percent is produced by growers of non-certified seed potato. The National Government performs, through the National Seed Service Agency, the certification of seed potato. This process includes field inspections throughout the growing season and a postharvest control to detect virus infection and nematodes in tubers. Such post-controls are applied to all categories of seed potato. After haulm killing, samples of tubers from all the seed crops are collected by certification inspectors and sent to the DV lab with an unknown number.

The growers of non-certified seed potatoes also use the postharvest control test to determine the real sanitary conditions of their seed crops. They collect tuber samples from their own seed crops following sampling instructions and take them to the DV lab.

Virus infection is detected by ELISA (Clark and Adams, 1977) on tubers. Nematological diagnosis is performed by peeling the tuber periderm with a portion of cortex, grinding these tissues and inspecting them with a stereomicroscope. Other services of DV to seed potato growers include:

1. virus testing during the growing season;
2. nematode diagnosis in soil samples during pre-planting period using the centrifugal-flotation technique (Jenkins, 1964);
3. monitoring of aphid population to determine haulm killing date; and

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4. advising on seed-borne disease epidemiology.

In 1985, DV generated a new potato project, Atlantida Semillas, which produces pre-basic seed. The pre-basic seed is the first progeny of in vitro cultivated plantlets, grown under controlled greenhouse conditions. The International Potato Center (CIP) has helped DV by providing in vitro pathogen-tested cultivars and training personnel. Atlantida Semillas has commercialized pre-basic seed in the Argentine seed potato market, with excellent results.

Diagnosticos Vegetales has an experienced professional team. All professionals were trained at the National Agronomic Research Institute, INTA, and at CIP. This company was built with private resources, without any government subsidy or credit support.

Antecedents

The National Agronomic Research Institute (INTA) at Balcarce developed a postharvest control laboratory to detect virus infections on potato seed for the benefit of potato production. It began work in the early 1970s under the direction of Dr. Butzonitch. Virus tests were performed by planting tuber samples in greenhouses and evaluating 7-week old plants visually and with the A6-clone reaction. This contribution from INTA was essential in confirming that the post-control test was a very important tool that gave more exact data on the real sanitary status of a seed lot. Seed growers also understood the advantages of this quality control and they were interested in using this technology.

The first private experiences in potato postharvest control to detect virus diseases started in 1979. The private laboratories applied the methodology used by INTA. The National Certification Agency observed the activity of the companies, and the National Government regulated their activities and used their services in the certification process.

In 1981, DV personnel participated in a training course on "Practical Virology" led by Dr. L. F. Salazar and his research team at CIP-Lima. During the course, it became clear that ELISA would be a useful tool for the Argentine seed potato program if it were applied to the potato control tests. After the course, active communication with CIP was very important for the future of DV. Furthermore, the participation of the DV professionals in other courses and training at CIP permitted the growth of DV.

At the end of 1981, after consultations with Dr. Gugerli in Switzerland, preliminary trials were run and laboratory equipment was adapted to apply ELISA on tubers for commercial purposes. The experimentation steps were periodically communicated to the Certification Agency to demonstrate that ELISA was a sensitive and specific technique for virus detection, so that it would be an essential tool in the seed program. As ELISA was an objective test, it eliminated the subjectiveness and doubt of symptom evaluation. The application of ELISA on tubers produced results in 20-25 days, so it was fast enough for the demands of the seed market. Virus diagnosis through combined symptom evaluation and A6-clone test was slower than the application of

ELISA on tubers, but ELISA's limited use in the world and the lack of experience with this technique in Argentina prevented its immediate use.

During 1982, at the request of the Certification Agency, a comparative assay between ELISA and the traditional methodology used in Argentina was performed. After the evaluation of the assay in May 1983, the Certification Agency and the National Service of Plant Sanitation authorized DV to apply ELISA on tubers for the postharvest control of virus diseases. The company, which was founded for this purpose, was qualified to work under Law No. 20,247 of Seeds and Phytogenetic Creations, which regulates the activity of private laboratories in support of government services.

The seed growers approved the immediate commercial development of the method. In the first year, DV analyzed 400 samples; in the second year, it analyzed 1500, and at the beginning of its third year, demand was stabilized between 1800 and 2000 samples. Today, the use of ELISA for postharvest virus control tests is widespread. In 1984, DV was given permission to perform diagnosis on parasitic nematodes affecting potato.

Conclusions

Training opportunities, and cooperation of national and international research centers were essential for the development of private enterprises.

When an official certification service cannot afford a modern, complete and continuous service, private companies can take the risks to complement the efforts of the official activities in a seed potato production program. Seed potato growers valued the private-public interaction in the certification process because this interaction permitted a fast adjustment of the certification process to technological changes and conserved the ethical framework. The advances produced by this interaction allowed to overcome technological and economic barriers such as seed importation.

The Brazilian Approach

A. N. Dusi¹

About 170,000 ha of potatoes are grown a year with a production of 2,000,000 tons. Approximately 50% of the seed come from the formal seed production system.

The virology laboratory at the Centro Nacional de Pesquisa de Hortalicas of EMBRAPA - CNPH (National Research Center for Vegetable Crops) supports the breeding programs by characterizing and diagnosing viruses. It also produces some of the antisera for cucurbit and solanaceous viruses. Specifically on potato, it cooperates with:

1. the potato breeding program for PVY and PLRV resistance;
2. indexing of the pre-basic seed, advanced clones and potato germplasm for viruses and viroids;
3. production of antisera. It also provides technical assistance to other government and private biotechnology companies involved in seed-potato production.

In the early 1980s, CNPH/EMBRAPA established a program on potato virology to provide Brazil with modern seed-potato production techniques. The main objective was to reduce seed imports by increasing the internal production of high quality seed. These programs depended on reliable virus detection systems.

The first phase was on staff training. The virologist was first trained at the University of Brasilia (UnB), which provided the laboratory facilities. During this period (1982-86), CNPH set up its Virology Laboratory with international funds (GTZ) and CIP's technical assistance. CIP's experts came to CNPH and Brazilian virologists had the opportunity to be trained at CIP. In 1984, the pre-basic seed production began at CNPH and the Serviço de Produção de Sementes Básicas of EMBRAPA (Basic Seed Production Service) was involved in the process. The micro-tubers were produced at CNPH and sent to SPSB for screenhouse and field multiplications.

The antisera production was initiated at CNPH and CNPFT (Centro Nacional de Pesquisa de Fruteiras de Clima Temperado/EMBRAPA - National Research Center for Temperate Climate Fruit Crops) and the first attempt was on latex flocculation techniques. Antisera were produced for PVY, PVX, and PVS, and a kit for laboratory and field detection of these viruses was released. CIP also participated in a joint PSTVd survey as well as in the first report of APMV detection on potatoes in Brazil. Antisera used were as produced at CIP and as previously agreed. CNPH later sent back the antisera produced in Brazil to other countries through CIP.

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By 1987, a collaborative program with the Canadian International Development Agency (CIDA) started on seed-potato production. It provided CNPH with facilities for viroid detection by Return Polyacrylimide Gel Electrophoresis (R-PAGE). Before this, all PSTVd work in Brazil was done by CIP with NASH. Membranes were sent to Peru for testing. Today, some private companies still have CIP's support on viroid indexing, but CNPH tests its own material by R-PAGE. CIDA also provided some other equipment and chemicals which permitted the production and sensibilization of antisera for ELISA and its use on a larger scale. A training program involving Brazilian and Canadian specialists was also carried out.

Antisera are available at CNPH for detection of PVS, PVX, PVY, and APMV by ELISA (Table 1). These antisera are being successfully used at CNPH and by other public and private companies to index their material. The success of this work is due to the integration of researchers from CNPH, CIP, CIDA, JICA in addition to the collaborative work and consultancy of Dutch scientists.

Table 1. Crude antisera produced at CNPH for ELISA.

Virus	Vol.(ml)
APMV	118,0
PVS	33,0
PVX	127,0
PVY	136,0

Antisera production for PLRV has so far been unsuccessful. Despite the high specific titer in some of the antisera produced, non-specific reactions occur in such high levels that these antisera are not reliable for diagnostic use. Today CIP still intermediates the importation of PLRV antisera and alkaline-phosphatase, which assures the continuation of the CNPH antiserum production program. Besides CIP and CIDA, CNPH is developing cooperative programs with Italy, the U.S., West Germany, and Hungary.

Future goals are to increase certified seed production in Brazil; to integrate government and private companies; to continue the research on PLRV antisera; and to breed for virus resistance.

We can summarize the Brazilian approach on virus and viroid detection in potatoes in two main points:

1. Access to different sources for: information, training, funds.
2. Commercial production of seed-potato: joint government/private company programs.

CNPH is now beginning to work on virus detection in sweet potato.

Virus and Viroid Detection: Strategies in Cuba

L. Lago and S. Perez¹

Many potato diseases are known to be caused by viruses (Salazar, 1982). There is historical evidence that the virus diseases increased their importance after its introduction in different continents.

"The incidence of the diseases increased, as tubers from a diseased crop were used as seed for the next. This was called degeneration, and was ascribed either to fatigue or deterioration of the crop resulting from continuous vegetative propagation, or to unfavorable conditions of climate and soil" (Van der Want, 1987). Now we can understand that those effects resulted from the action of infectious agents, which are more dramatic in the tropical conditions where the vectors are present over the year.

Potato plants can be infected with virus in different ways: mechanical and biological agents can propagate the infection in the field. Once the tubers are infected and used as seed they rise to virus infected plants that produce virus infected tubers again (Beukema, 1979).

The main methods used to control virus disease are based on the prevention of infection: detection of infection, reducing the number of infection sources and other crop management.

Since the beginning of the century, inspection programs for seed-potato production have been developed in Europe, United States and Canada, but in the beginning were not very successful in developing countries.

Over the years, official inspection services were created in different countries. The inspection systems became more complicated due to increasing knowledge about transmission through the tuber to the progeny. In the 1970s phytosanitary requirements for seed potato production were established. At that time the spread of the virus was studied and also the economic importance of the potato viruses in our production (Table 1).

Since that time, prevention of infection or spread of potato virus in the potato production systems have been studied and introduced through phytosanitary practices for controlling the so-called "degeneration of potato."

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Viruses are important in connection with their influence on yields (t/ha) of the potato and the opportunity to permit the longer and efficient multiplication of seedstocks with a low virus content.

Table 1. Increase of the viral infection after 1 reproduction (Lago et al., 1972).

Pro vince	Percentage of virus disease	
	1st reproduction (PVX+PVY+PLRV)	2nd reproduction (PVS+PVY+PLRV)
Pinar del Rio	0.35-5.40	6.0-29.0
Habana	0.35-5.40	15.0-30.0
Villa Clara	0.38-3.05	44.0
Matanzas	0.60-2.80	4.6

In most of the developing countries, the integrated management of potato virus diseases (diagnostic techniques, "rouging," chemical treatment against vectors, haulm destruction date, etc.) is very difficult to achieve as a result of the social structure and the potato production system. Figure 1 shows our results over the last 30 years.

Potato virus and viroid diseases in Cuba

The main viruses are PLRV, PVY and PVX (Table 2).

Table 2. Economic importance of potato virus disease in different varieties in Cuba.

Variety	Disease	Yield in t/ha	
		healthy	diseased
Red Soda	PLRV	25.257	10.062
Viking	PLRV	13.515	7.144
Cariboo	PLRV	18.268	12.425
Red Soda	PVY	25.257	10.002

The percentage of dissemination depends on the origin of the variety planted (from Canada, Holland or France).

We have found PVS and PVM in different varieties in the field, but these viruses are of lesser importance.

The PSTVd disease is not found at all in Cuba.

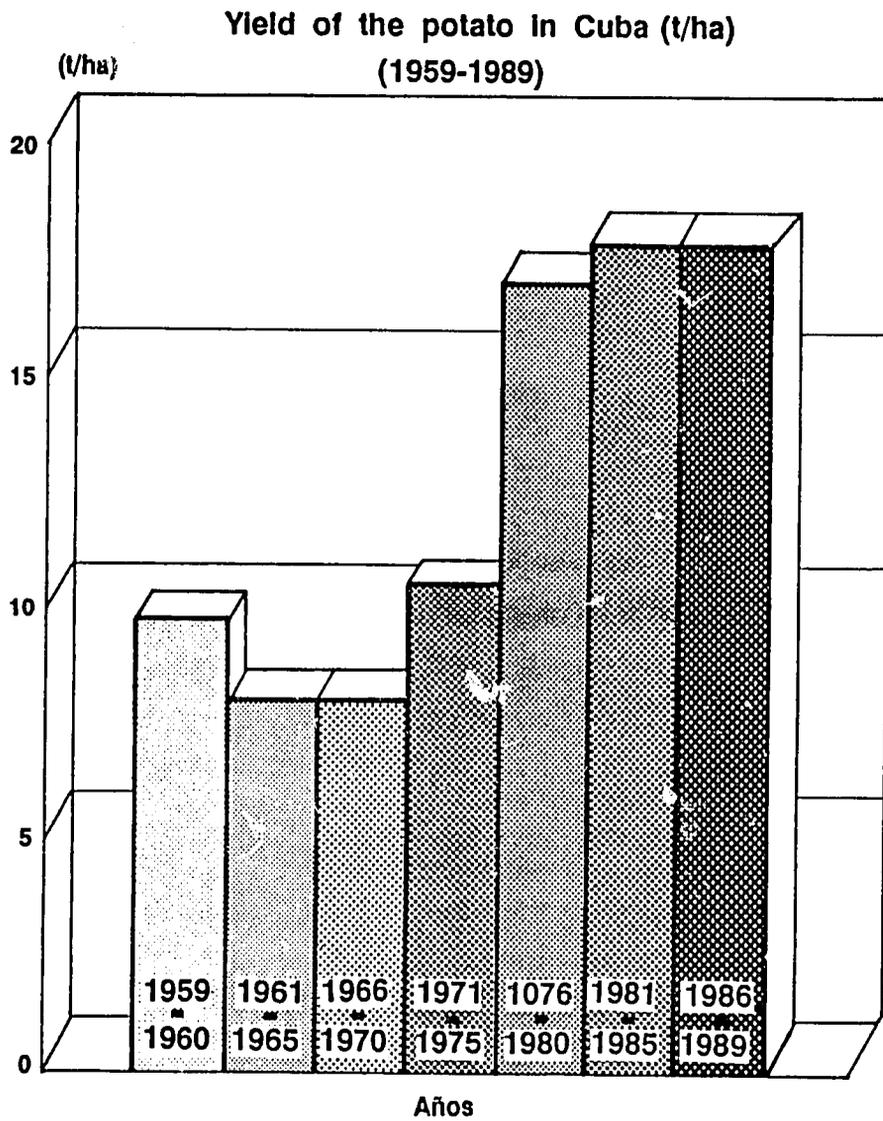


Figure 1. Yield of the potato in Cuba (T/ha) 1959-1989.

Present status of diagnostic techniques for the potato virus and viroid diseases in Cuba

In this point we use two ways for diagnosis:

1. To prevent the introduction of quarantine agents.
2. To prevent the spread of existing phytopathological agents.

In 1970, the Plant Protection Service (PPS) was organized at the Ministry of Agriculture which had as its principal target the potato crop, because we imported nearly 16,000 tons of potato seed per year. It presently has 14 provincial laboratories with facilities like 2 electron microscopes, 7 automatic systems (SUMA) for ELISA test. Those laboratories are managed by 12 specialists in connection with virus diagnosis including potato.

At the same time the PPS provides the Certification Service for all kinds of analysis to make sure that the seed potatoes are at the minimum level of infection in accordance with their class parameters.

The main effort in our country has covered in three areas of work:

1. Introduction and application of different diagnostic techniques for potato virus and viroid diseases (electron microscopy, serology, test plant, etc.).

Different institutions are working in that field in order to introduce into potato production the best techniques for phytopathological diagnosis (Table 3).

Table 3. Introduction and application of different diagnosis techniques.

Technique	Application level
Electron microscopy	Only in special samples
Test plants	At the potato station
Serology	
-microprecipitation	In the provincial laboratories
-immunodifusion	In small numbers of samples
ELISA test	At the potato station for Pre Basic and Basic categories
	In the post harvest control of those categories.

2. Production of diagnostic reagents.

The application of diagnostic techniques depends on the financial support for the potato production system and the opportunity to introduce these techniques in specific areas.

That is the reason why we are trying to produce our own diagnostic reagents (Table 4).

Table 4. Production of diagnostic reagents.

Technique	Reagents	Production level
Serology	Rabbit polyclonal antibody anti PVX	Commercial use
	Monoclonal antibody anti PVX (conjugate)	Commercial use
	Rabbit anti PVY	Commercial prep.
	Rabbit IgG anti PVY	Commercial prep.
	Monoclonal anti PVY	Development
	Polyclonal anti PLRV	Development
	Monoclonal anti PLRV	Development
NASH	Probes for PVX detection	Specialized lab use
	Probes for PVY detection	Specialized lab use
	Probes for PLRV detection	Specialized lab use
	Probes for PSTVd detection	Developing
	Reagents for non-radioactive probes	Specialized lab use
	Colloidal Gold	Specialized lab use
	Photobiotin	Specialized lab use

3. Development and optimization of the diagnostic system (Table 5).

Table 5. Development and optimization of the diagnosis techniques.

Technique	Topic	Level
Immunological Diagnosis	Ultramicro ELISA test (PVX,PVY,PLRV)	In use
	Latex technique	Developing
NASH	Non-radioactive NASH techniques	Developing
	Colloidal Gold	Developing
	Photobiotin	Developing
	PSTV hybridization test	Developing

Strategies for potato virus and viroid diagnostic

We consider that our viral diseases diagnosis must include:

Visual diagnosis

Used by the inspector to check the seed potato areas. At the same time we are training members of the "rouging team" about symptom identification of different varieties.

Test plants

Our personal opinion is that the test plants diagnosis is more useful as a technique integrated with serology, electron microscopy and NASH to check the potato varieties after meristem culture at the starting point in the in vitro micro-propagation system or in the in vitro collection of virus-free material.

Electron microscopy (EM)

Should only be used after meristem culture or to check different plant material from other countries but not in the routine work in the diagnostic system. We have enough people trained to identify virus using EM.

NASH (Nucleic Acid Spot Hybridization)

The procedure is based on a method of Owens and Diener (1981) for viroid detection and involves the hybridization of ^{32}P -labelled complementary cDNA probe with virus in crude sap spots previously immobilized on a nitrocellulose membrane.

Baulcombe et al.(1984) described a nucleic acid spot hybridization method (NASH) for the detection of Potato Virus X (PVX), Potato Virus Y (PVY) and Potato Leafroll Virus (PLRV) in crude sap samples.

In general, NASH is considered good or better than ELISA test in terms of sensitivity and reproducibility and it permits the handling of a large number of samples.

Nucleic Acid Hybridization is not used in virus diagnosis in Cuba. This technique up to present is associated with very specialized laboratories working in virus identification and molecular virology studies. Recently, big efforts are in progress for adapting NASH with non radioactive probes to be used in diagnosis. In our strategy the idea is to use this technique in the quarantine service because of its specificity in identifying virus strains, and during the first steps of the micropropagation program to select the material after the clean-up process.

Serology

From 1969 until 1982 most of the serology tests were carried out using the precipitation test, and the antiserum was provided by other countries. Then we started to prepare our own antiserum in cooperation with different countries and institutions like CIP in Lima.

The ELISA test (Clark and Adams, 1977) and their modifications like ultramicro ELISA system are the most common serology tests in Cuba.

The main use of the ELISA test is in the Potato Basic Seed Program with nearly 60,000 analyses per year for PLRV, PVY and PVX.

In the near future we may work with other viruses to cover all potato viruses and viroid diseases.

In our country as in other tropical countries it is impossible to produce healthy seed potatoes with a long scheme of multiplication; that is the reason why our scheme takes 5-6 years between Pre-Basic and consumption with a big number of samples for diagnosis in the first category of the scheme (Pre-Basic) to ensure that we multiply the "virus free" material.

Some remarks about the diagnostic techniques

Infections early in the growing season result in the symptoms being expressed in the leaves, but the late infection does not always show up in the haulm even when the tubers might be infected.

In addition to visual observation during the growing season the ELISA test is commonly used in the certification program in our country.

The diagnostic techniques will be applied at different levels of the scheme of propagation and according to the category (Table 6).

The strategy is to apply the ELISA test involving more laboratory tests in the Pre Basic and Basic categories, at a specific laboratory in charge of that production. On the other hand, high quality categories like Super Elite (R-I) and Elite (R-II) involve more field inspection and sampling for ELISA test which will be covered by the provincial laboratories.

In our case the postharvest control is one complementary procedure to the certification process.

Postharvest control has limitations, but in our strategy serves as a method to check or correct the field inspections. Actually it is commonly used for Pre-Basic and Basic category sampling with 100% of type plants to be checked by ELISA test.

Table 6. Application of diagnostic techniques in the seed potato scheme.

Category	Remarks
Virus-free material	Electron microscopy Test plants ELISA test
Pre Basic	Permanent "rouging" ELISA test for PLRV,PVY,PVX
Basic	Permanent "rouging" Inspection 3 times ELISA test (samples for PLRV,PVY,PVX) Post harvest control
R-I (Super Elite)	Post harvest control (Florida test) using ELISA test.
R-II (Elite)	-perspective-
Other categories	diagnosis when the problems are found.

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Strategies for Collaboration Between the Private Sector and IARC's.

R. F. Davis and C. L. Sutula¹

Introduction

Collaboration has been defined by Webster's Dictionary as "working jointly with others esp. in an intellectual endeavor." There have always been good reasons for collaboration between scientists, namely to utilize unique strengths of two or more individuals to achieve a goal of mutual interest. In the 1990s, as scientists become increasingly specialized in their areas of expertise, collaboration will be more important than ever to achieve broad objectives most effectively.

The advantages of collaboration are perhaps more obvious than the strategies for collaboration. To develop strategies for collaboration, it is important to first evaluate and understand the goals, strengths, and limitations of those involved. As this process proceeds, the critical components of effective collaboration involve selection of a specific project, commitment of priorities and resources for that project, and continued attention to the project from start to finish.

The private sector and International Agricultural Research Centers (IARCs), have a common goal of providing for the needs of those individuals that they serve. Although they are both oriented toward service, the means by which they approach this goal are very different. The private sector is very focused on customers, to the point of being defined and driven by customer needs and demands. The IARCs, on the other hand, are more oriented toward the pursuit of basic knowledge, and obtain funding through granting agencies. While the orientation of the IARCs allows greater flexibility to conduct research and develop technology, it allows for less focus on delivering technology to the end-users.

Among the strengths of the IARCs are their unique and specialized missions. In addition, IARCs are well equipped and staffed with competent scientists with crop-oriented expertise. Strengths of the private sector include access to many resources, especially a knowledge of customer needs. The private sector knows how to reach their customers, how to produce products that meet the customers' needs at a practical cost, and how to distribute products effectively. They have the ability to respond rapidly and effectively to market demands, and to locate and employ whatever resources are necessary to meet those demands.

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Agricultural diagnostics

As a model for examining strategies for collaboration, let us take the area of agricultural diagnostics. In the broad sense, this involves tests intended to detect compounds and organisms with agricultural significance. These include nutrients, microelements, insects, plant pathogens, herbicides, fungicides, insecticides, toxins, and plant constituents. Reagents to detect these compounds and organisms are produced and sold as components, tests, or kits.

There are basically two reasons for using agricultural diagnostics aimed at detecting plant pathogens: 1) to determine, in a crisis situation, what pathogen is associated with the disease; and 2) to use the test, in a preventative mode, to produce and/or maintain pathogen-free plant material. From a market perspective, these two testing modes have very different profiles. Crisis testing requires an urgent response, is not price sensitive, and is oriented mainly to the fresh market. Due to the crisis nature, it tends to be a volatile and fluctuating market with low sample volumes. This market is best handled by specialists, who can provide accurate as well as timely answers.

Prevention testing tends to be a more stable market, involving continuous programs to monitor plant health in a population of high-value plants that may be several years away from fresh market sales. The laboratory conducting such tests can be instrumental in helping the grower achieve goals of improving plant quality. Because of the higher volume and less immediate need of this type of testing, the price tends to be very critical, and must remain low as a per test cost.

The agricultural diagnostics market is unique. Its value is about 100-1000 times smaller than the human diagnostics market. Applications for this market are generally not well developed, as they are in the case of pregnancy tests, for example. The key focus is on service, rather than profit. The market is very fragmented: by crop type, by state and country, and by cultural practices. The testing 'window' or time frame is very limited for many crops, and testing is conducted on samples of low unit value. Finally, the market is evolving from a 'no charge' system which involves a highly developed practice of 'swapping' raw test reagents.

Collaboration between CIP and Agdia, Inc.

Agdia, Inc. is a privately held company with the business goal of providing diagnostic products to the agricultural community. Agdia has demonstrated particular expertise in taking technology and developing diagnostic tests which are convenient and acceptable to growers. Methods have been developed at Agdia to efficiently produce these easy-to-use kits, with a high degree of quality control and reproducibility. Agdia's products include ready-to-use Pathoscreen Kits for ELISA, Agdia 1000 Reagent Sets for ELISA and IFA, nucleic acid hybridization tests for viroids, custom testing services, and technical support. The average cost of testing with Agdia products ranges from about \$0.08 to \$1.00 per well for ELISA tests, depending on the size and format of the kit ordered. The success of Agdia in this area is evidenced by several points: 1) Agdia has been in business since 1981; 2) since that time, Agdia has experienced a 10-fold increase in growth; 3) the

number of products has increased from a few tests for pathogens of potatoes, to the current offering of test systems to detect over 150 pathogens, including viruses, bacteria, and viroids; 4) products are shipped to all states in the U.S.A., all provinces in Canada, and to more than 60 other countries worldwide; 5) since its inception, Agdia has collaborated with over 70 scientists worldwide on specific projects; and 6) Agdia has earned a reputation as a quality producer of diagnostic products.

As an example of developing strategies for collaboration, let us examine the advantages of collaboration between CIP and Agdia. CIP is located in an area well suited for studying potato diseases. Scientists at CIP have developed diagnostic reagents and methods for several pathogens of potatoes. The research effort that is required to produce these reagents and develop diagnostic methods is significant. Collaboration with a private company like Agdia, Inc. can help CIP achieve its mission at the customer level. In fact, because of our worldwide experience in agricultural diagnostics, we can position and extend CIP's reach beyond its mission, by using CIP's approach to diagnostic methods in other crops. Agdia, Inc. has an excellent track record for collaboration, and in fact, is a working example of successful, private-sector collaboration with scientists around the world.

Strategies for collaboration between CIP and Agdia, Inc.

The above discussion highlights one example of collaboration between the private sector and public institutions. But, in fact, Agdia is very interested in establishing collaboration with CIP. As two organizations with emphasis on diagnostics for pathogens of potato, there is a natural advantage to complementing, rather than duplicating, efforts. The first step in defining a strategy for collaboration is for CIP and Agdia to get to know each other. Through this knowledge of existing programs and goals, areas can be identified where collaboration would be mutually beneficial. An effective strategy to a successful collaboration would be to define one specific project for collaboration, make a commitment to the project, allocate the resources necessary to complete the project, and follow the project through to its completion. The ultimate success of the collaboration will depend on the attention given to each of these steps. Agdia would like to collaborate on specific projects, in which our expertise in application of technology, in development of tests or formats, and production of diagnostic tests, could be of value to CIP.

As an example of a specific project that might be developed, Agdia has recently introduced a broad-spectrum test for all aphid-transmitted members of the potyvirus group. This is based on a monoclonal antibody to which we have acquired a license for commercial use. This antibody is useful for detection of the potyviruses which occur in potato and sweet potato. If this test is of interest to CIP clients, we would like to discuss this as a collaborative project, in which the goal would be to introduce this useful test in a format acceptable to CIP clients in developing countries.

In addition to specific projects, Agdia would be willing to exchange biomaterials and samples, train personnel, and perform contract research to help advance the program at CIP.

Conclusion

There is a great deal to be gained by strengthening collaboration between the private sector and the IARCs. One way to identify opportunities is to open channels of communication between interested parties, and through mutual understanding of what each party can offer, ideas for collaboration often become obvious. In addition, a better understanding of the nature and constraints of each organization will facilitate negotiations of specific mechanisms for collaboration. However, sustained and successful collaboration can only occur through development of specific projects, in which both parties can benefit. Through well conceived and executed collaborative efforts, both the private sector and the IARCs will be better able to meet the needs of the international agriculture community in the 1990s and beyond.

Approaches to Developing Resistance to Viruses through Breeding

A. O. Mendiburu¹

Even though the Planning Conference refers to virus and virus-like diseases of potato and sweet potato, this presentation will of necessity have a heavy bias towards the first crop, since what will be said has mostly been inspired in experiences with potatoes. However, since both crops are asexually propagated and are polyploid, much of what applies to potato also applies to sweet potato.

In preparing this presentation, I soon came to the conclusion that I could hardly contribute to clarifying specific aspects of breeding for virus resistance here at CIP, where for so many years a comprehensive program has been conducted to produce excellent populations and clones with high frequencies of combined resistance genes (Mendoza, 1980, 1987, 1989). Rather, it was considered more advantageous to base the presentation on our own practical experience emerging from the last 40 years of potato breeding at INTA's Balcarce Agricultural Experiment Station, located in the South Eastern part of the Province of Buenos Aires (SEPBA), Argentina. At Balcarce we could claim a measure of success in breeding for virus resistance (Huarte et al., 1986; Mendiburu and Huarte, 1987).

Some of the potato varieties we bred -- notably Serrana INTA (B70.178.2 = CIP 720087) and Achirana INTA (B71.240.2 = CIP 720088) are considered to be highly resistant to some important viruses and have sustained that resistance in widely divergent environments (Huarte et al., 1988). Today, the aim will be to attempt an interpretation of the basic success of varieties capable of permanent production under warmer than "normal" conditions (Plaisted, 1974) and, on the basis of these elements, to discuss the possible implementation of a cooperative breeding effort involving CIP and local breeding programs located in warmer than normal (putative) potato producing areas.

Degree of environmental control (DEC)

In a previous paper (Mendiburu and Huarte, 1987; see also Bryan, 1974) we enumerated four contrasting strategies adopted by different countries in Latin America to cope with prevailing conditions for potato production. These include "normal" climates particularly with regard to temperature during tuberization combined with short days (Andean regions) or with long days

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(e.g. Southern Chile), where tuber seed production is permanent and resistance to viruses is largely dispensed with. Strategies for warmer than normal climates also include two kinds of situations. One is characterized by utilization of imported seed produced in normal climates of foreign virus-susceptible cultivars, and the other is distinguished by permanent local seed tuber production of locally bred, highly virus-resistant, adapted cultivars. The latter two situations occur simultaneously in the SEPBA region. A fifth strategy may be added as a challenge for future work to include regions where potato is not yet produced due to lack of adaptation of available cultivars.

At this point, it might be useful to state that a high level of virus resistance is but one aspect of varietal breeding. Under some conditions, it may be a fundamental one, while in other instances, it may be superfluous.

We may use the concept of "Degree of Environment Control (DEC)" to illustrate circumstances where virus resistance is important to sustain potato production (Mendiburu and Huarte, *op. cit.*).

In Figure 1, DEC is defined in terms of easiness of virus dispersal on seed, in the absence of genetic resistance. For one particular virus, DEC values range from 0 (conditions are so favorable for virus dispersal that 100 percent infection is reached within one growing season) to 1 (environment is so favorable for seed health, either because of ecological or technological conditions, or both, that there is no virus dispersal on seed even when a susceptible variety is grown). For a totally susceptible variety, the seed quality is directly proportional to DEC, i.e., $y = DEC$; whereas for an immune variety, seed quality (always considering one particular virus) is constantly at a maximum, i.e., $y = 1$. Intermediate situations have been developed on the basis of some function of the genetic resistance of a genotype (variety) to the virus under consideration. Examples representing high, medium and low levels of genetic resistance are given in Figure 1.

Figure 1 shows that virus resistance is of fundamental importance when varieties are to be grown under unfavorable environmental conditions, in particular when ecological conditions for clean seed tuber production are poor.

It is also true that virus resistance is more or less superfluous when ecological conditions for seed tuber production are favorable.

Without a doubt, one of the most important contributions that could be made to solve the food problem in large areas of the world would be to expand potato cultivation to warmer areas. It is in this context that potato breeding has a definite goal: to release varieties adapted to regions where it has not been possible up to now to produce potatoes on a permanent basis. For this, it is meant to produce, year after year, not only a crop of consumption potatoes but also a crop of high-quality tuber seed potatoes to ensure the sustainability of the production system.

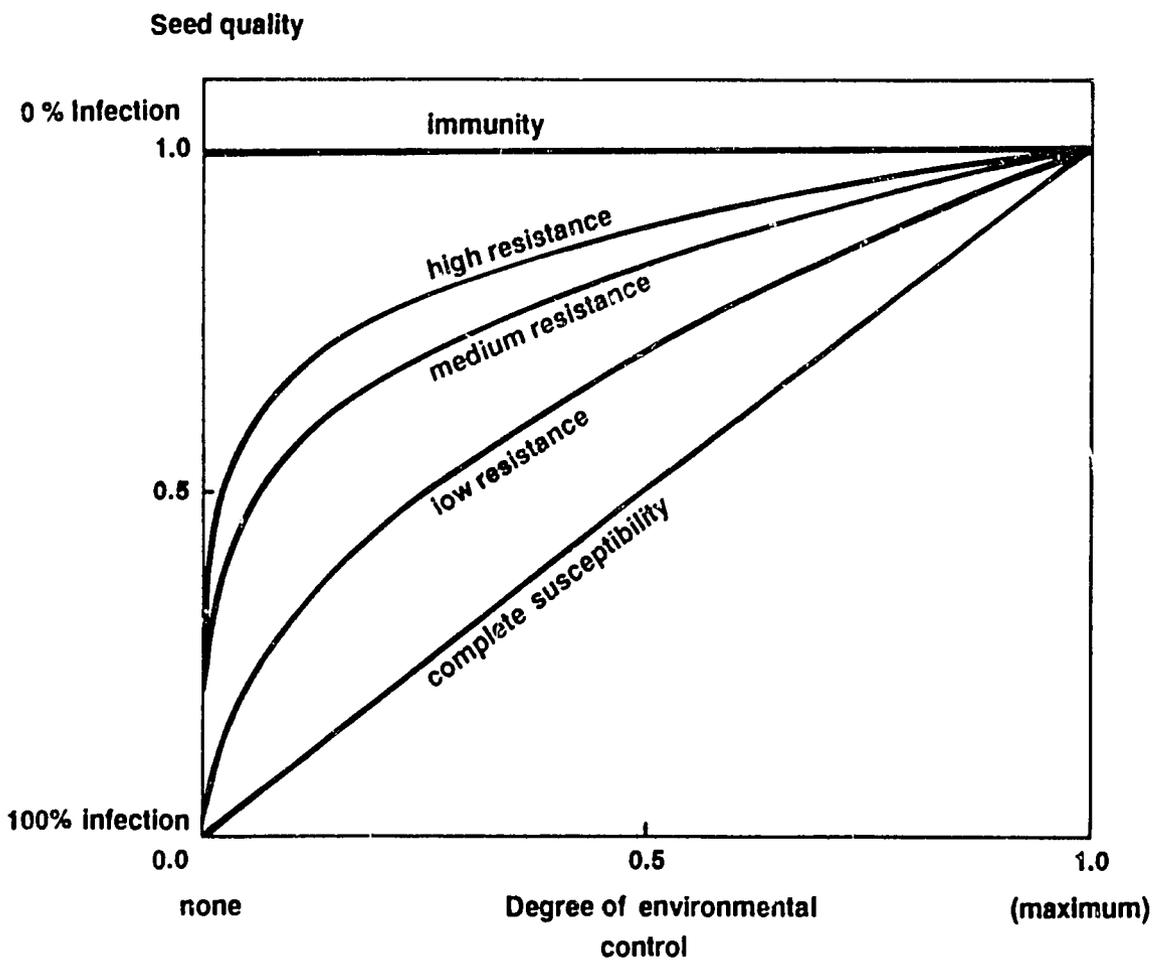


Figure 1. Level of tuber seed quality (measured in terms of freedom from virus infection) in relation to genetic resistance of cultivars to a given virus and to degree of environmental control (DEC) attained (measured in terms of easiness of virus dispersal on seed). (Modified from Mendiburu and Huarte, 1987.)

One important achievement of the Balcarce Breeding Program has been to obtain varieties sufficiently adapted to local conditions to be capable of permanent production. The northern-hemisphere varieties were, and continue to be, cultivated in the SEPBA region, but they are dependent on tuber seed imports. Ecological conditions for potato cultivation in the SEPBA region are unfavorable, thus preventing permanent production when northern hemisphere cultivars are utilized. However, conditions are favorable enough to permit permanent cultivation of locally-bred cultivars.

Relevant factors determining potato production strategies include, among others, the length of tuber dormancy, soil temperature during tuberization, photoperiod, storage, and seed. Among these factors, the most important appears to be the availability of good quality seed. In normal potato producing areas, the ecological conditions favor the production of high quality seed. As is shown in Figure 1, in warmer areas a higher level of genetic resistance to viruses is necessary to attain a comparable level of seed quality. Furthermore, with warmer temperatures, several other requirements for adaptation to permanent production emerge. For example, under the conditions which prevail in the Balcarce region, locally adapted varieties require a much longer dormancy period than northern-hemisphere cultivars. In the terminology suggested by Wurr (1978), locally-bred, adapted varieties may be described as possessing a physiological clock which starts "ticking" late, and then "ticks" slowly. In contrast, northern-hemisphere cultivars have a physiological clock that starts "ticking" early and "ticks" fast. Local varieties have been selected to adjust to the annual cycle which is characteristic of the region and at the time of planting the seed tubers exhibit a normal physiological age which, in conjunction with virus resistance, vegetative cycle, etc., would permit permanent production under prevalent environmental conditions.

Breeding varieties when genetic resistance to viruses is an important factor

Even though the development of cultivars which are capable of permanent production under warmer than normal conditions, as is the case in the SEPBA region, entails local breeding; it also represents a clear instance of capitalizing on international cooperation. So, Huinkul MAB, the first successful local variety in the SEPBA region, was selected from some 2,000 seedling tubers brought from the New York State potato breeding program, where they had been discarded because of their late-maturing characteristic (Millan, 1972). The development of Serrana INTA and B71.240.2 was based on crosses made between Huinkul MAG or some of the descendent clones (contributing adaptation, long tuber dormancy and field immunity to PVY) and clones developed at the Max Planck Institute (contributing resistance to PLRV, PVX and late blight).

Based on this kind of experience, a cooperative breeding scheme can be suggested which includes both centralized and decentralized activities. The centralized portion of the scheme is aimed at capitalizing on the breeding effort at CIP, which has produced populations and materials with high levels of combined resistances to viruses and to other biotic and abiotic constraints to potato production. In view of the complicated nature of selection under favorable

ecological conditions, when multiple requirements of particular environments have to be met, breeding in situ is considered essential for success if genotypes sufficiently adapted to provide for permanent production, are to be selected and maintained. This decentralized part of the scheme involves conducting a breeding program in each target area. The following inputs are considered relevant for success:

1. large numbers of progenies to increase the probability of selecting adapted genotypes;
2. seedlings transplanted to pots (clay or styrofoam) for high tuberization temperatures;
3. field evaluation;
4. tuber seed increase in target areas (the production of good quality seed, year after year, is a test for adaptation of the selected clones.)

Once the program is under way possibilities may be explored to provide for exchange of materials and improved and increased interaction among participants.

Conclusion

1. When conditions for potato production are favorable, high levels of genetic resistance to viruses may not be important. The same may be true when conditions are unfavorable but an ecologically favorable area is located nearby (or within the country), even if it is small.
2. When conditions for potato production are unfavorable and it is difficult to produce healthy seed nearby, virus resistance becomes fundamental. A large "in situ" breeding program may have to be organized to cope with simultaneous selection of many important attributes so as to obtain varieties for permanent production (sustainability of the production system).
3. However, if the program is successful, the adapted varieties may compete advantageously with unadapted ones maintained by external seed production.

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Genetic Engineering for Virus Resistance

J. Dodds, J. Jaynes, and R. Beachy¹

Introduction

Plant virus diseases have traditionally been controlled by hygienic agronomic practices for example, by using pathogen-tested propagules, controlling the insect vectors that spread the pathogen, and incorporating genes for virus resistance. These practices can in most instances substantially reduce crop losses normally attributed to virus infections. However, changing agronomic practices and changes in virus strains often lead to significant increases in virus spread, and the search for genetic resistance begins anew. In the majority of cases, sources of disease resistance are unavailable to the plant breeder, or if available, require many plant generations to incorporate the resistance trait into the desired cultivar. Furthermore, most of the resistance genes are effective against only a limited number of virus strains.

In instances where genetic resistance is unavailable, a cross-protection approach has been taken. First described by McKinney (1929), cross protection refers to the observation that tobacco plants infected with a mild strain of tobacco mosaic virus (TMV) were less susceptible when subsequently inoculated (challenged) with a more severe TMV strain. The large body of work conducted in the study of cross-protection during the last 55 years has been described in outstanding detail by others (Hamilton, 1980; Ponz and Bruening, 1986).

Two mechanisms have been proposed recently for the use of molecular methods to confer virus resistance. These are the expression of coat protein and the use of interfering molecular sequences (antisense) to disrupt virus replication.

Use of coat protein (CP) to confer virus resistance

In a review article in 1980, R. Hamilton predicted that the development of techniques to genetically transform plants might make it possible to produce plants that were similar to those that were cross-protected against virus infection. However, the molecules responsible for cross-protection were not determined. With the development of techniques for plant transformation (reviewed by many authors including Chilton, 1983; Caplan et al., 1983; and Fraley et al., 1986), came the opportunity to test a number of approaches to confer protection to transgenic plants. The TMV-tobacco system was a logical choice for the initial experiments for several reasons:

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1. There was exhaustive literature on the biology, molecular biology, and biophysics of the virus. Such a background made studies of the molecular biology of protection feasible.
2. The full sequence of TMV-RNA had been published earlier by Goelet et al. (1982), and facilitated cDNA cloning of selected sequences of TMV.
3. Cross-protection was first demonstrated against TMV in 1929 and has been applied to protect glasshouse crops of tomatoes. This precedent provided a basis for comparing cross-protection with protection produced in transgenic plants.
4. The transformation and regeneration of plants was demonstrated to be relatively easy in tobacco. Although the first report of successful protection of transgenic plants was in tobacco against TMV infection, other recent reports also documented that a similar approach provided protection against alfalfa mosaic virus (AMV) and other viruses.

The first published reports of the expression of viral cDNAs in transgenic plants (Beachy et al., 1985; and Bevan et al., 1985) described the expression of sequences of TMV-RNA that encoded the capsid protein (CP). In the report by Bevan et al., a cloned cDNA was obtained which represented the CP gene of the OM strain of TMV (Meshi et al., 1982). The cDNA contained the entire CP coding sequence flanked on the 5' end by 4 nucleotides of TMV-RNA, and on the 3' end by most of the non-translated region (205 out of 207 nucleotides) of the genome. This included the highly structured tRNA-like region (in brome mosaic virus such a tRNA-like region is the site to which replicase binds to initiate synthesis of complementary strand RNA (Miller et al., 1986). This cDNA was ligated to the promoter from cauliflower mosaic virus that causes the expression of the 35S RNA viral transcript (35S promoter) (Guilley et al., 1982), and the 3' regulatory sequences from the nopaline synthetase (NOS) gene of the Ti-plasmid. The chimeric gene was transferred to the intermediate plasmid, pBIN 6, (Bevan, 1984) and used in plant transformation reactions with the *A. tumefaciens* strain LBA 4404 (Hoekema et al., 1983). Tobacco plants regenerated from transformed leaf pieces expressed the chimeric gene and accumulated at least 6 different RNA molecules that were either less than or greater than the predicted size. Plants accumulated coat protein to the level of about 170 ug/gm fresh weight, which represents approximately 0.001% (w/w) of soluble leaf protein. However, when R1 progeny of the transgenic plants were inoculated with TMV, they were found equally to be as sensitive to infection as non-transgenic plants (Bevan and Harrison, 1986). Powell-Abel et al., (1986) reported that transgenic tobacco plants expressing the TMV CP were resistant to infection by TMV. Plants expressing the TMV CP sequences (CP+) either escaped infection, or developed disease symptoms significantly later than plants not expressing the gene.

Furthermore, they also found that each of the different CP expression transgenic lines tested were similarly resistant. This important result demonstrated that resistance was due to expression of the gene rather than to changes in the plant caused by tissue culture of plant regeneration. By contrast, the transgenic TMV CP (+) plants described by Bevan et al. (1985) were not resistant

to infection by TMV (Bevan and Harrison, 1986). The authors suggested that the level of gene expression in these plants was too low to produce resistance.

The experiments of Powell-Abel et al. (1986) were carried out in a tobacco cultivar that is a systemic host for TMV *Nicotiana tabacum* cv. Xanthi. Resistance was therefore manifested as a delay or avoidance of systemic disease development. Resistance might have resulted from lack of infection, or lack of short-distance spread, long-distance spread, or from combinations of factors. Plants that did not develop systemic symptoms did not accumulate TMV, indicating that they were resistant to infection and/or virus spread, rather than suppressing symptom development *per se* (Nelson et al., 1987).

In agreement with the experiments with TMV, transgenic plants that were CP(+) for expression of an AMV (alfalfa mosaic virus) capsid protein gene were resistant to infection by AMV (Tumer et al., 1987; Van Dun et al., 1987; Loesch-Fries, 1987). Likewise, CP gene expression has been reported for potato virus X (Hoekema et al., 1989) and Y (Stark and Beachy, 1989; Lawson et al., 1990). In the case of tobacco, Powell-Abel et al. (1986) reported that increasing the concentration of TMV substantially reduced the delay in systemic spread of the virus in tobacco plants. An example of results of one such experiment is presented in Figure 1. In Figure 1a, CP(+) plants were inoculated with increasing concentrations of TMV and disease development was recorded over a two-week period. All the control plants inoculated with the U₁ strain at 0.001 ug/ml developed systemic symptoms within 6 days past-infection (d.p.i.) (not shown). Transgenic CP(+) plants exhibited a delay in disease-symptom development unless inoculated with 20 ug of TMV/ml. In a similar type of experiment, disease symptoms were recorded at 5 d.p.i. on plants inoculated with increasing concentrations of TMV. As shown in Figure 1b, CP(+) plants required approximately 10⁴ higher concentrations of TMV to produce systemic symptoms in the same period of time as the controls. This level of resistance is therefore referred to as being 10⁴ greater than control plants. It is proposed that this type of assay be used to assess protection whenever appropriate.

It is noteworthy that PVX and PVY resistant (engineered) potato plants are already being commercially field-tested in several places. Similar studies using coat protein of PLRV (Potato Leafroll Virus) are underway at several institutions.

Use of molecular interference sequences (antisense) to disrupt virus/viroid replication

It has recently been found that bacteria regulate expression of some genes in a novel way. Under conditions where the cell would repress the biosynthesis of a particular protein, an additional level of control is exerted. This new type of control is called "micRNA" control (mRNA-interfering complementary RNA). This micRNA is complementary to the 5' end of the gene and when it is produced has the ultimate effect of reducing the amount of messenger RNA by annealing to it, thus removing it from normal protein synthesis.

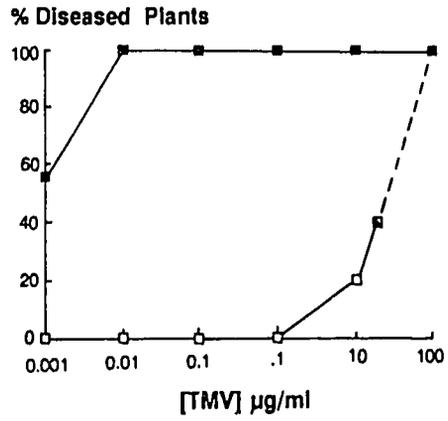
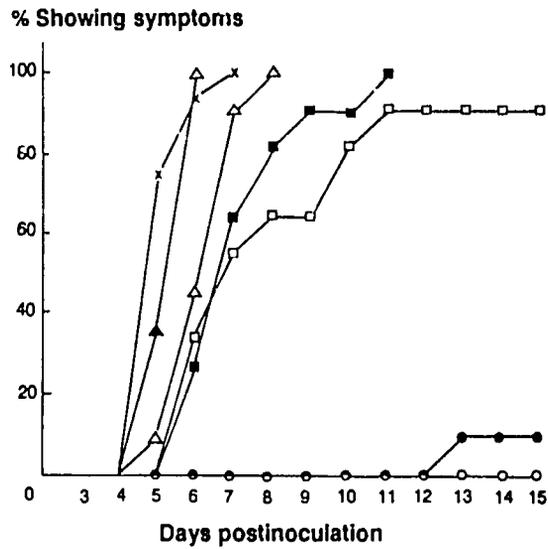


Figure 1. Relative resistance or susceptibility of transgenic and non-transgenic tobacco plants to TMV. Seedlings (3-4 leaf state) were inoculated with increasing concentrations of TMV and observed for systemic symptoms of disease (i.e., vein clearing, followed by chlorosis). A. Disease development in plants inoculated with increasing concentrations of U₁-TMV. x-x, control plants (transgenic, but not expressing the TMV CP gene) inoculated with 0.1 µg TMV/ml. Similar disease pattern was observed when plants were inoculated with 0.01 and 0.001 µg TMV/ml. The remaining seedlings were transgenic and expressed the TMV CP gene, inoculated as follows: O - O, 0.001 µg TMV/ml; ● - ●, 0.01 µg TMV/ml; □ - □, 0.1 µg TMV/ml; ■ - ■, 1.0 µg TMV/ml; △ - △, 5.0 µg TMV/ml; ▲ - ▲, 20 µg TMV/ml. (C. M. Deom and R. N. Beachy, previously unpublished). B. Disease development by 5 days post inoculation with increasing concentrations of TMV on plants not expressing the TMV CP gene, ■ - ■, or on those expressing the CP gene □ - □ (E. Anderson, previously unpublished).

This mode of gene regulation may offer a mechanism to control the expression of viruses in animals and plants, and therefore, the diseases they cause. Animals and plants have evolved very precise and elegant mechanisms which allow the regulated expression of their genes. Many viruses have "co-evolved" in order to exploit the eukaryotic cell by mimicking the general structure of plant and animal genes. Of course, the end result of this is disease which, in the case of vertebrates, is combated by the vastly complex immune system. But many viruses can eventually circumvent this response (by slightly altering a surface protein, for example, thus making the virus "invisible" to the immune system) and cause persistent infections, or totally overwhelm the system (e.g. the AIDS virus) and cause death of the animal.

It may be possible to use micRNA to block viroid replication. Viroids are single-stranded, circular ribonucleic acids (RNAs) of a few hundred nucleotides. Viroids are the smallest self-replicable structures known and represent the lowest level of life. They are the causative agents of a number of plant diseases and elicit mild to lethal symptoms depending on the fine structure of the viroid and the susceptibility of the host plant.

A great deal of effort is now being expended to elucidate the general mechanisms of replication and etiology of disease symptoms of the viroids. Indeed some information has been obtained which allows one to construct a possible mechanism of replication of the viroid within the plant cell. Figure 2 illustrates a scheme for replication of the viroid of potatoes (potato spindle tuber viroid, PSTVd). What is significant is the fact that in order for replication to occur, the host cell RNA polymerases must recognize and bind to the viroid for initiation and subsequent replication of the viroid to occur. Thus, if we endowed plants with the ability to produce regulatory RNAs complementary to specific regions of the viroid molecule, the plant may become resistant to infection.

Similarly it may be possible to produce antisense sequences that would interfere with replication of virus nucleic acid molecules. Recently several reports documented that antisense RNAs complementary to cucumber mosaic virus (Cuozzo et al., 1988) and TMV (Powell et al., 1989) were less sensitive to CMV or TMV infection. However, the degree of resistance was much lower than in plants that express CP genes.

Conclusions

The expression of viral CP genes can confer protection to infection to the virus from which the CP coding sequence was obtained. Thus far, protection has been conferred against TMV and AMV, PVX, PVY and PLRV, and other viruses. Research is ongoing in a number of laboratories around the world to extend the experimental approach to control other virus diseases. Recent field experiments have demonstrated that transgenic tomato plants are protected against infection by TMV, and add support to the suggestion that such plants will be important in agriculture.

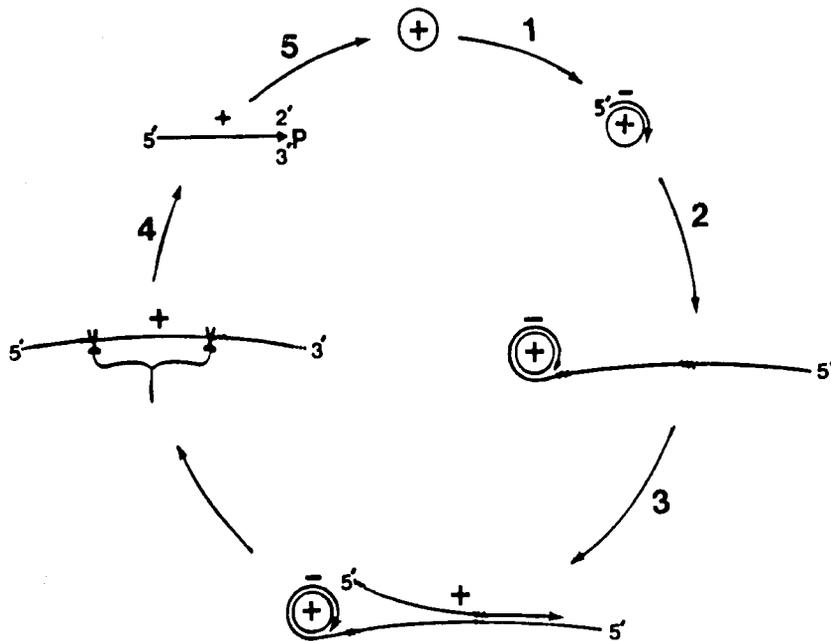


Figure 2. Hypothetical pathway of potato spindle tuber viroid replication. The cycle begins with the infecting circular plus strand (marked "+"). Step 1: initiation of minus strand synthesis. Step 2: Copy of a multimeric minus strand (marked "-"). Step 3: Production of multimeric plus strands. Step 4: The multimeric plus strands must be cleaved to produce unit length molecules. Step 5: Circularization of the PSTV molecules.

The basic cellular and molecular mechanisms responsible for the engineered protection are now well understood, although the results of experiments indicate that an early step in infection is affected: it has been suggested that CP expression in transgenic plants interferes with virus uncoating. Sherwood and Fulton (1982) hypothesized that (classical) cross-protection also blocks an early event in infection.

The use of antisense sequences, although elegant in concept, has still to be proven in an *in vivo* system.

Clearly, over the last 5-10 years tremendous advances have been made in both molecular biology and virology. The melding together of these two disciplines has already allowed the production of plants showing engineered virus field resistance. However, the value of these approaches should be not be overestimated, because the resistance has not been tested over a long enough period to be clearly shown as sustainable. Also, major administrative hurdles have to be overcome to allow routine commercialization and international distribution of these materials. The role of plant protection also needs to be further defined. Despite these problems, the techniques offer a unique opportunity to confer virus/viroid resistance to genotypes already showing other positive attributes.

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Sources of Resistance to Viruses in Andean Potato Cultivars Maintained at CIP

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The cultivated potato collection maintained at CIP by asexual propagation either in the field or through in vitro culture, comprises at present about 3,500 Andean cultivars. Originally, the collection contained almost 15,000 accessions of native Andean cultivars collected throughout Latin America. This number was reduced by means of identifying duplicate samples of the same cultivar, which are now being maintained only as true seed.

Through the years, CIP scientists have been evaluating this collection for numerous traits of importance in potato breeding. The data presented here concentrate on evaluations made on the most important virus diseases affecting the potato crop, namely PVX, PVY and PLRV.

Screening for resistance to viruses in the Potato Collection began when CIP became an International Center in 1972. In that year, L. Salazar started a systematic evaluation of virus reactions in native cultivars from Peru that were donated to CIP by the Peruvian Potato Program.

By 1974, Salazar had identified 532 accessions in the collection that were susceptible to PVX by their reactions in *Gomphrena globosa*; 35 that were hypersensitive with top necrosis reactions to the strains X₃ and X₄; and 276 accessions that were free of PVX. In 1975, R.A.C. Jones took over these evaluations and received 518 accessions from Salazar which included some new introductions to the collection. Out of the 518, Jones found 195 accessions that became infected with both strains, 138 infected with one strain, and 185 accessions were considered as potential sources of PVX resistance. This number of accessions was reduced with the identification of duplicates of the same cultivar or were found to be susceptible in further tests.

At present, there are 97 accessions with resistance to PVX in the collection, 20 of which are hypersensitive (Table 1). Although most of these cultivars are *S. tuberosum* subsp. *andigena* (ADG), there are a few accessions of *S. stenotomum* (STN), *S. goniocalyx* (GON), *S. phureja* (PHU), *S. x chaucha* (CHA), *S. x juzepczukii* (JUZ), and *S. x curtilobum* (CUR). With the exception of two accessions from Bolivia, all the rest are from Peru. However, it should be emphasized that only a small portion of the potato collection was screened for PVX resistance. Numerous accessions obtained from new collecting expeditions throughout Latin America remain to be screened.

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Table 1. List of Andean Cultivars from the Potato Collection with Resistance to Potato Virus X.

CIP number	Species ^a	Country	PVX ^b	PVY	PLRV	Plants Used ^c
700001	ADG	PER	R	S	-	PUnt
700070	ADG	PER	R	-	-	PUnt
700079	ADG	PER	R	-	-	PUnt
700111	ADG	PER	R	-	-	PUnt
700119	ADG	PER	R	-	-	PUnt
700150	ADG	PER	R	-	-	PUnt
700174	ADG	PER	R	S	-	PUnt
700187	ADG	PER	H	-	-	PUnt
700191	ADG	PER	H	-	-	PUnt
700213	ADG	PER	H	-	-	PUnt
700223	ADG	PER	H	-	-	PUnt
700235	STN	PER	R	S	-	PT
700355	ADG	PER	R	S	-	PUnt
700361	ADG	PER	R	-	-	PUnt
700395	ADG	PER	R	S	-	PUnt
700397	ADG	PER	R	-	-	PUnt
700407	STN	PER	H	-	-	PUnt
700485	ADG	PER	H	S	-	PUnt
700498	CHA	PER	R	S	-	PT
700505	ADG	PER	H	-	S	PUnt
700520	ADG	PER	H	S	-	PUnt
700536	ADG	PER	H	-	S	PUnt
700571	ADG	PER	H	-	S	PUnt
700577	ADG	PER	R	S	-	PUnt
700595	ADG	PER	R	S	-	PUnt
700602	ADG	PER	R	S	-	PT
700608	ADG	PER	R	-	S	PUnt
700611	ADG	PER	R	S	-	PUnt
700629	ADG	PER	R	-	-	PUnt
700658	ADG	PER	R	-	-	PUnt
700675	ADG	PER	R	-	-	PUnt
700697	ADG	PER	R	-	-	PUnt
700724	ADG	PER	H	-	-	PUnt
700744	ADG	PER	R	-	-	PUnt
700771	ADG	PER	R	-	-	PUnt
700790	ADG	PER	R	-	-	PUnt
700806	ADG	PER	R	S	-	PUnt
700818	ADG	PER	R	S	-	PUnt
						(...)

^aADG = andigena, CUR = curtilobum, CHA = chaucha, GON = goniocalyx, JUZ = juzepczukii,

PHU = phureja, STN = stenotomum, TBR = tuberosum.

^bH = Hypersensitive, R = Resistant, M = Moderately, S = -Susceptible

^cPT = Pathogen tested (X, Y, LR free), PUnt = Pathogen untested

CIP Number	Species	Country	PVX	PVY	PLRV	Plants Used
700826	CHA	PER	R	S	-	PUnt
700918	ADG	PER	R	S	-	PUnt
700932	ADG	PER	R	-	-	PUnt
700937	ADG	PER	R	-	-	PUnt
700961	ADG	PER	R	-	S	PUnt
700971	ADG	PER	R	S	-	PUnt
701016	ADG	PER	H	S	-	PUnt
701112	ADG	PER	R	-	-	PUnt
701172	ADG	PER	R	-	-	PUnt
701179	ADG	PER	R	-	-	PUnt
701187	ADG	PER	R	S	-	PUnt
701208	ADG	PER	R	-	-	PUnt
701231	ADG	PER	R	-	S	PUnt
701233	ADG	PER	R	-	-	PUnt
701274	ADG	PER	R	-	-	PUnt
701286	ADG	PER	R	-	-	PUnt
701287	ADG	PER	H	S	-	PUnt
701396	CHA	PER	R	-	-	PUnt
701463	ADG	PER	R	-	-	PUnt
701477	ADG	PER	H	-	-	PUnt
701515	ADG	PER	H	S	-	PUnt
701532	ADG	PER	R	S	-	PUnt
701540	ADG	PER	R	-	-	PUnt
701664	ADG	PER	R	-	-	PUnt
701751	ADG	PER	R	-	-	PUnt
701869	ADG	PER	R	-	-	PUnt
701875	ADG	PER	R	-	-	PUnt
701895	ADG	PER	R	-	-	PUnt
701986	ADG	PER	H	S	S	PT
702005	ADG	PER	R	-	-	PUnt
702040	ADG	PER	R	-	-	PUnt
702124	ADG	PER	H	-	-	PUnt
702139	ADG	PER	R	-	-	PUnt
702159	ADG	PER	H	-	-	PUnt
702217	ADG	PER	H	-	-	PUnt
702256	ADG	PER	R	-	-	PUnt
702261	ADG	PER	R	-	-	PUnt
702311	ADG	BOL	R	-	-	PUnt
702318	ADG	BOL	R	-	-	PUnt
702397	ADG	PER	R	-	-	PUnt
702407	CHA	PER	H	S	-	PT
702443	JUZ	PER	R	S	-	PT
702455	CUR	PER	R	S	S	PT
						(...)

CIP Number	Species	Country	PVX	PVY	PLRV	Plants Used
702460	ADG	PER	R	-	-	PUnt
702462	ADG	PER	R	-	-	PUnt
702467	GON	PER	R	-	-	PUnt
702514	ADG	PER	R?	S	-	PUnt
703313	STN	PER	R?	-	S	PT
703321	STN	PER	R?	S	-	PT

The search for resistance to PVY in the Potato Collection also began in 1972. In 1973, Salazar identified 568 accessions that were susceptible and 800 accessions that were free of PVY. These evaluations were made by serology (microprecipitation test) and indicator hosts. In 1974, this work was passed on to A.M. Hinostroza de Lekeu, who received 573 of the PVY-free accessions after they had been exposed to infection in the field with high aphid populations. She selected 41 accessions with high probability of resistance to PVY. However, Salazar (1978) believed that there might be more accessions without PVY infection in that group because Hinostroza de Lekeu's evaluations were based mainly on symptomatology.

Of the 41 accessions which were considered as having high probability of resistance to PVY in 1977, 5 were found to be susceptible in pathogen-tested plants. The remaining 36 cultivars with resistance to PVY are listed in Table 2.

In 1974 and 1975, Jones initiated field exposure trials to test for PLRV resistance. He used some accessions from the potato collection as well as other clonal materials. Those that showed some potential resistance were further studied using open-pollinated seedlings that were exposed to infective aphids. Five Peruvian *andigena* cultivars ("Hualash" 700031, "Yana Imilla" 700178, "Casa Blanca" 700485, "Ccompis" 700921, and "Huagalina" 703748) produced seedlings with low percentages of plants with PLRV symptoms. One of the susceptible checks used was the hybrid "Renacimiento" 720026, which produced almost 100% of infected seedlings (Jones, 1977).

In 1977, C. R. Brown started a large-scale effort to identify new sources of resistance to PLRV in the Potato Collection of Andean cultivars. About 3,000 accessions that showed no symptoms of either PLRV or PVY were selected in the field at Huancayo. Tubers of these accessions were planted in the field and scions of PLRV-infected plants were later grafted to each plant. Accessions showing severe symptoms of mosaic and rugosity were excluded from further evaluations. In 1979, S. A. Rizvi continued the screening work using 1,112 accessions, selecting 27 with potential PLRV resistance (Rizvi, 1980) (Table 3). It should be noted that re-evaluations made on pathogen-tested plants of 10 of these accessions confirmed their resistance to PLRV.

These 27 accessions with PLRV resistance are again mainly ADG, but there are 4 accessions of PHU, 1 of JUZ and 1 accession of *S. tuberosum* subsp. *tuberosum* (TBR). Seventeen of these accessions are from Peru, 3 from Bolivia, 6 from Colombia, and 1 from Chile.

Table 2. List of Andean Cultivars from the Potato Collection with Resistance to Potato Virus Y.

CIP Number	Species	Country	PVX	PVY ^d	PLRV	Plants Used
700005	ADG	PER	-	R	-	PUnt
700024 = 701200	ADG	PER	S	R	-	PUnt
700075	ADG	PER	-	R	S	PUnt
700528	ADG	PER	S	R	S	PUnt
700530	ADG	PER	S	R?	S	PUnt
700575	ADG	PER	S	R	-	PUnt
700682	ADG	PER	-	R	-	PUnt
700729	ADG	PER	-	R	-	PUnt
700787	ADG	PER	S	R	-	PUnt
700862	ADG	PER	-	R	-	PUnt
700953 = 701026	ADG	PER	-	R?	S	PUnt
700980	ADG	PER	-	R	-	PUnt
701102 = 700854	ADG	PER	-	R?	-	PUnt
701209	ADG	PER	S	R	S	PUnt
701213	ADG	PER	-	R	S	PUnt
701374	ADG	PER	S	R	S	PUnt
701442	ADG	PER	-	R	-	PUnt
701758	ADG	PER	-	R	-	PUnt
701761	ADG	PER	S	R	S	PUnt
701860	ADG	PER	-	R	-	PUnt
701940	ADG	PER	S	R	S	PUnt
702013 = 700686	CHA	PER	S	R?	-	PUnt
702072	ADG	PER	-	R	S	PUnt
702081	ADG	PER	-	R	-	PUnt
702089	ADG	PER	S	R	S	PUnt
702174	ADG	PER	S	R	-	PUnt
702245	ADG	PER	-	R	S	PUnt

^dAll of these PVY-resistant accessions are tetraploid ADG, except for one diploid that is PHU and another triploid that is CHA. Again, all of these accessions are from Peru with the exception of one from Colombia.

Table 3. List of Andean Cultivars from the Potato Collection with Resistance to Infection by Potato Leaf Roll Virus.

CIP Number	Species	Country	PVX	PVY	PLRV	Plants Used
700426	ADG	PER	S	S	R?	PUnt
700921	ADG	PER	S	S	R?	PT
701106=OCH 4692	ADG	PER	-	-	R?	PUnt
701589=CUS 392	ADG	PER	-	S	R?	PUnt
701754=HJT 5658	ADG	PER	-	-	R?	PUnt
701980	JUZ	PER	S	S	RM	PT
702317	ADG	BOL	-	S	R?	PUnt
703232	ADG	PER	S	S	RM	PUnt
703243	ADG	BOL	-	-	RM	PUnt
703266=CUP 199	ADG	PER	S	S	R	PT
703292	PHU	COL	S	S	R	PT
703295	PHU	COL	-	S	R	PT
703346	ADG	BOL	-	S	R	PT
703362	TBR	CHL	-	-	R	PUnt
703506	PHU	COL	-	-	R?	PUnt
703531=CCC 4383	ADG	COL	S	S	R?	PUnt
704220=CCC 4575	ADG	COL	S	-	R?	PUnt
704221=JAK 170	ADG	PER	S	S	R?	PUnt
704222=OCH 4744	ADG	PER	S	-	R?	PUnt
704223=OCH 4222	ADG	PER	S	S	R?	PUnt
704224=OCH 5150	ADG	PER	S	S	R?	PUnt

Finally, Table 4 shows a total of 12 accessions in the Potato Collection that combine resistances to more than one potato virus disease. Six of them combine resistance to PVX and PVY, 3 to PVX and PLRV, and 3 to PVY and PLRV.

As is demonstrated in the tables, there are numerous Andean cultivars that possess resistances to the most important potato virus diseases. For the frequency of cultivars that have been found resistant in the screening tests made by CIP scientists, the tetraploid *S. tuberosum* subsp. *andigena* appears to be a good source of resistance to viruses. However, its utilization in potato breeding has been minimized because of the presence of some undesirable agronomic characters such as late maturity, long stolons, deep eyes, etc.

Table 4. List of Andean Cultivars from the Potato Collection with Combined Resistance to Potato Viruses.

CIP Number	Species	Country	PVX	PVY	PLRV	PLANTS USED
700455	ADG	PER	R	R	-	PUnt
700775	ADG	PER	R	R	-	PUnt
701172 = 701122	ADG	PER	R	R?	-	PUnt
701225	ADG	PER	R	R	-	PUnt
701299	ADG	PER	R	R	-	PUnt
701535	ADG	PER	R	R	-	PUnt
700031 = 701535	ADG	PER	H	S	r?*	PT
700523	ADG	PER	R?	S	RM	PT
701171	ADG	PER	H?	S	R?	PT
700754 = CUS 644	ADG	PER	-	R	R	PUnt
702867	DG	PER	S	H	RM	PT
703294	PHU	COL	S	R	RM	PT

* Resistant in seedling test.

Most of these accessions have not been re-evaluated in pathogen-tested plants because virus eradication has only been made in a low percentage of them. However, the availability of new techniques for in vitro thermotherapy will certainly increase the number of pathogen-tested accessions in the near future. Furthermore, new biotechnological developments might facilitate the transfer of valuable genes from these native Andean cultivars into other potato cultivars with adaptation and other traits needed outside the Andean region.

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Resistance to Sweet Potato Viruses

J. Moyer and L. F. Salazar¹

Virtually every sweet potato breeding program is an evaluation trial for resistance or tolerance to local sweet potato virus strains. Sweet potato lines in these programs may accumulate local viruses, thereby ensuring that virtually all material is exposed to these viruses prior to release to the growers. This phenomenon has generally resulted in the absence of devastating virus diseases in sweet potato production areas with active sweet potato breeding programs. On an international basis, it also argues for the involvement of local programs in the preliminary evaluation of cultivars to be recommended for a given geographic area. Concomitant with this fortuitous situation is the scarcity of information regarding resistance to specific viruses. There are nearly 300 citations which address sweet potato viruses; however, of these, very few examine resistance to specific viruses.

Most report cultivar or breeding-line reactions to virus diseases prevalent in a particular locale which is valuable information on a local or regional basis but may not be widely applicable. This situation is not due to any overt omission or deficiency in breeding programs. In fact, breeding strategies have been proven for sweet potatoes that are very effective for introducing pest resistance into sweet potato genotypes (Jones et al., 1976). As efforts have increased to combat sweet potato viruses, it is logical to assume that the evolution of research on sweet potato viruses is now nearing the point where the proportion of effort on resistance should be expanded. The primary deficiency in sweet potato virus research with regard to expanding our research effort on resistance has been that the etiology of many of the important virus diseases has not been completely understood. Thus, it has not been possible to compare results from one test to another in the same or at different geographic locations. Many of the viruses or virus diseases are known only by the symptoms they cause in specific cultivars, resulting in the identification of some viruses as being very awkward. The implication for breeding programs is that detection systems are too cumbersome to be practical for evaluating large numbers of genotypes.

In spite of these problems, there are two diseases cited in the literature where resistance or perhaps more precisely, tolerance, has been introduced into commercial cultivars (Hahn et al., 1981; Jones et al., 1976; and Miller et al., 1960). In both of these instances, breeding programs were initiated to develop cultivars which were resistant to or at least highly tolerant of specific diseases. One of the diseases, internal cork disease (Nielsen, 1952), has relatively little effect on

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plant growth and yield. The primary effect was the objectionable necrotic, cork-like lesions which developed in the interior of the fleshy, edible roots (Nielsen, 1952). The etiology of internal cork disease has been linked to strains of sweet potato feathery mottle virus (SPFMV), but Koch's postulates have not been strictly completed (Cadena-Hinojosa and Campbell, 1981). The other disease is called the sweet potato virus disease and is caused by the synergistic interaction of selected strains of SPFMV and an as yet unidentified white-fly transmitted agent (Schaefer and Terry, 1976). This disease causes severe stunting of vine growth and root production (Hahn, 1973).

The breeding programs which identified resistance to internal cork relied primarily on artificial inoculation techniques, as experience had shown that natural infection did not result in a disease incidence sufficient to permit reliable evaluations. In this case the researcher used the labor-intensive procedure of core-grafting (Nielsen, 1952) tissue pieces from infected roots into roots of the tester clones (Nielsen and Pope, 1960). These roots were sprouted for plant production and subsequent production of roots. The roots were stored following harvest at the optimal temperature for lesion development and then evaluated for severity of lesion development. In one study, 32 clones were found to exhibit a wide range of reactions. The very sensitive clones exhibited severe lesions in nearly all of their roots. The most tolerant clones, while still infected, exhibited few or no lesions in the roots (Nielsen and Pope, 1960). This investigation also demonstrated that the tolerance to symptom expression was highly heritable. In one study, 36 progeny were evaluated from a resistant clone which had been selfed. Five of the clones exhibited symptoms, 10 were symptomless carriers and the agent was not detected in 21 of the clones (Nielsen and Pope, 1960). Empirically we now know that high levels of tolerance are highly heritable since nearly all new cultivars released in the United States since the release of 'Centennial' (6) have been tolerant to symptom expression.

Similar results were achieved by researchers investigating resistance to the sweet potato virus disease complex (Hahn, 1981; Schaefer and Terry, 1976). Although two viruses are involved in this complex, one vectored by aphids and the other by *Bemisia tabaci*, some progress was obtained in field trials which relied on natural infection. The natural infection compared reasonably well with results obtained with the same clones which had been core-grafted in subsequent trials. The clones were evaluated by subjectively assigning a numerical rating to each clone based on a combined assessment of disease severity and incidence. The results of these studies demonstrated that tolerance to symptom expression was a highly heritable trait (Hahn, 1981).

Tolerance to both of these diseases has been identified in sweet potato germplasm. It was possible in one instance to make use of natural infection for preliminary evaluations. While evaluations for symptom expression were conducted with relative ease, both research groups have conducted some evaluations of material to determine if symptomless clones carried the causal agents. In both cases many symptomless clones were carriers of the pathogen(s). Although it is

highly desirable to identify clones that are immune to infection, it may not be necessary in some instances. However, this aspect should be carefully considered in programs developing elite material to serve as the source of resistance to a disease or a specific virus. Elite breeding programs should carefully monitor the occurrence of pathogens in clones which are symptomless carriers (tolerant) to avoid the inadvertent exposure of more sensitive clones when these elite clones are distributed into new geographic areas.

Research is currently in progress at CIP to identify sweet potato clones which are immune to infection by SPFMV. The justification for selecting SPFMV as the first virus for which a resistance program would be developed to select clones which would be immune to a specific virus is several fold. SPFMV is worldwide in its distribution; although it does not by itself cause any serious diseases, it is involved in several notable disease complexes; further probes are available for efficient evaluation of clones for the presence of the virus (Abad and Moyer, 1988; and Moyer and Cali, 1985). Although research is still underway, considerable progress has been made to select clones which are resistant to one or more strains of SPFMV. A total of 1641 accessions in the CIP germplasm collection were examined for expression of virus symptoms which resulted in the selection of about 10% of the accessions. These were further tested for the presence of SPFMV by grafting to *Ipomoea setosa* and by ELISA. The remaining 6% were graft inoculated and further tested by ELISA and symptom expression, which reduced the number to 1% of the original (Fig. 1). These are currently being inoculated with a representative number of strains of SPFMV to determine the breadth of the resistance. Investigations are planned to determine the basis of the resistance, the heritability of the resistance and the stability of the resistance under field conditions.

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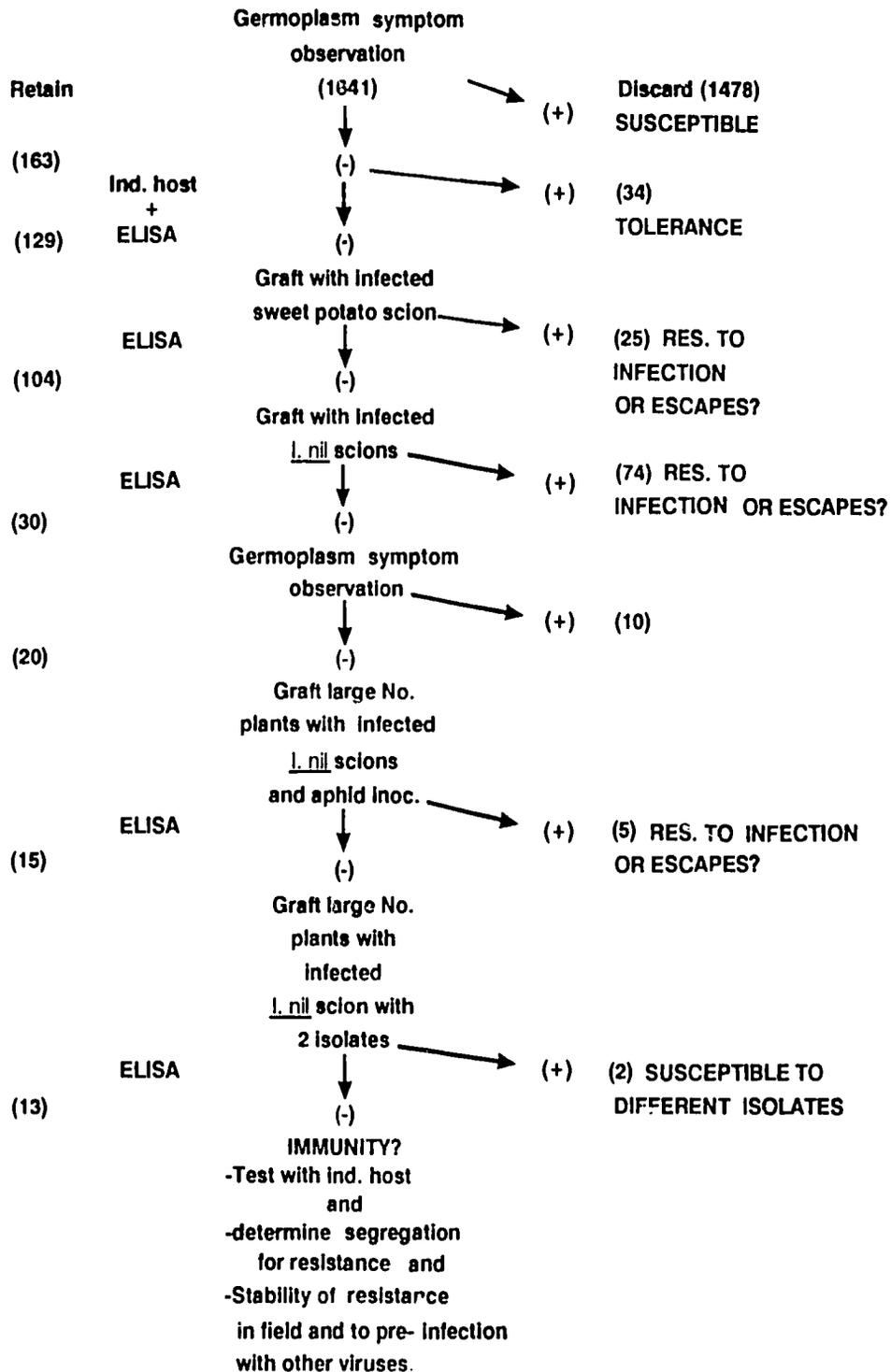


Figure 1. Search for genetic resistance (immunity) to SPFMV in CIP germplasm accessions.

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Variability of PVX and PVY and Its Relationship to Genetic Resistance

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Potato Virus X (PVX) and Potato Virus Y (PVY) are two of the most important viruses affecting the potato crop on a worldwide basis. Recent information on the incidence of potato viruses in developing countries indicate that PVX becomes more important in countries where certified seed has restricted the introduction of PVY and PLRV sources of inoculum and where aphid activity is low. PVY becomes more important in countries where mix cropping potato with other crops is a traditional farming system. The importance of PVX and PVY has increased for countries where potato is grown in large fields, since studies at CIP by Jayasinghe (1989) have shown that in certain cultivars, previous infection by PVX and PVY breaks the resistance to PLRV, making it necessary to breed for resistance to PLRV in a background of immunity to PVX and PVY.

Breeding for resistance to PVX and PVY is the best alternative to control these viruses. In general, breeding for resistance to diseases is one of the major accomplishments of plant breeding. However, durability of the resistance is a great concern. Our work and hopes at CIP are directed toward a durable resistance to PVX and PVY. For this, the work has to be accomplished within the framework of a breeding program that involves, besides the definition of an strategy, research to determine the methodology to adopt on:

1. sources of heritable resistance
2. suitable screening techniques
3. transfer of resistance to advanced breeding materials or adapted cultivars
4. management of genes for resistance.

To select a good source of heritable resistance for the breeding program, to make use of an appropriate population of the pathogen in our screening techniques, to be able to have a good detection technique to test our breeding populations, and to be able to manage in the future the genes for resistance, we need to know our enemy, especially its variability. The type of variability that we are interested in and the technique used to study it are:

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Variability
antigenic
genomic

Technique
serology
hybridization
pathogenicity

What is the experience at CIP with this kind of PVX and PVY variability?

Variability of PVX

According to Torrance et al. (1986), PVX isolates can be classified by using three monoclonal antibodies into the major serogroups I, II, III and IV. At CIP, in work mainly with Andean isolates and using NCM-ELISA (Fernandez-Northcote and Lizarraga, 1988), PVX isolates are classified in the serotypes PVX^O (common) and PVX^A (Andean). It appears that a third serotype might be present in Africa (Table 1).

When isolates from PVX^O serotype (PVX-GUA 2 and PVX-NZ) and from PVX^A serotype (PVX_{cp} and PVX_{HB}) were tested with three cDNA probes prepared by D.C. Baulcombe from a PVX^O British isolate (strain-group 3), they hybridized with all strains but more strongly with the PVX^O serotype (Baulcombe and Fernandez-Northcote, 1988). One probe, pPVX13, did not hybridize with the cp strain and showed only a weak reaction with HB, the other PVX^A serotype.

In collaborative work with M. Querci at CIP (Querci, Salazar and Fernandez-Northcote, paper in preparation), using probes that she has prepared at CIP for a PVX^A isolate (PVX_{cp}) serogroup III, results were the reverse of those obtained with Baulcombe's probes. That is, the Andean isolates from serogroups I-II and IV, which are in the common serotype PVX^O, reacted weakly, while the isolates in the PVX^A serotype (serogroup III) reacted strongly. This has been confirmed also by comparing simultaneously Querci's and Baulcombe's probes in a non-radioactive NASH.

In summary, there are two important serotypes: PVX^O and PVX^A. This antigenic difference is correlated with a genomic difference as detected by NASH, and it affects their detection if proper antibodies or probes are not used.

What is the variability of PVX with regards to pathogenicity?

Cockerham (1955) described 4 strain-groups according to the interaction with two dominant genes for hypersensitivity, N_x and N_b. According to the interaction with genes for immunity, I consider two pathotypes for PVX (Table 2). Almost all strains of PVX are pathotype 1 since they do not break the immunity to PVX given by the dominant genes R_{x_{and}}, R_{x_{acl}} or R_{x_{tub}}. However, there is one isolate, HB from Bolivia, which breaks that immunity. I consider this isolate as a different pathotype: pathotype HB.

What is the relationship of serogroups, serotypes, strain groups, and pathotypes? Although Torrance et al. (1986), reported that their serogroups did not correlate well with Cockerham's

serogroup IV with strain group 3. They used only a few isolates for serogroups II and III to give an association. Our work associates serogroup III with pathotype HB, and serogroup I-II and IV with pathotype 1 (Table 2). The pathotype HB belongs to the PVX^A serotype, serogroup III, which is recognized by MA 67, which we can use to detect PVX^A isolates and, indirectly, the pathotype HB.

Table 1. Worldwide distribution of serogroups and serotypes of PVX according to their reaction to monoclonal antibodies 58, 59 and 67 in NCM-ELISA.

Percent Isolates ^a	Geographic area	Serogroup	Serotype
39	Peru, Bolivia, UK, Kenya	I or II	PVX ^O
54	Peru, Bolivia, Chile, Guatemala USA, UK, Netherlands, India Kenya	IV	PVX ^O
7	Peru, Bolivia	III	PVX ^A
1	Kenya	----	PVX ^{AF}

^aFrom a total of 149 isolates tested.

Table 2. Strain groups and pathotypes of PVX.

Resistance Level ^b	Gene	Pathotypes of PVX				
		1				HB
		Strain Group ^a				
		1	2	3	4	
s	nx, nb, rx	s ^c	s	s	s	s
H	Nx	R	s	R	s	s
	Nb	R	R	s	s	s
	Nx, Nb	R	R	R	s	s
I	Rxand	R	R	R	R	s
	Rxacl	R	R	R	R	s
	Rxtub	R	R	R	R	s

^aBased in Cockerman (1955) for levels s and H.

^bs = susceptibility, H = hypersensitivity, I = immunity.

^cs=susceptible, R = resistant.

At present, only one isolate from Bolivia, the HB isolate, has been tested as a breaking strain for the immunity to PVX. In Bolivia, PVX^A is localized mainly in the Lake Titicaca area (Fernandez-Northcote and Lizarraga, 1988). This knowledge should allow us to design for Bolivia a strategy for the management of genes for resistance to PVX.

Our studies of the variability of PVX give us information on the proper IgG and conjugates to be used in ELISA, the most routinely used test for the detection of PVX, and proper cDNA probes to be used in radioactive and non-radioactive NASH, for worldwide detection of PVX for both quarantine and breeding for resistance.

Variability of PVY

Potato virus Y can be grouped into the PVY^O, PVY^N and PVY^C groups of strains according to their effect on *Nicotiana tabacum* 'White Burley' and aphid transmissibility (Fernandez-Northcote, 1980).

We expect a greater variability of PVY in the Andean region, the center of origin of the potato, as it has been shown above in the case of PVX. Isolates obtained from this region gave us the following distribution (Fig. 1) (Fernandez-Northcote, in preparation).

It is possible to detect differences between PVY^O and PVY^N groups of strains using double diffusion in agar with sodium dodecyl sulfate. All isolates tested so far have been recognized in DAS-ELISA by polyclonal antibodies when a PVY^N strain was used to prepare the antibodies (Fernandez-Northcote and Gugerli, 1987). There is not at present a set of MA that would selectively detect each of the three groups of strains of PVY. However, studies with European isolates by Gugerli and Fries (1983) and with our Andean PVY gene pool by Fernandez-Northcote and Gugerli (1987) show that MA-C9 detects an epitope common all over the world. A few PVY isolates are not detected by this MA. The epitope detected appears to be highly conserved among the virus population and is probably important for virus survival.

All PVY isolates tested, both European and Andean, hybridize with the cDNA probe pPVY15 developed by D.C. Baulcombe (Baulcombe and Fernandez-Northcote, 1988) from a British PVY^O isolate.

It appears that in the case of PVY, evolution has been faster than in PVX. PVY deviants appearing because of geographical, vector and host (i.e. tobacco, tomato, pepper, etc.) pressures, have become at present different although related potyviruses. In relation to the breeding program, this is important because we can expect a different PVX strain to overcome the gene for immunity. In the case of PVY, we may expect not a different strain but a different PVY-related potyvirus.

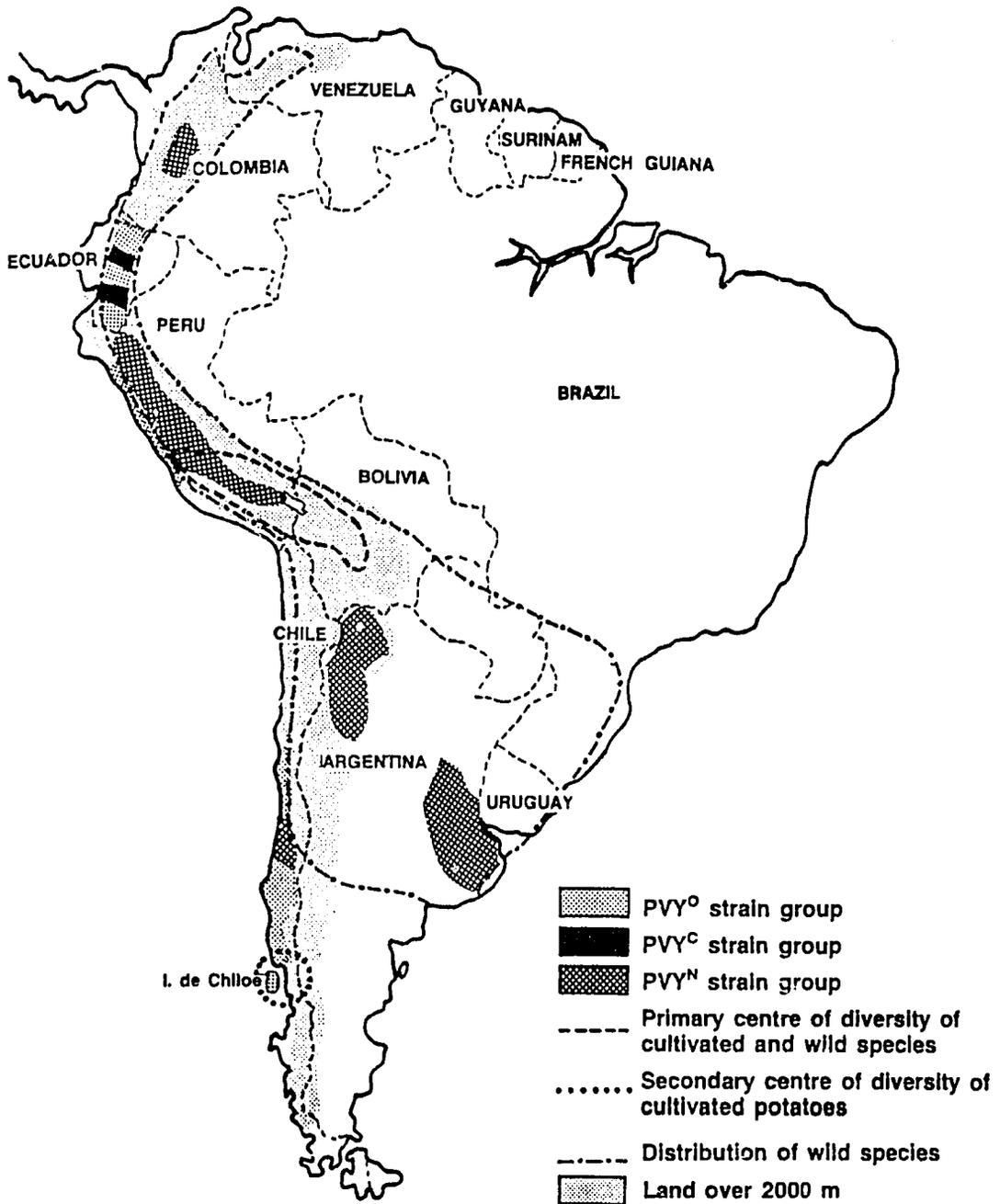


Figure 1. Distribution of potato virus Y strains, and potato, in South America.

A selected group of immune progenitors have been challenged under controlled conditions with a broad spectrum of PVY isolates mainly from the Andean region as well as with other potyviruses detected in potato or other natural hosts of PVY. These studies have confirmed previous observations with a few PVY European isolates (Cockerman, 1970) that the immunity to PVY from *andigena* and *stoloniferum* is effective against a wide spectrum of PVY isolates from the three recognized groups of strains PVY^O, PVY^N and PVY^C (Fernandez-Northcote, 1983). The sources of immunity to PVY were also immune to other potyviruses tested (Table 3).

Table 3. Reaction of PVY sources of immunity to PVY and related potyviruses after mechanical and graft inoculation.

Virus	Origin	Immunity from <i>S. stoloniferum</i>		Immunity from <i>S. tuberosum</i> ssp. <i>andigena</i>		Controls	
		Bzura	V-3	V-2	XY13.14	Conchita	Molinera
PVY ^O	Peru	I*	I	I	I	h	S
PVY ^N	Peru	I	I	I			
PVY ^C -Arran	Holland	I	I	I	I	h	S
PVA	Holland	I	I	H	H	R	S
PVV (PVY ^C -GL)	Holland	I	I	Ha	Ha	S	S
PVV (PVY ^C -AB)	Ireland	I	I	Ha	H	h	S
PVV (UF)	Peru	I	I	I	I	S	S
PTV	Peru	I	I	I	I	I	I

*I = immunity; H = hypersensitivity; Ha = hypersensitivity only after grafting; h = low hypersensitivity, virus becomes systemic; R = resistant, no infection after mechanical inoculation but after grafting; S = susceptibility.

However, there was an important exception, the *andigena* source was not immune to PVA, it was hypersensitive. It was also hypersensitive to potato strains of PTV (PVV) but in this case only after grafting. Thus, there is a difference in the immune genotype between these two sources of immunity. Progress made so far in the number of advanced clones selected with immunity to PVY in different parts of the world, should lead us to develop a strategy to decide which gene to

deploy in any given area. We should not deploy the gene for I to PVY from *andigena* in an area where we know that PVA is or could be widespread.

What is the experience on the stability of the immunity to PVX and PVY under natural conditions and the natural variability of both PVX and PVY?

After field exposures, six at La Molina-Ica, Peru, and two at Las Brujas, Uruguay, no clones with immunity to PVX or PVY were infected with these viruses (Table 4). However, clones with a lower level of resistance such as hypersensitivity were infected in various percentages (Fernandez-Northcote, Hernandez, Crisci and Vilaro, unpublished).

Table 4. Percent of plants infected with main potato viruses after field exposures, six at La Molina-Ica, Peru, and two at Las Brujas, Uruguay.

Clone	PVY			PVX			PLRV		
	R*	Las		R	Las		R	Las	
		Ica	Brujas		Ica	Brujas		Ica	Brujas
V2	I	0		I	0		RR	67	
V3	I	0		I	0		RR	0	
Bzura	I	0	0	I	0	0	S	100	53
LT-4	I	0		S	50		S	100	
Serrana	H	0	7	H	30	17	RR	0	27
CEX 69.1	H	20	60	S	0	25	S	70	75
MEX 750815	H	0	20	S	10	0	S	0	47
Tomasa C.	S	88		S	13		RR	10	
DTO-33	S		60	RR		44	S		87

*R = reaction after inoculation under greenhouse conditions. I = immunity, H = hypersensitivity, RR = relative resistance, S = susceptibility.

Data in Table 5 from another set of experiments at La Molina and Ica, Peru, again show the stability of the immunity to PVX and PVY (Garcia, and Fernandez-Northcote, 1990).

Table 5. Percent of infection after three field exposures in the central coast of Peru in 1988-1989.

Clone or cultivar	Reaction to		Percent of infection			
	PVX	PVY	PVX	PVY	PVS	PLRV
V-2	I*	I	0	0	30	40
V-3	I	I	0	0	30	10
LT-8	I	I	0	0	30	20
Bzura	I	I	0	0	10	30
LT-9	I	I	0	0	40	30
Loman	R	H	50	60	40	30
Serrana	H	H	30	40	0	20
P.Crown	S	H	40	30	40	30
Cew-69.1	S	H	50	70	30	50
Rosita	S	S	40	90	60	50
Tomasa C.	S	S	40	60	60	30

* Reaction after inoculation under greenhouse conditions. I = immunity, H = hypersensitivity, S = susceptibility.

Concluding remarks

As a conclusion, I would like to emphasize that in our breeding program for resistance to PVX and PVY, studies on the variability of these viruses have allowed us to select proper sources of heritable immunity, to select an appropriate strain for our screening techniques, to select appropriate strains for the production of IgG and conjugates for ELISA and for cDNA probes for the detection of these viruses, not only to monitor our breeding materials but also for quarantine purposes, and finally to predict the future stability of immunity to PVX and PVY.

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Variability of, and Resistance to Potato Leafroll Virus (PLRV)

U. Jayasinghe¹

Potato leafroll is the most important virus disease in potato and it can cause yield losses up to 80% in susceptible cultivars. This can be even higher when the plants are simultaneously infected with other viruses such as PVY and PVX of common occurrence in potato (Peters and Jones, 1981). Potato leafroll virus is a Luteovirus (Matthews, 1982; Casper, 1988) having a particle with a diameter of 26 nm. The virus is restricted to the phloem sieve tubes and companion cells of the infected plant (Shepardson et al., 1981; Barker and Harrison, 1986), and it is transmitted by more than 10 different aphid species; *Myzus persicae* is its most efficient vector (Harrison, 1984).

Variability of PLRV

Very little work has been done with respect to the variability of this virus. Earlier reports indicated the existence of strains which cause symptoms of varying severity in infected *Physalis floridana*, in *Solanum tuberosum* and in *Montia perfoliata* or showing differences on their ease of transmission by the aphid *M. persicae* (Webb et al., 1951; Tamada et al., 1984). In experiments conducted at CIP using 3 different virus isolates from different parts of the world, the symptoms produced in infected *F. floridana* were not consistent. In the first experiment clear differences could be seen where the Peruvian isolate 29 produced the most severe symptoms, whereas isolate from China produced the least severe symptoms. These results, however, were never reproduced in other experiments. In one occasion isolate 29 produced the least severe symptoms. There was no variation in the symptoms caused by the different isolates on other hosts of PLRV such as *Nicotiana benthamiana*, *Datura stramonium*, *N. clevelandii*, *Gomphrena globosa* or *Lycopersicon esculentum*. Wild *Solanum* species were not useful to distinguish PLRV isolates.

Polyclonal antisera used in double diffusion or ELISA serology were not useful to identify the different strains of PLRV (Kojima, 1981; Tamada et al., 1984). However, Massalski and Harrison (1987) were able to distinguish PLRV isolates differing in aphid transmissibility using monoclonal antibodies produced against a British isolate of PLRV. Recently we have tested seven monoclonal antibodies produced against a British isolate of PLRV with different virus isolates available at CIP. Since it was not possible to purify PLRV the results could not be quantified. To summarize the results, the highest absorbance value for every virus was assigned a value of 10, and the remaining absorbance values were calculated in relation to this value. The results indicate that a large variability of surface epitopes is found in the PLRV particles

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(Table 1). Based on the reactions with the monoclonal antibodies we can group the PLRV isolates at CIP into three serogroups.

Group number one includes the Korean, 029 from Peru and El Salvador isolates, the group number two includes the PLRV isolates from China, Kenya, Uruguay and the British. However, in this group the Chinese isolate is slightly different to the rest of the members since it reacts strongly with the monoclonal PM-10. In fact only the Chinese isolate reacts strongly with PM-10 monoclonal. The third group includes isolate 10 from Peru. These preliminary results indicate the existence of variability among the different PLRV isolates tested.

Table 1. Reaction of different PLRV isolates with some PLRV monoclonals prepared against the British isolate.

PLRV Isolate	Monoclonals					
	1	4	5	7	PM-6	PM-10
Korea	10	6	6	4	5	2
029 (Peru)	10	6	9	6	8	3
El Salvador	10	5	8	4	5	1
China	7	2	10	1	4	7
British	9	7	10	4	1	2
Uruguay	9	5	10	6	2	3
Kenya	8	3	10	1	5	1
010 (Peru)	4	6	7	1	10	2

Resistance

So far in both wild and cultivated *Solanum* species immunity to this virus has not been found. However, it is reported that the resistance to PLRV is polygenically controlled, but it may be controlled by a small number of genes (Ross, 1958; Brown, 1980). Many cultivated and wild *Solanum* species have been reported as resistant to this virus. The majority of these reports refer to field observations or glasshouse inoculation using aphids (Davis et al., 1975; Simpson et al., 1952; Mackinnon, 1969; Ross, 1978). This type of resistance to PLRV is called relative resistance, and it can only be established after comparing the percentage of infection of the test clone with that of susceptible controls (Davidson, 1973). For obtaining this data frequent field exposure trials have to be done. Field exposure trials depends on the natural aphid population, and often these trials have to be repeated to obtain reliable results. The necessity to have field trials to determine leafroll resistance extends the time scale of a breeding programme for PLRV resistance by some years. Many attempts have been made to determine the levels of resistance in potato genotypes under glasshouse conditions (Davidson, 1973; Chuquillanqui and Jones, 1980).

Though the data obtained under glasshouse conditions are important the final result has to be obtained after a field exposure trial for the reasons which will be explained later.

In both wild and cultivated *Solanum* species which are resistant to PLRV, many components of the resistance can be identified. The various components of resistance to PLRV are:

1. resistance to infection
2. resistance to multiplication
3. tolerance to infection
4. resistance to virus transport (translocation)
5. hypersensitivity
6. aphid antibiosis
7. aphid antixenosis

Resistance to PLRV infection

Genotypes showing this component of the resistance do not become infected when inoculated with a standard number of viruliferous aphids. Considering the time and cost of field exposure trials to assess this resistance, we at CIP have developed a greenhouse method to rapidly determine the levels of resistance to infection (CIP, 1985). The levels of resistance obtained in this glasshouse test is comparable to that of field exposure trials with certain limitations. Using this methodology a major part of the clones from the CIP pathogen tested list have been screened for PLRV resistance to infection and only a few of these clones showed a high level of resistance. This data however, has limited value since only one species of aphids was used in the trials. Potato leafroll virus is transmitted by more than 10 different aphid species and under field conditions the plants are exposed to the available natural aphid population, where *M. persicae* may not be the most abundant aphid species present (Fig. 1) as shown for Ica, a town located 340 km south of Lima, Peru. Temperature has an influence on the efficiency of transmission of PLRV by *M. persicae* (Cervantes, 1988). The effect of temperature on other aphid species in transmitting PLRV is not documented. Therefore, under the conditions prevailing in Ica *M. persicae* may not be the most efficient vector. Our preliminary experiments also indicate that aphid populations of the same species collected in different localities can vary greatly in their efficiency to transmit PLRV. Though field exposure trials can give more accurate data on the level of resistance to infection of a genotype in a given locality, at times obtaining this data can be a difficult task because of interactions with other viruses, and because resistance to infection in potato genotypes can be overcome due to interactions with other viruses (Jayasinghe et al., 1989).

Resistance to multiplication and tolerance

Using ELISA we can determine the concentration of PLRV in infected tissues at any time after infection. This method therefore can be used to determine clones which have a very low PLRV concentration after infection.

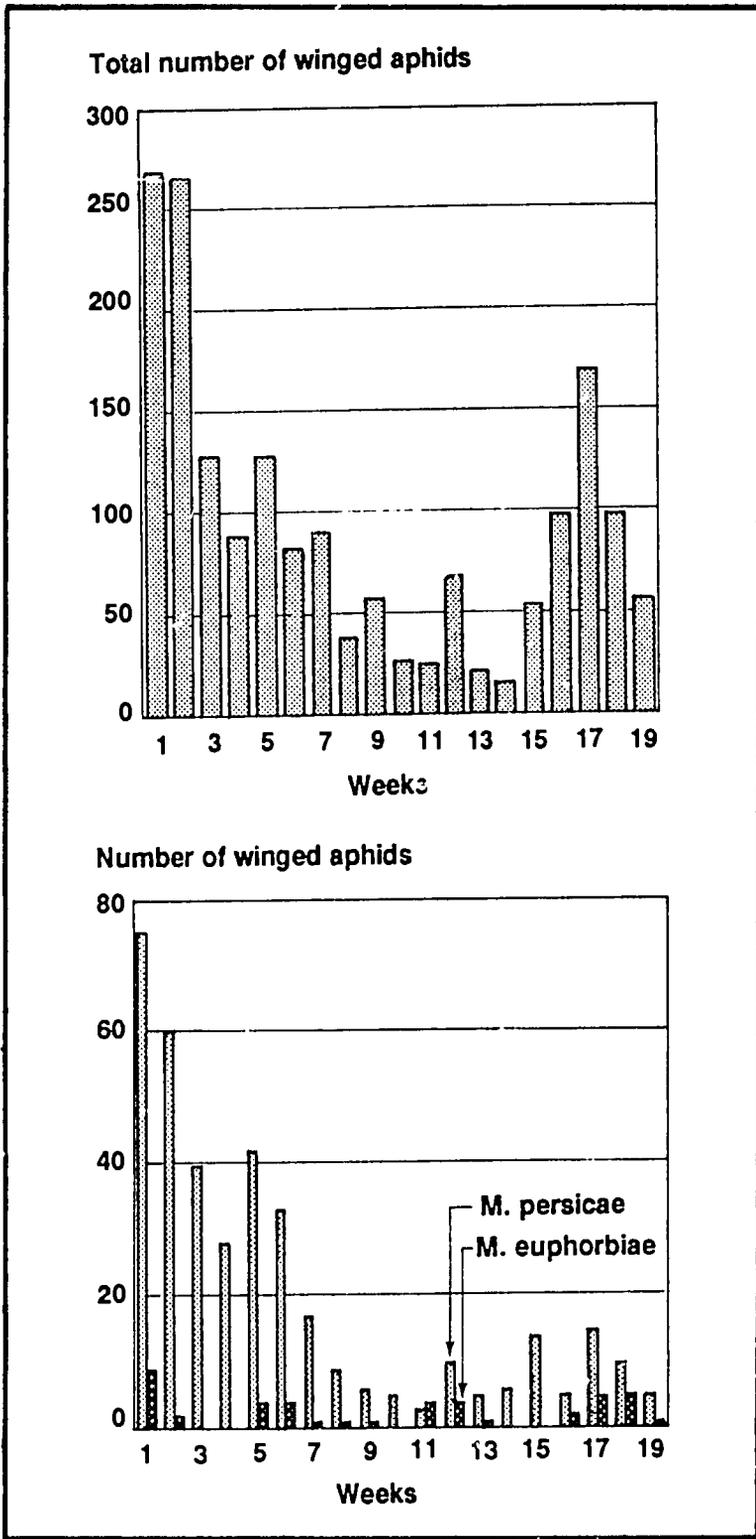


Figure 1. Aphid population data from Ica, Peru, 1987 (May-September).

In clone B-71-240.2, the maximum concentration of PLRV, reached approximately 4 weeks after infection, is about 5 times less than that of DTO-28 (Fig. 2). This is an indication of resistance to PLRV multiplication in clone B-71.240.2. So far, we have only been able to identify two clones in the pathogen-tested list with this component of resistance. PLRV-infected clones having this component of resistance do not show symptoms under field conditions. Hence they are tolerant to PLRV. It is important to note that clone B-71-240.2 has only resistance to PLRV multiplication; it does not have any resistance to PLRV infection. This means that two separate mechanisms of resistance are involved in these two components. Recently, we have analyzed two clones originating from the Scottish Crop Research Institute. These two clones have an even greater level of resistance to multiplication than clone B71-240.2. According to Barker (personal communication, 1989) this component of resistance to PLRV is governed by a single dominant gene.

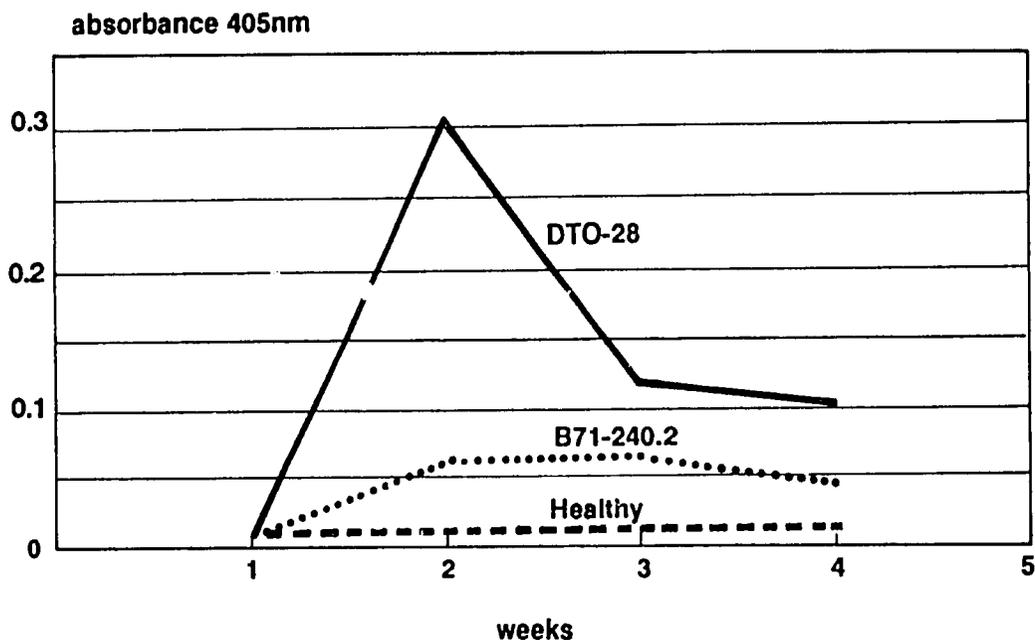


Figure 2. Rotative concentration of PLRV after inoculation at weekly intervals in clones DTO-28 and B71-240.2.

Resistance to multiplication is also modified due to interaction with other viruses. Experiments have shown that the concentration of PLRV in clone B-71-240.2 infected only with PLRV is less than in plants infected simultaneously with virus SB-22 (Fig. 3) or PVY. Clones showing low absorbance values in ELISA for PLRV are also poor sources of virus for the vector *M. persicae* (Barker and Harrison, 1986) because in these clones only a few phloem companion cells are infected and the virus is present in negligible amounts in the phloem. Using microscopy it has been shown that, upon infection with PVX these resistant clones have a larger proportion of companion cells infected with PLRV than when they are infected with PLRV alone (Barker, 1987). Reports also indicate that in plants infected with both PLRV and PVX or PVY, PLRV can also infect mesophyll and parenchyma cells and its concentration is higher than when infected with PLRV alone (Barker 1987; Barker and Harrison, 1982; Atebekov et al., 1984).

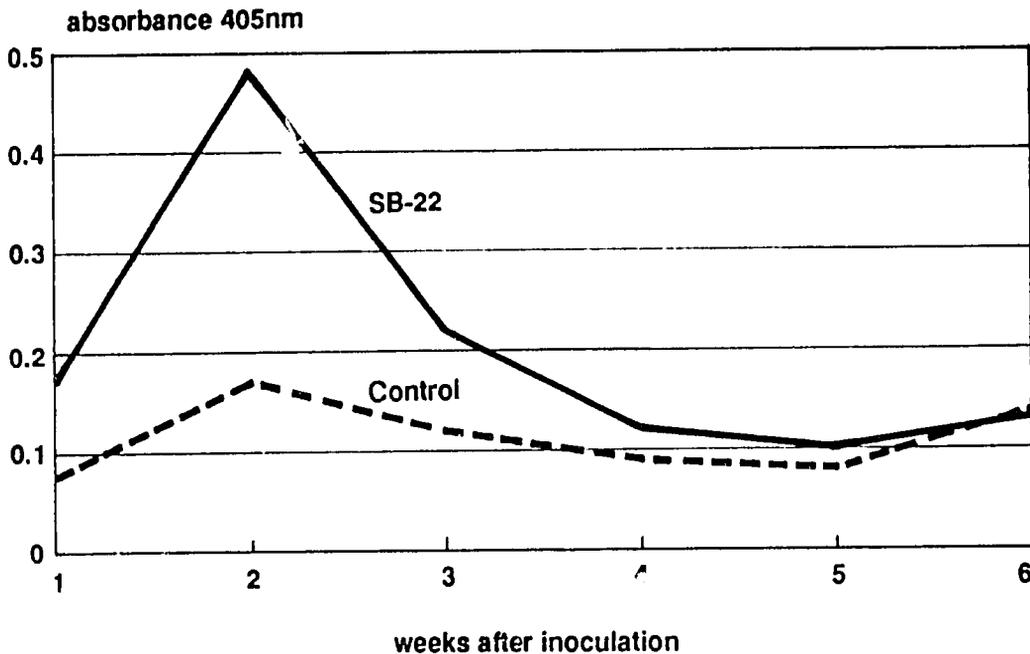


Figure 3. Concentration of PLRV in clone B-71.240.2 with and without infection with virus code named SB-22.

Hypersensitivity/intolerance to PLRV

Certain German and Polish clones such as Apta and Carla, when infected with PLRV, produce as primary or secondary symptoms stem necrosis, premature death of plants, lack of tuber setting, poor or absence of sprouting and wilting. These symptoms are generally different to those that we see in the field with infected *Solanum tuberosum* ssp. *tuberosum* or *S. tuberosum* ssp. *andigena*.

Phloem necrosis is a common microscopic symptom in PLRV-infected plants. However, in the cultivars Apta and Carla the effect is severe and immediate, causing rapid wilting of the infected plants. It has also reported that hypersensitivity is governed by a single dominant gene which is modified by minor genes (Butkiewicz, 1978; Zadina and Novak, 1983). Clones having hypersensitivity are considered as self-eliminating for PLRV infection due to the absence of tuber sprouting caused by severe phloem necrosis.

Resistance to PLRV translocation

When a virus infects a potato leaf, it does not move to other tissues immediately. First, it multiplies in the infected cell and, when the concentration of the virus reaches a certain level, the virus begins to move to other cells. PLRV infects the phloem companion tissue but long distance transport occurs via the sieve tubes (Barker and Harrison, 1986). The speed at which the virus translocates initially depends on the rapidity with which the virus enters the sieve tubes. Therefore, in clones having resistance to multiplication, the rate of translocation should be slower.

As mentioned, earlier interaction with viruses PVX and PVY will lower the resistance to PLRV multiplication and accelerate the rate of translocation. Our preliminary experimental results do not show any differences in the rate of translocation of PLRV in the potato clones studied. However, in *Solanum acaule* where strong resistance to multiplication occurs, a strong resistance to translocation also occurs. This indicates that the level of resistance to multiplication is greater in wild *Solanum* species than in the cultivated clones. The resistance to translocation may also indicate the rapidity at which plants respond to infection by producing callus tissue in the phloem, so preventing virus translocation. This resistance is an important component since it may directly determine the time of infection and number of tubers infected.

Aphid Antibiosis and Antixenosis

These two topics are purely approaches to insect resistance in plants, therefore, they represent indirect but important approaches to PLRV resistance. Any factor which affects the vector may contribute to PLRV resistance. Antibiosis includes all the adverse effects exerted by a plant on the biology of aphids. It may be a factor which reduces the rate of growth, oviposition, the number of nymphs produced, or even aphid mortality. The advantage of this component is its effect on the aphid population build-up within a field and, prevention of spread of PLRV.

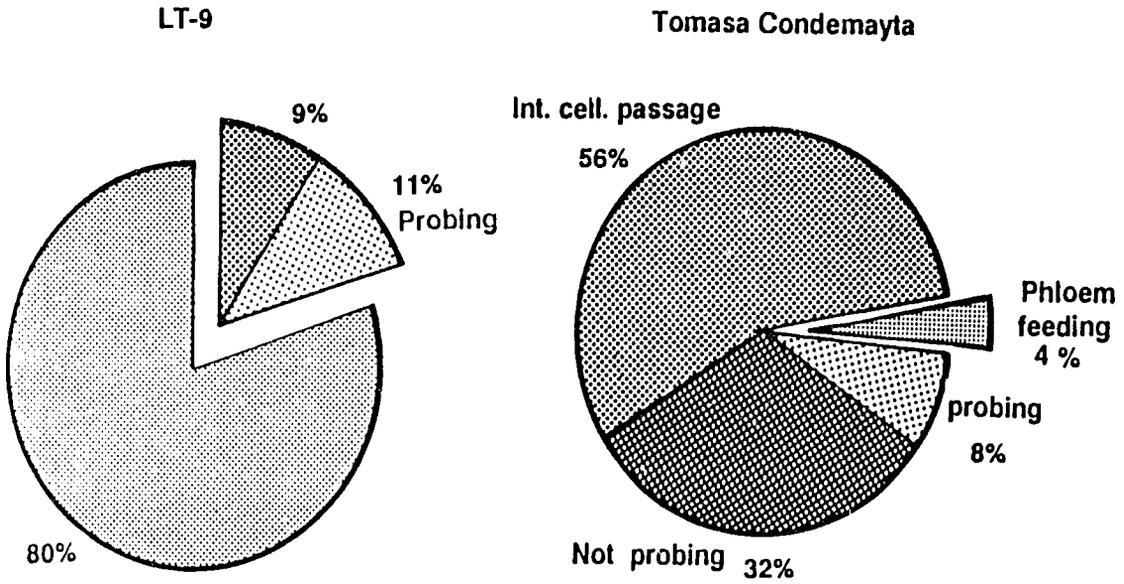
Antixenosis or non-preference is avoidance of plants by aphids as hosts. This non-preference may be due to toxins in the plant, or to the presence of volatile repellants produced by the plant or even due to non-glandular-type hairs. In the potato cultivar Tomasa Condemayta, this component of resistance to PLRV is exhibited very strongly in Peru. Viruliferous aphids inoculated onto Tomasa Condemayta move away from the plant without inoculating PLRV. Our previous experiments with Tomasa Condemayta show that this clone does not possess resistance to PLRV multiplication, hypersensitivity, tolerance, or translocation, which are resistance components directed toward the virus. However, Tomasa Condemayta possesses resistance to infection and antixenosis or non-preference by the aphid vector.

Resistance to infection can be direct or indirect due to the resistance to the vector. To differentiate between direct and indirect resistance in Tomasa Condemayta, we utilized an electronic system for monitoring the feeding behavior of aphids as described by Brown and Holbrook (1976). The wave pattern of the feeding behavior obtained in our experiment is similar to that reported by Brown and Holbrook. From these wave forms, we calculated the time an aphid spends in non-probing, probing, intercellular passage, ingestion of parts other than the phloem, and ingestion of phloem from its host. The experiment was repeated 20 times with *M. persicae* and *M. euphorbiae* as vectors and Tomasa Condemayta as the PLRV-resistant clone and clone LT-9 as the susceptible clone.

Results obtained (Fig. 4a) indicated *M. persicae* on clone LT-9, spent, on average, 13% of the time non active, 6% of the time probing, 11% of the time feeding on the phloem. On the other hand, in Tomasa Condemayta most of the time the aphids were inactive, and the time spent probing is similar to that of LT-9, less time was spent on intercellular passage and phloem feeding was only 5%. It is important to mention that among the 20 aphids tested on Tomasa Condemayta, only one aphid could reach the phloem, whereas in the case of LT-9, the majority of the aphids reached the phloem. Results obtained (Fig. 4b) indicated *M. euphorbiae* on Tomasa Condemayta spent, on the average 80% of the time non active, 11% of the time probing and 9% of the time was spent in intercellular passage of the stylets. None of the aphids *M. euphorbiae* tested could reach the phloem in Tomasa Condemayta. Both aphid species tested spent considerable time non active on Tomasa Condemayta and many short-duration probes are done before a deeper penetration of the stylets can occur. Deeper probes are also abandoned more frequently than in the case of LT-9.

To confirm the phloem feeding of *Macrosiphum euphorbiae* in Tomasa Condemayta aphids were allowed to feed on the ventral side of the leaf using an entomological cage. Then the leaf tissues were processed for sectioning.

(a) *Macrosiphum euphorbiae*



(b) *Myzus persicae* Sulz.

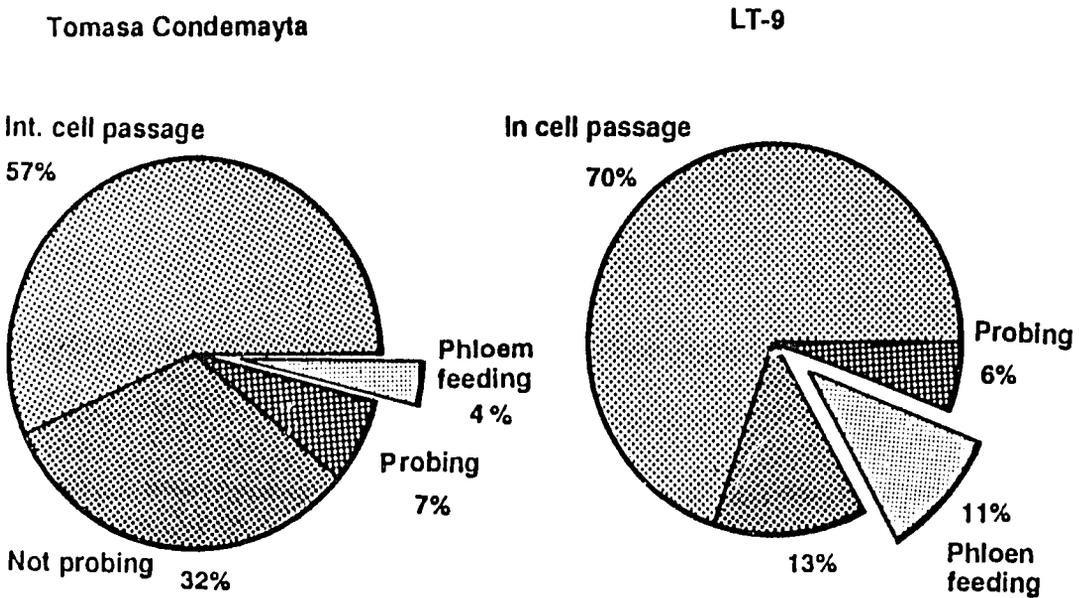


Figure 4 a and b. Feeding habits of aphids (a) *Macrosiphum euphorbiae* (b) *Myzus persicae* on clone LT-9 and cultivar Tomasa Condemayta.

Cut sections were stained with safrannin and fast green. For both vector and host combinations the number of branches in a stylet and its destination were recorded. The results show that in LT-9, the stylets ending in phloem were greater than in Tomasa Condemayta (Table 2). The number of probes ending in palisade cells and mesophyll cells are greater in Tomasa Condemayta than in LT-9 an indication of unsuccessful probes is greater in Tomasa than in LT-9.

Table 2. Comparison of number of branches in a probe and their destination.

<i>Aphid</i> /Clone	No. of branches	Percentage of branches ending in				
		Phloem	Xylem	Palisade	Mesophyll	Epidermis
<i>M. persicae</i>						
LT-9	102	62.7	3.9	30.4	0.9	5.8
T. Condemayta	137	22.38	8.3	58.3	11.01	0
<i>M. euphorbiae</i>						
LT-9	105	48.57	1.0	50.47	0	0.95
T. Condemayta	128	21.09	3.0	60.93	12.5	3.16

These results indicate that aphids find it difficult to locate the vascular bundles specially the phloem cell, the site of PLRV multiplication. Published reports indicate the presence of chemicals, or a sugar gradient which is detected by the chemo-receptors located at the tip of the stylets guiding the stylets toward the phloem tissue. The reason why the aphids cannot locate the phloem tissue in Tomasa is not known. Normally under field conditions colonization of the recently-grown potato crop is done by winged aphids whose primary objective is to locate a suitable host and produce young. The winged aphids' difficulty in locating the phloem in Tomasa Condemayta discourages their feeding. Further research is needed to elucidate the gene inheritance to see the possibility of incorporating this resistance to PLRV resistance breeding program.

Concluding Remarks

Genotypes which show resistance to PLRV possess one or many of the components of resistance described in Table 3. In the case of cultivars Serrana and Mariva the only component of resistance present is resistance to infection. In the case of cultivar Tomasa Condemayta, the component of resistance is aphid antixenosis. These three cultivars show resistance to PLRV infection under field conditions. However, the mechanism of resistance in each case is different. In the case of Mariva and Serrana, the mechanism is directed towards the virus, whereas in the case of Tomasa Condemayta it is towards the aphid vector, indirectly give resistance to the virus. Therefore, resistance to PLRV infection is of two types, direct and indirect. The existence of

more than one component of resistance in a genotype is not uncommon. In *Solanum acaule*, all the components of resistance discussed earlier are present. This makes *S. acaule* very resistant to PLRV. In the absence of immunity and dominant resistance genes, breeding for PLRV resistance is not as straightforward as it is for PVX and PVY. Therefore, the best approach would be the integration of various components of resistance mentioned above to give adequate control for this disease.

Table 3. Relation of some selected potato genotypes to components of resistance to PLRV.

Genotype	Resistance to			
	Infection	Multiplication	Antixenosis	Symptoms
Mariva	Yes	No	No	Yes
Serrana	Yes	No	No	Yes
T. Condemayta	Yes	No	Yes	?
B-71-240.2	Moderate	Yes	Yes	No
Mex-32	Yes	No	Yes	Yes
<i>S. acaule</i>	Yes	Yes	Yes	?

? = data not available

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Breeding for Resistance to Potato Viruses Y, X, and Leafroll: Research Strategy, Selection Procedures, and Experimental Results

H. Mendoza, E. Fernandez-Northcote, U. Jayasinghe,
L. F. Salazar, R. Galvez, and C. Chuquillanqui¹

Introduction

The cultivated potato *Solanum tuberosum* L. is susceptible to the attack of a large number of pests and diseases. A compendium of potato diseases (Hooker, 1981) lists 23 viruses, 38 fungi, 6 bacteria, 2 mycoplasmas, and 1 viroid that infect this crop plant. Among these pathogens the four most distributed, on a world-wide basis, are late blight (*Phytophthora infestans*), potato leafroll virus (PLRV), potato virus Y (PVY) and potato virus X (PVX). Other plant parasites of major economic importance are more localized within geographical zones. (Mendoza and Sawyer, 1984).

The potato viruses in general and PLRV, PVY, and PVX in particular, are the most important agents of the degeneration of the crop. Infected seed tubers transmit viruses from one generation to the next, reducing, to a variable extent, the yield potential of the crop.

Most temperate countries, so as to avoid the consequences of virus infections, have seed programs producing seed tubers that are in an excellent sanitary condition. Conversely, in most developing countries seed programs either do not exist or do not produce enough high quality seeds to supply the farmer's demand. Consequently, the farmer will either be forced to buy costly imported seed or to use locally-available seed which frequently has a medium-to-high degree of viral infection which usually cause variable yield reductions.

Moreover, in many developing countries, the establishment of seed production programs often has serious economic and environmental limitations and their output may not help in solving the problems. Under these conditions, the availability of varieties with resistance to the three major potato viruses, PLRV, PVY, and PVX, is the most logical route to improve seed quality and crop yields in quantity, quality, and stability.

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In the last 5 to 6 years, the International Potato Center (CIP) has placed a strong emphasis on breeding for virus resistance in order to minimize the importance of these pathogens in seed production.

General Breeding Strategy

The breeding strategy at (CIP) maximizes the utilization of the genetic resources contained in the genus *Solanum*. These include commercial cultivars and breeding lines, primitive cultivated species, and a set of selected wild species containing valuable traits for resistance or tolerance to stresses, pests, and diseases.

To utilize these genetic resources a population-breeding strategy based on a cyclic-recurrent selection scheme with progeny testing is followed. This strategy pursues an overall germplasm improvement which besides maintaining a wide genetic variability is also directed to an increase in the frequency of genes controlling valuable attributes and to their recombination. These selection criteria were applied from the onset of the population development. Further, selection for adaptation yield, earliness, and tuber-quality attributes is performed in a genetic background of resistances or tolerances to major pests, diseases, and stresses. CIP is developing advanced populations from which several National Potato Programs of developing countries have already released new commercial varieties and/or these countries are in the final process of testing advanced clones characterized by a high yield and resistance or tolerance to two or more pests, diseases or stresses.

Within this context efficient seedling screening techniques play a major role in the outcome of the population breeding strategy. Selection will change gene frequencies and consequently modify the genotypic structure of the population. The magnitude of change will depend on the precision in identifying and isolating the individuals carrying the attributes under selection. Any errors or "escapes" during the process of screening, could, depending on their importance, alter the response to selection. This justifies the statement that "a breeding effort can only be as efficient as the screening procedures permit" (Mendoza, 1987). This paper on Breeding for Resistance to Potato Viruses Y, X, and Leafroll presents a specific example of recurrent selection to illustrate the overall population breeding strategy at CIP.

Genetic Considerations

The cultivated potato *Solanum tuberosum* L. is an autotetraploid with $2n=4x=48$ chromosomes. Thus, the transmission of attributes from parents to offspring involves a tetrasomic inheritance. In the context of this paper and for the inheritance of the immunity to potato viruses Y and X a random chromosome segregation ($=0$) and the presence of only two allelic forms at one locus are assumed.

It is known that the immunity to the potato viruses Y (PVY) and X (PVX) are each controlled by a dominant major gene i.e. Y controls PVY immunity and y susceptibility. Likewise,

X controls PVX immunity and x susceptibility. Further, the assortment of these two loci is independent. At each locus there are five possible genotypes but only two possible phenotypes (Table 1).

Table 1. Genotypic and phenotypic structures at one autotetraploid locus.

Structure	Genotype		Phenotypes
	PVY locus	PVX locus	
Quadruplex	YYYY (Y ₄)	XXXX(X ₄)	Immune
Triplex	YYYy (Y _{3y})	XXXx (X _{3x})	
Duplex	YYyy (Y _{2y2})	XXxx (X _{2x2})	
Simplex	Yyyy (Yy ₃)	Xxxx (Xx ₃)	
Nulliplex	yyyy (y ₄)	xxxx (x ₄)	Susceptible

In the case of resistance to the potato leafroll virus (PLRV) there is much less information about the genetic nature of its control. The scarce available information suggests that its inheritance is polygenic and both additive and non-additive gene effects are involved.

The genetic resources presently utilized at CIP in the breeding for virus resistance or immunity are the following:

1. Genes for resistance to PLRV are at low frequency in some *S. tuberosum* ssp. *tuberosum* cultivars with *S. demissum* and/or *S. phureja* in their genetic background and also some *S. tuberosum* ssp. *andigena* clones.
2. Genes for immunity to PVY and PVX are scattered in *S. tuberosum* ssp. *andigena* and *S. stoloniferum* derived *S. tuberosum* ssp. *tuberosum* cultivars for the former and *S. tuberosum* ssp. *andigena* and *S. acaule* derived *S. tuberosum* ssp. *tuberosum* cultivars for the latter. In both cases the genes for immunity are mainly at the simplex stage i.e. Yyyy, Xxxx. This means that the transmission of these resistances to the offspring is in a medium frequency, i.e. 50%, as it is schematically represented.

$$\begin{array}{ccc}
 Y Y_3 \times y_4 & & X x_3 \times x_4 \\
 | & & | \\
 11 : 1S & & 11 : 1S
 \end{array}$$

If both immunities are considered together the frequency of transmission is further reduced, i.e. 25%.

$$\begin{array}{ccc}
 Y Y_3 X x_3 & x & y^4 x_4 \\
 & | & \\
 & 11 : 3S &
 \end{array}$$

In a variety breeding program these gene frequencies are acceptable because what is being looked for is a single genotype. However, in the pursuit of germplasm improvement this frequency is from medium to low and needs to be upgraded to obtain a significant increase in the proportion of individuals carrying the immunities at the population level and hence, to increase the probabilities of selecting immune individual genotypes.

Selection

If, in a given population, one selects individuals presenting certain types of attributes, and discards those not having these attributes, one is attempting to modify the genetic structure of that population. To what extent selection can modify the genetic make up of the population depends, among other factors, on how efficiently one can identify and isolate the desirable phenotypes.

When the selected individuals are entered into a new reproductive cycle, the resulting population should, due to the changes in gene frequency brought about by selection, have a different genotypic structure with respect to the original. Therefore, the extent of success in this task depends on the efficiency of the screening procedures. If the screening is perfect i.e. if no escapes occur, then the realized progress will coincide with the expected progress. Conversely, if a certain percent of escapes take place, a number of susceptible individuals will be included into the next cycle of selection and the realized progress of selection will be decreased. Let's assume a tetraploid locus for the control of PVY immunity, containing the alleles Y and y with frequencies $f(Y)=p$ and $f(y)=q$, where $p + q = 1$. A certain selection pressure represented by s will be applied against the recessive genotype $yyyy$.

When $s = 1$, i.e., when screening eliminates all the recessive individuals, the new frequency of y will be q_1 .

$$q_1 = \frac{q - q^4}{1 - q^4}$$

and the change in the frequency of y , q will be

$$q = q_1 - q = \frac{(1-q)q^4}{1-q^4}$$

q has a negative value which indicates that the frequency of **y** has decreased.

When the selection is not complete, i.e. when $s < 1$, then

$$q_1 = \frac{q - sq^4}{1 - sq^4}$$

and

$$q = \frac{sq^4(1-q)}{1 - sq^4}$$

From experience one can consider that the efficiency of the seedling screening for immunity to both PVY and PVX should be in the order of 90%. Therefore $s = .9$ (Mendoza, 1987).

Let's assume a population with genes **Y** and **y** with frequencies $p = .01$ and $q = .99$ respectively. After one generation of selection with $s = .9$ the new frequency of **y** will be $q_1 = .93$ and $q = -.06$

Under ideal conditions assuming, $s = 1$, i.e. no escapes occur during the screening, $q_1 = .74$ and $q = -.243$. It is evident that 10% escape decreases the change in gene frequency from $-.243$ to $-.06$.

However, this is not all that bad if one considers that during the first field evaluation for agronomical characters, of the seedlings surviving the screening, about 10% of the genotypes, immunes and escapes, are selected. This 10% retained are further evaluated and those finally selected are retested for immunity before using them as parents for the next cycle.

Breeding Strategy for Virus Resistance

In CIP's earlier years the breeding strategy increased the gene frequency for combined immunity to PVY and PVX without giving sufficient emphasis to agronomical characters. On the other hand, a separate population was assembled to breed for PLRV resistance but no significant progress was achieved. This approach has been modified because of the following reasons:

1. It has become evident that in a PLRV-resistant clone the expression of this attribute may be dependant on whether or not the clone is infected either by PVY, PVX, or by both; e.g. the percentage of infection with PLRV in the resistant clone Mariva increases significantly when the plants were already infected with PVY or PVX or both. This indicates that PLRV resistance should be introduced in genetic materials which are already immune to the other two viruses (Jayasinghe, et al., 1989).

2. From the breeding point of view it is important not only to upgrade gene frequencies for virus resistance *per se* but at the same time to test for general combining ability for yield and other attributes to select superior progenitors.

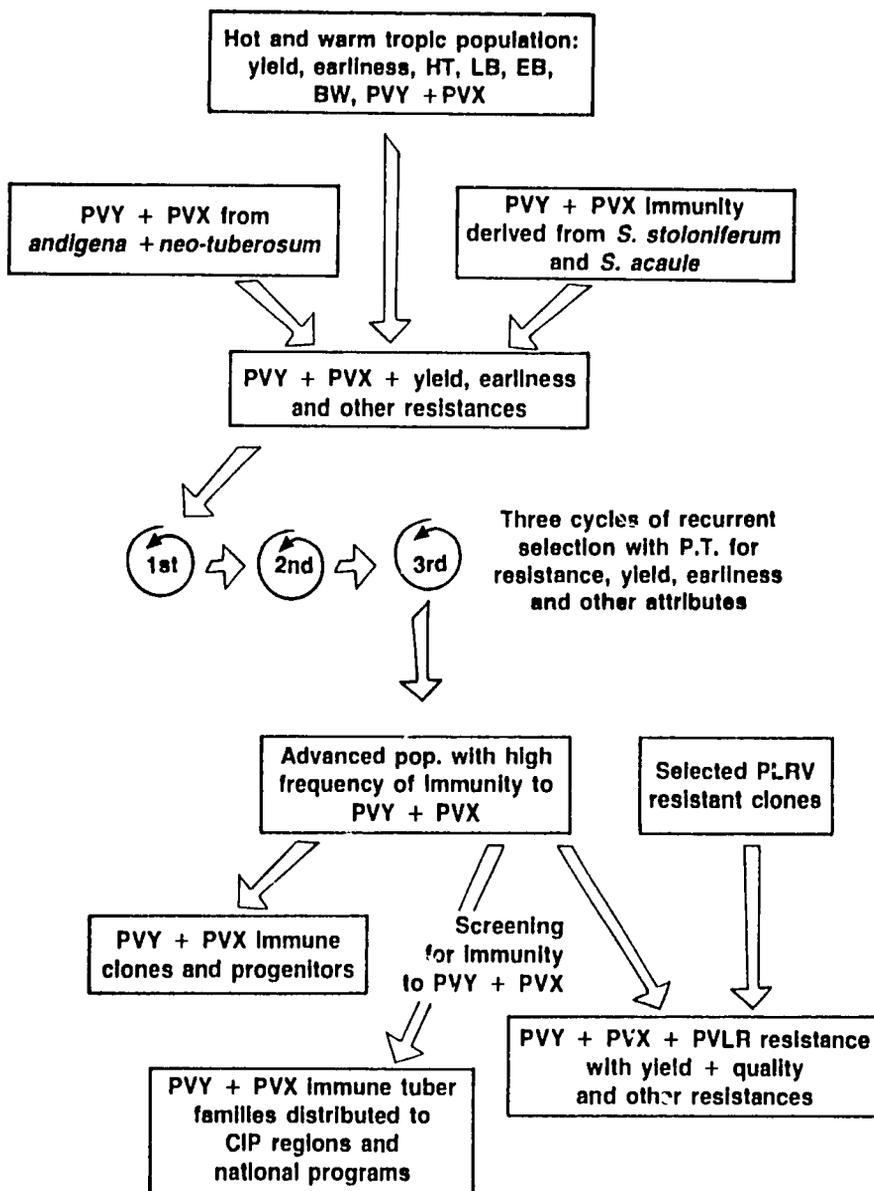


Figure 1. Breeding strategy for PVY + PLRV resistance.

Combining resistance with good agronomical performance is mandatory if resistance is to have an economic value. Otherwise, increasing only the gene frequencies for resistance become an academic exercise with little practical use.

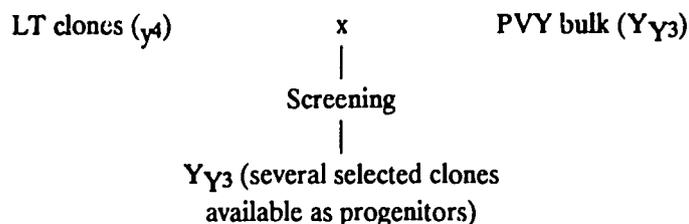
Because of the previous problems a new strategy is being designed (Fig. 1) including the following aspects:

1. All the breeding work is carried out on a genetic background of high yield, earliness, heat tolerance, good agronomical and tuber characteristics and other resistances.
2. The breeding is made in a stepwise fashion.
 - a. Breed for combined PVY and PVX immunity.
 - b. Combine PVY + PVX immunities with PLRV resistance.

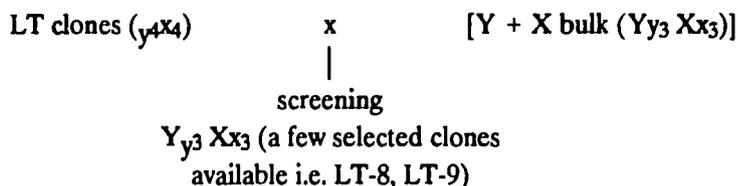
Breeding Procedures and Present Status

Introduction of PVY and PVY + PVX immunities into an advanced lowland tropic population. The clones from the lowland tropic (LT) population had the following attributes: high yield, earliness, and heat tolerance, and also resistances to early blight, late blight, and bacterial wilt scattered across the population most of which are susceptible to viruses. The clones with PVY and PVY + PVX immunity and, in addition, maturity, high yield, and fair tuber characteristics were developed rather late. The early development of these virus immune materials took place in the years 1975 and 1976. (Mendoza unpublished results).

The crosses between these two groups of materials were made in 1979 and are schematically represented as follows:

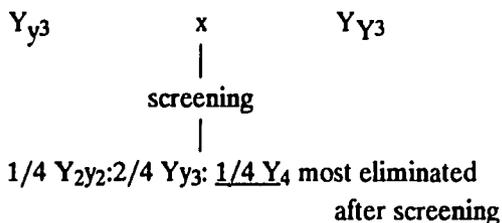


Likewise, the introduction of joint immunity to PVY and PVX is presented in the following scheme.



The selected clones immune either to PVY or PVY + PVX were tested during several seasons for yield, earliness, and agronomical and tuber characteristics. Then, they were progeny-tested to measure their parental value for yield and other attributes.

Increase of gene frequency for PVY immunity (Fig. 2). Selected PVY immune clones were intercrossed to increase the gene frequency of the Y allele as represented in the following scheme.



After the elimination the final relative genotypic frequency was:

$$1/3 Y_2y_2:2/3 Yy_3$$

Crosses	Immune genotypes to select as progenitors	Frequency of immunity
LT clones (y_4) x PVY bk. (Yy_3)	Yy_3	50%
$Yy_3 \times Yy_3$	Y_2y_2	75%
$Y_2y_2 \times Y_2y_2$	Y_4, Y_3y	97.2%
$\begin{bmatrix} Y_4 \\ Y_3y \end{bmatrix} \times y_4$		100%

Figure 2. Increase of gene frequency for PVY immunity.

All the clones selected from this population were field evaluated for yield, earliness, heat tolerance, and tuber characteristics. At harvest, a selection was made of 160 clones of which it was expected that 1/3 should be duplex i.e. YYyy. This was determined by performing testing of progenies produced either by selfing or testcrossing the resistant clones. Selfing the duplex genotypes produced a segregation of 35I:1S while the selfing of the simplex gave the ratio 3I:1S. Testcrossing the duplex gave the segregation 5I:1S while the simplex produced a ratio 1I:1S. These ratios are sharply distinguishable.

Table 2. Segregation ratios of the four PVY-and PVX- immune genotypes from matings between simplex genotypes.

Genotype	Tester	Segregation ratios
Y ₂ y ₂ X ₂ x ₂	y ₄ x ₄	25I:11S
Y ₂ y ₂ Xx ₃	y ₄ x ₄	5I: 7S
Yy ₃ X ₂ x ₂	y ₄ x ₄	5I: 7S
Yy ₃ Xx ₃	y ₄ x ₄	1I: 3S

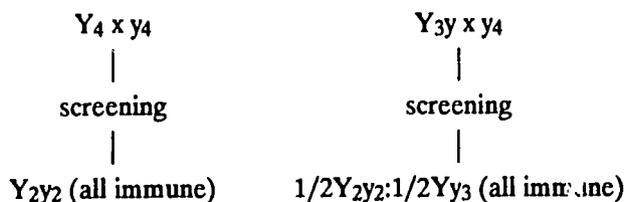
Following this process a group of PVY immune clones carrying the PVY allele in double doses were selected and tested for general combining abilities of several other traits. Finally 20 PVY immune duplex clones were retained and are intensively utilized by various CIP breeders, and progenies of them have been sent to CIP regions and national programs.

Further increase of gene frequency for PVY immunity. By the middle of 1988 the intercrosses of the PVY immune duplex genotypes had been performed. The genotypic and phenotypic segregation of the progenies should be the following:

Y ₂ y ₂ x Y ₂ y ₂				
screening				
1/36Y ₄ :	8/36Y ₃ y :	18/36Y ₂ y ₂ :	8/36Yy ₃ :	1/36y ₄
Quadruplex	Triplex	Duplex	Simplex	Nulli-plex
	35 immune			:1 Sus-ceptible
	(97.22%)			(2.78%)

This third step has two important implications. a) In intercrossing advanced clones duplex for PVY immunity 97.22% of the progenies are immune. b) Looking to the frequency of genotypes obtained from crossing two duplex, one can see that the frequency of quadruplex (Y₄)

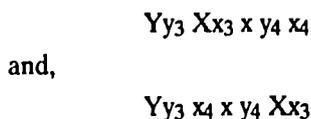
plus triplex (Y_3y) represents 1/4 of the progeny. By testcrossing one can easily separate them from the duplex and simplex.



The identification of these genotypes and their use as progenitors will permit minimizing the importance of PVY, i.e., one of these progenitors crossed to a PVY susceptible clone like CIP 378676.6 but resistant to late blight and early blight, will produce all progenies PVY immune and about 20% will also be resistant to both late and early blight.

At present the quadruplex and triplex genotypes are in process of identification.

Combination of immunities to PVY and PVX (Fig. 3). Two types of crosses have been made using lowland tropic adapted materials.



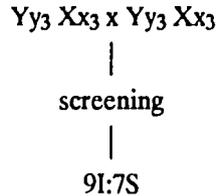
In both types of matings the frequency of immunity to PVY and PVX was 11:3S.

Crosses	Immune genotypes to select as progenitors	Frequency of immunity
LT clones (y_4x_4) x XY bk. (Yy_3Xx_3)		25%
	Yy_3Xx_3	
$Yy_3Xx_3 \times Yy_3Xx_3$	$Y_2yX_2x_2$ progeny test	56.2%
$Y_2yX_2x_2 \times Y_2yX_2x_2$	<div style="display: flex; align-items: center;"> <div style="border-left: 1px solid black; border-right: 1px solid black; padding: 0 5px; margin-right: 5px;"> Y_4X_4 Y_4X_3x Y_3yX_4 Y_3yX_3x </div> <div style="margin-right: 5px;">x y_4x_4</div> <div style="border-left: 1px solid black; border-right: 1px solid black; padding: 0 5px;"> Y_4X_4, Y_4X_3x Y_3yX_4, Y_3yX_3x </div> <div style="margin-left: 5px;">progeny test</div> </div>	94.5%
		100%

Figure 3. Increase of gene frequency for joint PVY + PVX immunity.

After seedling screening the PVY-and PVX-immune individuals were taken to the field. At harvest, in September 1986, 241 clones were selected. These clones were planted in May 1987 at the San Ramon station to evaluate for yield, earliness, and heat tolerance. At harvest, in August, 18 clones with high yield and maturity ranging from 75 to 90 days were selected. All these clones, were simplex immune to both viruses (Yy₃ Xx₃).

Increase in the joint frequency for PVY and PVX immunities (Fig. 3). In October 1987 the 18 selected clones were intercrossed to upgrade the frequency of the genes Y and X.



The gene frequencies for the joint immunity to the viruses has more than doubled increasing from 25% to 56.25%. Among the immune phenotypes the following genotypic frequencies should be present.

$$1/9Y_2y_2X_2x_2 : 2/9Y_2y_2X_2x_3 : 2/9Yy_3X_2x_2 : 4/9Yy_3Xx_3$$

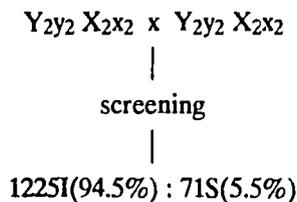
All these materials were evaluated at the seedling stage at La Molina during the summer 1988 and those selected were re-evaluated in the period from June to August 1988 at San Ramon. In both cases selection for yield, earliness, heat tolerance, and tuber characteristics was performed.

From the four genotypes the most important one to concentrate is the duplex at both loci, i.e. Y₂y₂X₂x₂. The identification of this one has to be made by joint screening, for PVY and PVX immunities, the progenies resulting from testcrossing all the selected immune clones.

The identification of the duplex genotypes at both loci does not present any problem, since the segregation ratios are very different.

The process of selection of the duplex genotypes is taking place at present.

Further increase in the joint frequency of genes controlling PVY and PVX immunities. The intercross of the duplex genotypes Y₂y₂X₂x₂ will further upgrade the gene frequencies for immunity to the two viruses.



As in the case of increasing the gene frequency for PVY, the intercross of duplex genotypes for both PVY and PVX has two important consequences:

- 1 By intercrossing advanced clones duplex for PVY and PVX immunity, 94.5% of the progenies will be immune, thus minimizing the importance of these two major potato viruses.
2. By examining the genotypic frequencies obtained by crossing two duplexes one can see that the frequency of quadruplex (Y_4X_4) and quadruplex at one locus but triplex at the other (Y_4X_{3x} and $Y_{3y}X_4$), and triplex at both loci ($Y_{3y}X_{3x}$) represents 6.25% of the progenies. By testcrossing the immune genotypes one can recognize the quadruplex or triplex since all the progenies will be immune to both PVY and PVX viruses. The identification of these genotypes should permit solving the problem of PVY and PVX on a definitive basis

Another important consequence of the availability of several progenitors with joint immunity to PVY and PVX is the combination of these immunities with other very important traits such as yield, earliness, tuber quality, and disease and stress resistances or tolerances.

During the 1988 summer season, 1379 hybrid combinations were obtained at CIP's experiment station in Huancayo. From these, 603 progenies combined several attributes as shown in Table 3. It was stated earlier in this paper that one of the objectives of the population breeding strategy was the combination of several traits. The data contained in Table 3 illustrates the achievement of that objective.

Table 3. Combination of traits including yield, earliness, diseases and stress resistances or tolerances. (Crosses made in 1989).

Traits ^a	No. of Progenies
Increase in gene freq. for Y + X	284
Yield, Earl., HT, Y + X	264
Yield, Earl., HT, LB, Y + X	161
Yield, Earl., HT, BW, Y + X	124
Yield, Earl., HT, PLRV, Y + X	400
Yield, Earl., HT, LB, EB, Y + X	81
Yield, Earl., HT, LB, BW, PLRV, Y + X	65
TOTAL	1,379

^aEarl. = Earliness, HT = Heat tolerance, Y + X = immunity to PVY and PVX, LB = Late blight, EB = Early blight, BW = Bacterial Wilt, PLRV = Potato leafroll virus.

Table 4. Top-performing, heat tolerance and PVY+PVX immune clones selected at San Ramon. Summer 1988 (Growing period: 90 days)

Clone	Yield (t/ha)	Earliness ^b
(Atlantic x 484.007).5	39.3	7
(484.007 x Atlantic).4	30.5	7
(LT-8 x 575049).19	24.9	5
(LT-8 x 484.001).9	24.6	7
(LT-8 x C83.119).15	23.9	5

^bEarliness: 1 = Very late, 5 = Medium, 9 = Very early.

The high yielding potential exhibited by the PVY-and PVX-immune hybrids in evaluations made under severe heat stress at CIP's San Ramon experiment station should also be underscored. Data in Table 4 show that during the summer season characterized by a mean temperature of 25°C and heavy rainfall, high yields of excellent tuber quality were obtained in a period of only 90 days. The Y + X1 immune clones have a good of performance stability as they do not generate even in the presence of heavy aphid populations.

Combination of resistance to PLRV with joint immunity to PVY and PVX. Beginning in 1986 a significant effort has been made at CIP not only to combine the resistance to PLRV with the immunities to both PVY and PVX but also to carry out research related to the virological aspects of resistance.

The information obtained from this work has shown certain new complexities involved in a breeding effort that includes PLRV. Despite this, the results are encouraging and the new knowledge acquired will permit certain readjustments in the original breeding strategy.

The results obtained can be summarized as follows:

1. In previous years only resistance to infection and resistance to multiplication were considered when making selections. However, recent research has identified other components in the resistance to PLRV in potatoes. To the resistance to infection and resistance to multiplication, three other components have been added: tolerance, antibiosis, and antixenosis:

The methodology commonly used to screen for PLRV resistance is based on exposing the plants to aphids carrying the virus. With the five above-mentioned components it becomes difficult to separate the host resistance to the virus and the indirect resistance to PLRV due to the aphid behavior in the host (CIP Annual Report, 1988).

2. The identification of the type of component(s) of resistance carried out in several of the PLRV-resistant progenitors will improve the establishment of the mating plans to stimulate the combination of the components of resistance.

3. The present availability of a group of 20 progenitors that transmit immunity to PVY and PVX has simplified the selection for resistance to PLRV at the same time as it has confounded interactions that may cause misjudgment of the parental value of the PLRV resistant progenitors.
4. The performance of the virus-resistant materials in various locations around the world, Peru, Uruguay, Brazil, Philippines, Taiwan, Tunisia, and Turkey has permitted the selection of high performing progenies and individual clones. For instance, in Ica, Peru, a group of about 400 clones from 1986 and 1987 crosses had undergone 4 or 5 field exposures under heavy aphid pressure, and a large number of infector plants were interplanted with the plants under test. About 20 clones have shown high yield and high to very high levels of resistance to PLRV, in addition to their immunity to PVY and PVX. Some of these clones also exhibited an excellent processing quality for both french fries and chips. (See Table 5).

Table 5. Top selected clones for processing attributes from an early maturing, heat tolerant, PLRV resistant, PVY and PVX immune population

Clone	DM ^c	SG	RS	Attribute
(B71-240.2 x 7XY.1).026	23.1	1.085	1.66	Chips
(Serrana x LT-9).041	23.5	1.093	2.20	Chips
(Serrana x LT-9).052	24.0	1.096	2.33	Chips
(Serrana x LT-9).057	20.9	1.084	2.00	French fries
(BR63.15 x 7XY.1).102	22.9	1.090	1.00	Chips
(Bzura x LT-9).105	25.7	1.106	1.66	Chips
(Bzura x LT-9).109	25.8	1.100	2.00	French fries

^cDM = Dry matter, SG = Specific gravity, RS = Reducing sugars: 1 = 0%, 2 = .1%, 3 = .25%, 4 = .5%, 5 = 2% or more. Less than 03 is adequate.

The yield capacity of the clones with resistance to PLRV and immunity to PVY and PVX is also high. Evaluations made at La Molina during the summer of 1988 under a mean temperature of 25°C showed high yield performance. Data in Table 6 show yield of up to 40 t/ha in a period of 90 days. Again, the most important point, is that these clones do not degenerate and they keep their yield potential for an extended period of time.

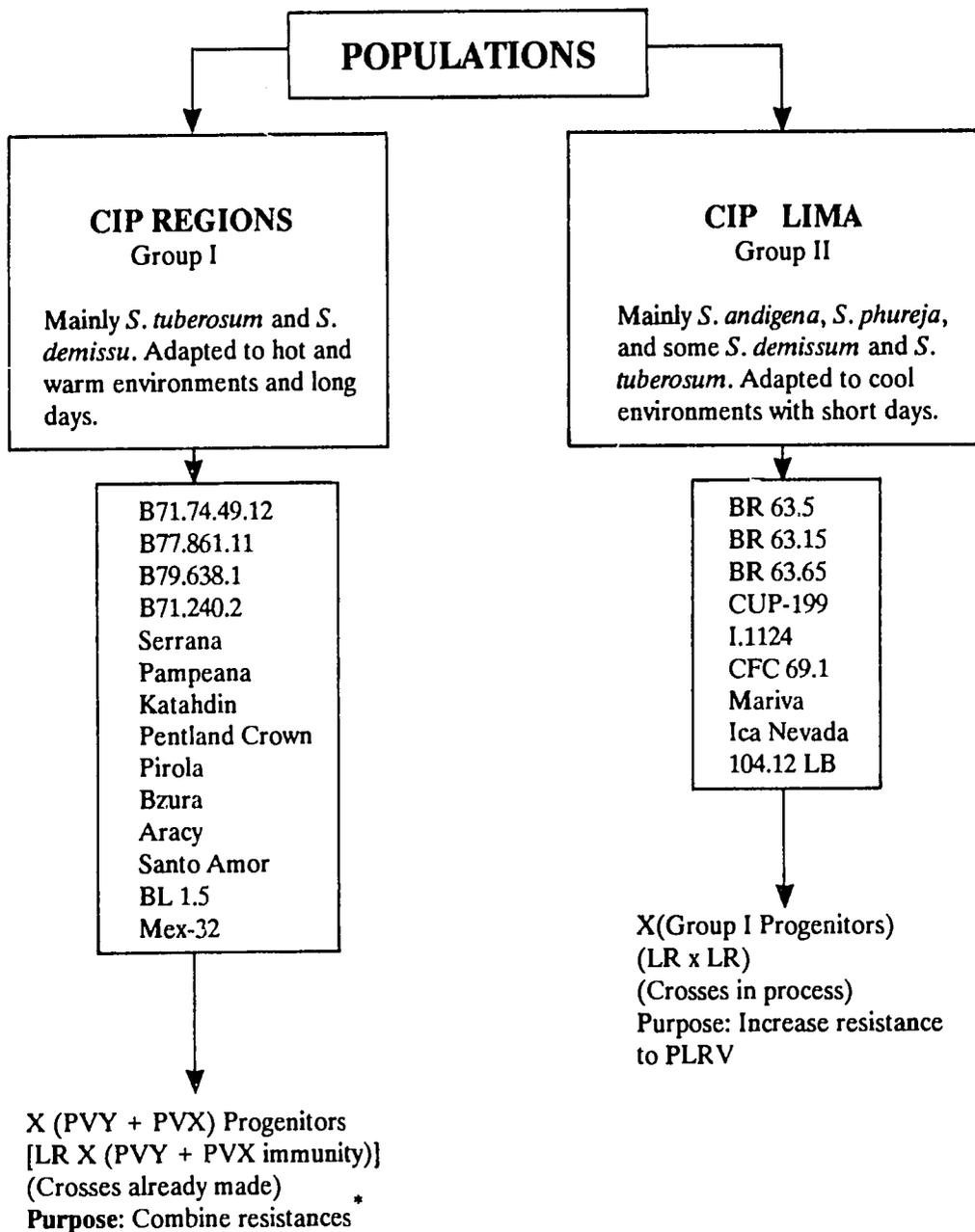
5. The performance of the virus resistant materials discussed in 4, has also permitted readjustment of the crossing schemes by separating the PLRV progenitors in the performance of their progenies in groups I and II according to their geographical adaptation (Fig. 4).

Table 6. Top performing, heat tolerant, PLRV resistant, and PVY + PVX-immune clones grown at La Molina. Summer 1988 (Growing period: 90 days).

Clone	Yield (t/ha)	Earliness ^d
(Pirola x LT-9).13	40.3	5
(Utatlan 69.1 x 7XY.1).9	35.7	7
(Serrana x 7XY.1).2	35.3	3
(LT-8 x Atlantic).9	24.6	7
(LT-8 x Katahdia).7	21.7	7

^dEarliness: 1 = Very late, 5 = Medium, 9 = Very early.

The Group I progenitors have a better adaptation to medium-to-long photoperiods and to relatively higher temperatures than the clones of Group II. Among Group I the B clones and the cultivars Serrana and Pampeana, all originating from INTA, Argentina, have a high level of resistance as do the Polish cultivar Bzura and the German Pirola. Among the rest, the Brazilian cultivars Aracy and Santo Amor and the Mexicans BL1.5 and Mex-32 have a medium level of resistance, and so do, the US cultivar Katahdin and the Scottish, Pentland Crown. All these clones have already been crossed to PVY + PVX-immune progenitors and a few resistant progenies have been identified (Tables 5 and 6). In addition, most of the progenies of these crosses have shown a good level of adaptation to growing under long days and warm temperatures. If the level of resistance is not sufficient in larger proportion of the progenies of the LR x (Y + X) crosses then it is necessary to backcross {LR x [LR x (Y + X)]} or to intercross [LR x (Y + X)] x [LR x (Y + X)]. In these cases all progenies are first screened for PVY and PVX immunity and then tested for PLRV resistance as well as for agronomical and tuber quality. Some of the progenitors of Group II, i.e., BR-63.15, CUP 199, CFC 62.1 and Mariva have shown, through the performance of their progenies, a good level of resistance to PLRV. However, those progenies are mainly adapted to growing under short days. Under longer photoperiods, the progenies show a lack of adaptation being in most cases from late to very late. As for their resistance a valuable cycle of selection in crosses Group II x Group I or reciprocals is in preparation trying to upgrade the resistance to PLRV by considering their different genetic origin.



* If level of resistance is low, the backcrosses of type [LR X (LR X YX)] and intercrosses of type [LR X (Y + X)] X [LR X (Y + X)] will be made.

Figure 4. PLRV progenitors utilized to combine resistance to PLRV with immunity to PVY and increased resistance to PLRV.

Concluding Remarks

The availability of potato varieties with a combination of immunity to PVY and PVX and resistance to PLRV would significantly simplify the process of seed production. By genetically controlling three of the major causes of seed potato degeneration, the farmers could keep their seed for a longer period of time without the threat of a rapid loss of its yield potential. Consequently, the yield stability could also be improved by reducing the constant loss of vigor and yield capacity to which susceptible varieties are subjected.

Another important aspect is that the availability of varieties resistant to the three main potato viruses can decrease the cost of production of this crop, since one of the most expensive production inputs is good quality seed.

Finally, the increase in gene frequency particularly for immunity to PVY and PVX is rendering possible the combination of these attributes with other important resistances such as late blight, early blight, bacterial wilt, etc. The output of this process will be the availability of new varieties tailored to better fit into the growing conditions of the developing countries and make the potato a food more economically available to a majority of people.

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Virus Detection Procedures for Seed Production

J. Hammond¹

Since pathogens that are transmissible through the planting stock, either tubers or true seed, are perpetuated and multiplied at each stage of propagation, the highest levels of sanitation must be applied to the initial stages of propagule production, the nuclear stock. Greater tolerance is allowed at successive multiplication stages.

It is not possible to grow large areas for seed production under conditions which exclude vectors, and continued visual inspection, roguing and testing must occur. However, even in field-scale production seed crops can be protected to a high degree by physical separation from ware crops, by being grown in areas where the vectors are scarce, and by manipulation of planting and harvesting dates to minimize vectored transmission and translocation of introduced virus to the tubers (Peters, 1987; Beemster, 1987). Vector access to the crop can be minimized by physical barriers such as periodic rows of non-viral or vector host plants taller than the crop in order to block lateral movement of vectors and act as a sink for viruses that are not continually transmitted. The use of treatments such as oil sprays to prevent aphid or whitefly transmission (Simons and Zitter, 1980) may be useful.

True potato seed (TPS) production has an advantage because fewer viruses are known to be transmitted through TPS than through tubers. PSTVd and PVT can be transmitted through TPS (de Bokx, 1987; Beemster and de Bokx, 1987). As the seed-transmitted viruses are not vectored by aphids, it should be possible to maintain TPS production free of these viruses provided that the original stock is not infected and care is taken not to introduce viruses from other infected crops by contact with machines and personnel.

True seed of sweet potato yield too much phenotypic variability to be useful for propagation, so either roots or stem cuttings are used to produce the next crop. Either method will result in the transmission to the next crop of any of the viruses present in the starting material.

Schemes for producing high quality propagules of both crops will be similar. To establish a satisfactory nuclear stock where none exists may take several years. First, the healthiest-looking plants should be selected, and if possible only those surrounded by other apparently healthy plants should be used. These plants may be tested while in the field by inoculation of sap extracts to a range of appropriate indicator plants, by the most sensitive serological tests that are available, and by grafting onto sensitive indicator clones. For these relatively few plants, the

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major effort of multiple testing is worthwhile to establish a good nuclear stock. The easiest and fastest type of test should be done first, to eliminate from further testing those plants that have any detectable infection. The type of assay to be used will depend on local expertise, equipment and reagents available. Commercial ELISA kits may be the most suitable means for initial testing; if these are not available, then gel diffusion, chloroplast or latex agglutination tests, serologically specific electron microscopy, dot-blot immunoassay or various types of ELISA may be used. A type of assay that has low strain specificity (i.e. broad cross-reactivity) should be used. Many polyclonal antisera are suitable for antigen-coated plate forms of indirect ELISA, which is a useful and sensitive qualitative test that is not typically strain-specific.

Once the obviously virus-infected samples have been eliminated, the remaining apparently healthy plants should then be subjected to secondary testing, which should include sap inoculation to indicator plants and/or grafting to indicator varieties for detection of specific diseases.

Those that are true to varietal type and apparently free of virus infection are selected. If none can be identified as apparently virus-free, then the least-infected plants should be treated by thermo or meristem-tip culture to rescue a proportion of plants or plantlets of higher health status. All individual tubers or plants derived from these selected superior plants should then be grown out and retested. Any progeny groups containing even a single infected plant should be discarded from the candidates for nuclear stock status, or subjected to further rounds of screening and selection. This procedure is called clonal selection (Vander Zaag, 1987), and plants in which no virus was detected form the nuclear stock.

Meristem and tissue culture can replace clonal selection to some extent. Once a clean stock has been established, tissue culture can be used to rapidly propagate the clone for the next stage. During this process, a proportion of plantlets should be selected at random and grown out under conditions favorable for virus replication. These plants should be retested to ensure that the tissue culture process has not resulted in inhibition of virus replication below the levels of detection in culture; otherwise apparently healthy (but latently infected) tissue culture could give rise to thousands of infected plants when they are grown in the field. Regenerated plants should also be examined for trueness to type.

The next level of production, basic seed, is on a field scale. A specified level of tolerance for particular virus or symptom types is set, typically on the order of 0.1% to 0.5% for each of severe symptoms (including severe mosaic, leafroll, stipple streak and crinkle) and mild symptoms; variation from varietal type has similar or lower levels of tolerance (Oosterveld, 1987). Basic seed production should be isolated from ware production, in fields not used for potatoes for 4-5 years previously, and should be visually inspected two or three times during the growing season. Obviously diseased plants must be rogued to prevent further spread. Only basic seed crops meeting tolerance requirements at the time of haulm destruction are used for raising certified seed; crops not meeting the tolerance for basic seed may be used for ware production.

The tolerances for certified seed are less strict than for basic seed, of the order of 1-2% for severe symptoms and 1-5% for mild symptoms (Oosterveld, 1987).

Lower standards may be appropriate at the start of a certification program to initiate the system. The influence of the level of infection in certified seed on that in the subsequent ware crop must be determined. The tolerance at each level of seed certification must take into account vector pressure and environmental conditions (Oosterveld, 1987).

Appropriate Testing Methods

Indicator plants should be grown initially in conditions most favorable for virus inoculation to be successful, and subsequently for virus development. Typically, diffuse light and medium to high humidity are advantageous for susceptibility to infection, while higher light conditions may favor disease development after inoculation. Viruses that are not mechanically transmissible, such as PLRV, must be graft-inoculated or transmitted by specific vectors; sensitive serological procedures are of greater importance for such viruses than for those that are mechanically easy to transmit. A range of indicator hosts are available for diagnosis of potato viruses (de Bokx, 1987; Beemster and de Bokx, 1987). Most of the sweet potato viruses can be detected by grafting onto *Ipomoea setosa*, although some may produce only mild symptoms and may not be reliably detected by this method. Serological methods should be applied to the grafted *I. setosa* to boost the chance of detection (Moyer and Salazar, 1989). A whitefly-transmitted agent that is not mechanically transmissible has been reported from several sweet potato-growing regions. It can be detected by grafting onto a sweet potato clone, Tib 8A, which is pre-infected with a strain of SPFMV, as the mixed infection results in severe symptoms (Rossel and Thottappilly, 1985). Additional testing by mechanical inoculation to *N. benthamiana*, *N. clevelandii* and *Chenopodium quinoa* is also advisable (Moyer and Salazar, 1989).

ELISA has become the standard method for detection of many viruses. Many variations of the basic ELISA technique have been developed, which differ in strain specificity (e.g. Koenig, 1981; Koenig and Paul, 1982). Indirect antigen-coated plate ELISAs are very simple, with broad reactivity (Lommel et al., 1982); and several antisera can be tested against samples in a single plate (Hammond and Chastagner, 1989). Lommel et al. (1982) reported that water was a sufficient diluent for sap extracts. Sap can be prepared by crushing leaves directly in plastic bags used to collect samples in the field, without cross-contamination, and diluting by addition of buffer. Appropriate dilutions will depend on the ELISA protocol used, but the ratio is typically 1:100 in antigen-coated indirect tests.

Some of the older and simpler techniques can also be applied to samples collected in the field. Microprecipitin tests can be carried out in 96-well plates, in which it is easy to overlay the samples with paraffin oil. Chloroplast and latex agglutination tests can also be done in microtiter plates. The latex test is much more sensitive than the chloroplast test, and extracts may need to be diluted in a 5-fold or 10-fold series because of the sensitivity to antigen excess (Maat and Huttinga, 1987).

In the absence of facilities for testing for PSTVd by nucleic acid hybridization, tomato inoculation tests are the simplest, and can be made more reliable by growth under continuous high light (Yang and Hooker, 1977). Alternative methods include several variations of polyacrylamide gel electrophoresis (e.g. Morris and Smith, 1977).

Another sensitive serological test that can be applied to samples collected in the field is a tissue blotting technique. Wang et al. (1985) showed that bean common mosaic virus could be detected with monoclonal antibodies when imbibed bean seed were blotted to nitrocellulose membranes. Cassab and Varner (1987) used a similar method to localize antigens in tissue sections imprinted on nitrocellulose.

For all these techniques appropriate tissue must be chosen, and preferably from multiple locations on the plant, as infections (especially primary ones) are not necessarily uniformly distributed. The timing of sampling is also important; for PVM and PVS the best leaf tissue is from 2-3 nodes below the top of the plant, and at the start of flowering (Goth, personal communication).

Although some viruses (PVX, PVY, PVS, PVM, and PLRV) often occur in sufficient concentration to be detectable in dormant tubers, more reliable results are obtained for most viruses from sprouts about 2-3 weeks after dormancy has been artificially broken (Maat and Huttinga, 1987). The rose end of the tuber generally has a higher virus concentration than the heel end (Beemster, 1987), although PLRV is more reliably detected from the heel end (Gugerli, 1980). Latex agglutination is recommended for detection of APLV and APMV because of the strain specificity observed in ELISA (Maat and Huttinga, 1987). Indirect ELISA, dot-blot or tissue-printing assays are less strain-specific than double-antibody sandwich ELISA.

Tuber testing can also be done on individual eyes cut out with a sterilized melon-baller, and grown out for symptom expression, host range inoculation, or serological assay; however, virus distribution may not be uniform, and a negative result from one eye does not mean that the tuber is virus-free. It is possible to recover healthy plants from infected stock, as some tubers may escape infection, or individual eyes can be grown out and tested separately. Several cycles of growth and retesting are necessary before such a plant is used for nuclear stock.

The nuclear stock should be retested each year before propagation of the next cycle; periodically, the nuclear stock should be regenerated from a single plant or stem cutting which is selected after thorough testing.

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Production of Seed Potato in Chile

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Introduction

The development of modern agriculture is based on the use of quality seed and adapted varieties which have a high yield potential and are adequately managed. Thus, if developing countries expect to increase the production or yields of crops as important as the potato, they must by all means develop or improve seed programs on a sound and efficient basis.

Chile's certified seed production program has operated uninterruptedly since 1959. The country has derived many benefits from this program, namely self-sufficiency in quality seed potato of the most important commercial cultivars; better production quality and higher yields both in the regions where certification is most important and at the national level; the growth and strengthening of the national seed industry; generation of foreign earnings through the exportation of seed potato; and collaboration in the training of Latin American professionals working in seed production programs.

This paper summarizes the most salient aspects of Chile's Certified Seed Potato Production Program.

Background

From its inception, this scheme included a strong private-sector component usually considered more efficient than the public sector in the development and implementation of seed programs. The contribution of private concerns such as SEGENTA Ltda., Semillas S/Z, ANASAC, POROSECOR and a number of individual seed producers in the provinces of Llanquihue, Osorno and Valdivia has gained wide recognition. However, the public sector plays a pivotal role in the scientific development and dissemination of new seed technologies through the research on and transfer of technology; the approval of laws and regulations; the supervision of the certification process and the supply of credit, etc.. INIA is well-known as a generator and disseminator of technologies, producer of basic seed, and as coordinator of the efforts undertaken by individual farmers, producer associations and private enterprises involved in seed potato production. Likewise, SAG's Seed Technology Unit has played an important role in the regulation and control of the certification and marketing of seed potatoes.

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Multiplication and Selection of Pre-basic and Basic Seed. Mass Reproduction and Negative Selection in the Production of Certified Seed

The use of the above-mentioned technical principles coupled with methods for the detection of virus and virus-like diseases (indicator plants, serology) have allowed the long-term preservation of the cultivars' varietal purity and identity, and the control of most important viruses (PLRV, PVY, and PVX). The Chilean program also benefits from its location in one of the most favorable world regions for the production of seed potato. The region is characterized by its cool moderate climate, its deep and permeable soils, and the absence of constraining plagues and diseases. Although the long and costly clonal reproduction and selection method employed in Chile has allowed the preservation of varietal purity and identity and the control of important viruses. It has failed in controlling important bacteria (*Erwinia* spp, *Streptomyces scabies*) and fungus (*Helminthosporium solani*) diseases. Therefore, INIA's potato program recently started using in vitro micropropagation, rapid multiplication, and serial tests for pathogen control in the production of prebasic and basic materials.

Constant Updating of Regulations

Since 1936, laws, regulations, and norms have been passed with the aim of improving seed certification in Chile, speeding up the production of certified seed, and enhancing the available methods of genetic, sanitary, and physiological evaluation. As a result of these measures and of the joint efforts of the Ministry of Agriculture and the Association of Seed Producers (ANPROS), a Seed Bill was passed in 1977. The corresponding regulations were approved in 1978 and the Seed Technology Unit (STU) was set up.

In 1983, STU approved the Supervised Certification Process that regulates visiting, sampling, and controls of private production plots. In 1988, both public and private sector enterprises were authorized to conduct seed certification activities under certain approved mechanisms specified by Decree Law 40. This government agency also keeps a record of varieties ready for certification, issues norms and instructions, controls seed certification, certifies seed following the requirements of the Organization of Economic Development and Cooperation (OECD) and the European Economic Community (EEC), and conducts seed-marketing control and post-control trials.

Managerial, technical, and facility development

The facilities available in Chile (store- and greenhouses, quality control laboratories, and selection centers) for the production of seed, have been gradually expanded and upgraded thanks to the continuity of the seed potato certification program. At the same time, the producers have improved their technical and managerial skills through the constant training of support professionals and the transfer of technology developed by private and public concerns. The success of a seed production program depends largely on the availability of a select group of producers who master the production, storage, and marketing of seed tubers, for them is the final

responsability for producing quality seed; they are not only selling an input, but a product bearing a seal attesting to the seriousness, honesty, responsibility and prestige of its producer.

Production

Over the last 20 years, an average of 6852 t of seed tubers were produced annually (Table 1).

In the last ten years, INIA has handed over to private-sector producers a yearly average of 480 tons of basic seed-potato (Table 1). A similar amount was produced by private-sector companies, mainly ANASAC and SEMILLAS S/Z.

Table 1. Area planted and production of basic seed by INIA and certified seed from the private sector in Chile (1979-1988).

Year	Basic Seed		Certified Seed	
	Area (ha)	Production (ton)	Area (ha)	Production (ton)
1979	34,5	333	444	3.655
1980	33,6	410	633	6.898
1981	18,2	366	758	6.815
1982	22,0	560	545	5.063
1983	22,5	539	445	6.877
1984	27,7	700	515	7.576
1985	31,1	775	794	11.222
1986	28,3	380	858	9.811
1987	21,0	310	618	5.548
1988	20,1	396	550	5.054
Average	25,9	477	616	6.852

Source: INIA, Estación Experimental Remehue, Osorno. Aguila, Armando, 1989, Curso INIA/CIP/PNUD.

The annual average production of certified seed-potato over the same period was 6,850 tons, to which we may add another 5,300 tons of seed-potato from producers not yet fully certified who are thus excluded from this process. Consequently, we reach a total production of about 12,000 tons of quality seed-potato with an estimated value of some US \$2.5 million. This production covers the needs of about 50% of the commercial potato area in Chile. Moreover, certified seed is the backbone of the "informal program" for the production of seed-potato by individual producers. The impact of the certification program is thus multiplied.

Conclusion

The best method for the control of virus and virus-like diseases is the establishment and development of either "formal" or "informal" seed production programs. Seed programs allow for the integration of resources, technology, and methods to control or diminish the effects of these diseases. For example, a seed production program could multiply highly-resistant varieties in zones or regions with a high-degeneration rate. This is the focus of our work, and is one of the achievements of the breeding program. A seed production program also needs to apply disease detection methods in order to eliminate infected material in multiplication lots. Pathology and biotechnology methods are being applied in this work. The improvement of techniques applied in seed production programs depends on the objectives of their application, the development achieved, and the resources available.

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Status of Seed Production in India and Nearby Countries

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The potato cultivation in countries of South Asia is characterized by two distinct climatic zones. These are highland temperate and lowland sub-tropical climates. In these countries most potato growers are aware that seed tubers from the highlands are usually healthier and produce more vigorous crops than tubers grown at lower elevations. As a result, in these countries, informal seed systems have developed that link seed potato producers in the highlands with growers in lower areas. Countries like India, Nepal, and Sri Lanka still have these traditional informal seed systems which are supplying a greater portion of seed requirements. However, in countries like Bangladesh and India, 30-40% of the crop is still raised from farmer's own seed produced in lowland areas. The major part of this seed is of local cultivars and is highly degenerated. Thus the production in these areas is very low.

A formal seed program among these countries was started in India in the mid-1960s. The Central Potato Research Institute had started production of basic seed, locally designated as "breeders seed," after establishing a seed-plot technique based on the observations that certain areas in the northwestern plains have a 75-90 day aphid-free period, and that crops grown during this period from the first week of October to the middle of December produce healthy tubers with a very low level of virus infection. The Institute established basic seed production units at three locations in the Indo-Gangetic plains and one at 9000 ft in Kufri-Fagu (Shimla hills).

Bhutan's National Potato Programs began the formal basic seed programs in the mid-1980s. Sri Lanka has had the formal seed multiplication in operation since the late 1970s, whereas Bangladesh started formal seed multiplication in the early 1980s. Efforts are being made to do away with imported seed and to become self-sufficient insofar as the production of basic seed is concerned.

Indian Seed Potato Programme

In Region VI, India is the only country which has a well developed seed potato program.

After the discontinuation of large-scale seed imports during the second World War, a scheme for the production of healthy seed was started in 1941. However, a sound seed production technology could be developed only in 1964, after studies on the aphid population built up in the

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plains and the introduction of serological techniques. This led to the establishment of a seed-plot technique and a technique for producing and multiplying virus-tested seed.

The procedure for the production of basic seed (as breeders' seed is called) is outlined below:

Procedure

After selecting true-to-type single tuber units, four tubers each are collected from a unit. They are tested in the glasshouse for freedom from the virus diseases, PVX, PVS, PVY, and PLRV.

The glasshouse-tested tubers are multiplied in the field for four years, in Stage I, Stage II, Stage III, and Stage IV.

In Stage I, all the plants are serologically tested for PVX and PVS. Healthy clones are harvested individually and this clonal produce of Stage I is multiplied in Stage II and each clone is tested serologically for PVX and PVS. In the following year (Stage III) healthy clones are bulked and planted and check testing is done at the rate of 300 plants per ha. The produce of Stage III is further multiplied for one more year (Stage IV). In this stage, serological testing for PVS and PVX is done for 150 plants per ha.

In all the multiplication stages, visual inspection is also carried out apart from serological testing to rogue out diseased and atypical plants. These phased multiplications of virus-tested stocks in the field are carried out using the seed plot technique.

Foundation Seed

Breeders' seed received by the various agencies are required to be multiplied in three stages for three successive years as foundation-I, foundation-II, and certified seed.

Independent seed-certification agencies were set up in various states under the National Seed Project and a World Bank-funded scheme, initiated in 1975. At present, 18 states have set up seed certification agencies, and six of them are certifying seed potato. The certification of the crop is being done according to the Minimum Seed Certification Standards fixed by the Central Seed Certification Board, Ministry of Agriculture, New Delhi for various categories of seed.

Seed Certification

The government of India declared seed as an essential commodity under the Essential Commodity Act, 1955. In 1983 the Government of India issued the Seed Control Order under which licensed dealers were authorized to deal in seed of recognized varieties.

Future Perspective

In 1976, the National Commission on Agriculture envisaged that by 2000 AD, the area planted in potato in India will have increased to 1.5 million ha (from the present 0.83 million ha) and yield up to 20 t/ha.

With the present rate of multiplication, the quantities of breeders' and certified seed required by 2000 AD are given in Table 1.

Table 1. Present and future seed requirements.

	Present	by 2000 AD
Certified seed	68,750 MT	125,000 MT
Breeders' seed	5,500 MT	10,000 MT
Indexed tubers	2.5 MT	4.5 MT
Area for breeders' seed	275 ha	500 ha
Area for certified seed	34375 ha	62500 ha

In 1988-89, CPRI supplied about 1,728 t of breeders' seed which met approximately one-fourth of the total requirements (Table 2).

Table 2. Breeders' seed production (tons) at CPRI regional stations (1988-89).

Cultivars	Plains	Hills	Total
Kufri Chandramukhi	454	5.0	459
Kufri Jyoti	290	64	354
Kufri Badshah	512	-	512
Kufri Sindhuri	166	-	166
Kufri Bahar	170	-	170
Kufri Lalima	33	-	33
Kufri Lauvkar	34	-	34
	1559		172

With a view to meeting the total seed requirement, the ICAR appointed a committee to develop a procedure to involve State Agricultural Universities in breeders' seed production. The Committee has recommended requiring various steps be taken to make the country self-sufficient in its requirements of seeds, mainly by involving the State Agricultural Universities and by a system of financial support. Moreover, the following steps are planned to enhance seed production and also to improve seed quality.

1. *Rapid multiplication.* Various rapid multiplication techniques such as planting sprouts, stem cuttings, in vitro multiplication, and tuberlet production are being developed in the Institute. The in vitro tuberlets will be multiplied in net houses for one season and the produce will be

multiplied in the field for one more season to produce breeders' seed. As and when this is implemented, the time taken for producing breeders' seed will be reduced to less than two years from the present four years. Furthermore, the quality of seed will also be enhanced considerably.

2. *Establishment of new seed production station.* The Institute has proposed the establishment of four breeders' seed production stations, each of 300-400 ha, to be located in the eastern plains (Bihar or West Bengal), north eastern India (Meghalaya or Arunachal Pradesh), the plateau region (Karnataka) and the northern hills (Himachal Pradesh).

Bangladesh

Bangladesh Agricultural Development Corporation (BADC) launched a scheme for the multiplication and distribution of improved potato seed in 1968-69. Since 1970-71, BADC has been producing certified seed locally and supplying it to the growers. The gradual increase of area, production, and yield from 1969 to 1987 is shown in Table 3.

Table 3. Area, yield, and total potato production in Bangladesh from 1954 to 1987.

Year	Area 1000 ha	Yield t/ha	Total production (1000 t)
54-59	30	5.8	177
59-64	54	5.6	324
64-69	69	8.5	591
69-74	81	9.6	781
74-79	91	9.4	844
82-83	109	10.4	1149
86-87	105	10.0	1059

About 4,000-4,500 tons of seed potatoes were imported every year to meet the requirements of the country. However, with the full implementation of the scheme in 1988, BADC has been supplying about 7,200 tons of certified seed by local production with contract growers. The rest of the seed requirement is met by the farmers' own production.

The total requirement of potato seed in Bangladesh is about 0.12 m tons for about 0.105 million hectares. With the involvement of BADC in potato seed production, only about 800 tons of foundation seed is now imported for the multiplication program.

System of Seed Multiplication

BADC is the only organization which is involved in systematic potato seed production. Presently, three types of seeds are produced in Bangladesh. These are pre-foundation and certified seed potatoes.

Pre-foundation Seed Production by the Potato Research Center (PRC), BARI. Production and maintenance of breeder seeds in sufficient quantity is being done at Debiganj. Pre-foundation seed is handed over to BADC for further multiplication.

Foundation Seed Production by BADC

BADC has been producing foundation seed potatoes with strict sanitary measures on its seed multiplication farms during the aphid-free period, for the last four years. The technique of multiplication of foundation seed is not much different from the certified seed production technique. Timely planting, more rigorous roguing, spraying, and timely pulling of haulm are fully ensured in the case of foundation seed potato production. BADC has been successful in the production of high quality foundation seed of potato varieties Cardinal and Kufri Sindhuri. Source-wise, availability of local foundation seeds is shown in Table 4.

Table 4. Source of foundation and certified seed in Bangladesh (tons).

Year	Foundation			Certified	
	PRC	BADC	Imported	BADC	Imported
81-82	20	103	390	2000	1500
82-83	40	59	378	1800	1300
83-84	25	75	401	2600	1600
84-85	35	128	540	2600	1100
85-86	45	208	608	3500	1300
86-87	48	215	926	4000	1000
87-88	45	447	870	6500	0

The Potato Research Centre (PRC), now redesignated as the Root and Tuber Research Centre (RTRC) has had scientists trained in CIP, Lima for in vitro maintenance and multiplication techniques with a view to strengthening and augmenting their efforts to utilize rapid multiplication technology for the production of pre-basic seed. Much-expected Canadian funding will provide manpower and infra-structural facilities for the seed program.

Bhutan

During 1980/81, the estimated area planted in potato was approximately 4,000 ha, producing approximately 25,000 t with an average yield of 6.85 t/ha.

The organized seed supply, which was not necessarily produced in the country and in no way inspected, ranged from 500-750 t/year. This quantity of seed could only cover 6-9% of the area. However, because of cultivation at high altitudes and the slow generation rate, the quantity of seed supplied did maintain production, but at a low level.

Since 1981, the Department of Agriculture, Royal Government of Bhutan, has been operating a Basic Seed Multiplication Farm at 3000 m.a.s.l. in Phubjikha. Initial efforts were to import a limited quantity of elite seed (10 t) in 1983 and multiply it to initiate a flush-out seed multiplication system. From 1983 to 1987, this farm has been able to produce and supply 80-100 t of good quality seed. This seed was given to certified growers in the districts for single multiplication using the seed-plot technique.

Since the 1987/88 season, the scientists and technicians trained by CIP, have been able to produce pre-basic seed tubers from in vitro plants in the net house. Through the bilateral funding from the Swiss Development Corporation/Helvetas, a well-equipped tissue culture laboratory was established in 1987/88. With the pre-basic seed produced, as well as that imported from PRI, Australia (in the initial stages - ca 1000 tubers), the Basic Seed Unit at Phubjikha has maintained the targets and improved the health standards of the seed produced not only at the unit, but in the whole valley, where farmers are contract growers for the program to produce foundation seed using basic seed supplied annually by the unit.

The Bhutan National Potato Programme with the support of Swiss Development Corporation (SDC)/Helvetas and CIP has been able to establish a sound seed program, producing about 2,000 t of certified seed.

Nepal

The National Potato Development Program (NPDP) has been supported, since 1978, by a project under bilateral cooperation, financed by the Swiss Development Corporation (SDC). This project is now in its third phase (1988-1992). Statistics of potato production are given in Table 5.

With the objective of improving the potato production per unit of land and inputs, NPDP, in the past, concentrated on production of improved seed of selected varieties. Experience showed that on-farm seed production is feasible and that, in certain areas, good health standards of the seed could be maintained over many years.

Table 5. Area, yield and total potato production in Nepal from 1979-1989.

Year	Area ('000 ha)	Total production ('000 t)	Yield (t/ha)
1979-80	51	278	5.4
1986/87	74	430	5.8
1987/88	81	560	6.9
1988/89	82	640	7.8

(Project Document of NPDP/SDC)

Quality seed tubers increased from about 485 t in 1982 to 800 t in 1988. However, the major portion of the seed needed for approximately 82,000 ha is available to the growers through the traditional channels.

In its third phase, the project has taken up the task of utilizing in vitro technology by establishing a well-equipped laboratory to produce in vitro plantlets and tuberlets which would be grown and multiplied in insect-proof glasshouses. The flow chart given in Figure 1 shows the planned seed production channels with the hope that by the year 1992, the project will generate 10,000 t of foundation seed to be supplied to certified seed growers who would produce the certified seed to meet the total seed requirements.

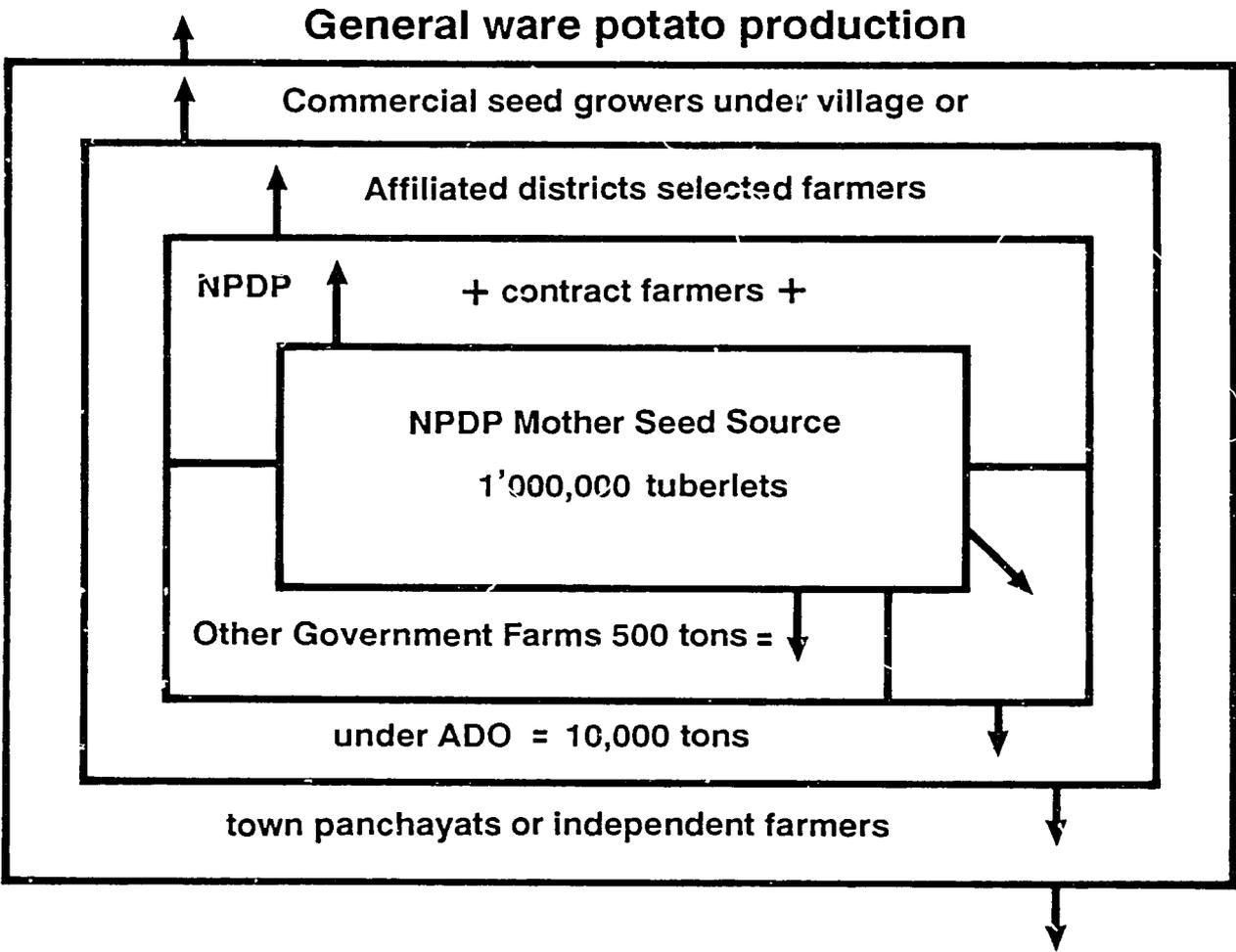
Sri Lanka

Currently, approximately 6,000 ha of potatoes are grown annually, producing around 90,000 tons of consumer potatoes. The total requirement of seed is approximately 13,000 tons per year, of which the departmental seed programs produce approximately 2,000 tons, while the farmers generate some 9,500 tons of seed on their own. The remainder is imported. The seed potato imports, which were approximately 5,000 tons in 1977/78, have now been reduced to 1,500 reflecting a positive approach to local seed production and self-reliance.

Seed Potato Production Strategies

Initially, the imported varieties, which had given promising results in research trials, were multiplied in the government seed potato farms located at around 2,000 m and once the seed was multiplied it was issued to farmers for producing potato for consumption. The locally produced seed was therefore supplementary to the imported seed, but was mainly diverted to the lower elevation plantings.

Figure 1. The planned scheme for the production of certified potato seed in Nepal.



The supply of seed potato, whether imported or local, had to be geared to the planting times of the different regions (Table 6).

The largest area, of approximately 2,600 ha is planted in the Badulla district during June-July in the paddy fields immediately after the harvest of the rice crop. Until the mid-1970s, the entire quantity of seed potato required for this planting was imported from Australia (variety Delaware). However, in 1978, there was a serious breakdown in imports and the farmers successfully embarked on a November-December planting of potato in the highlands (1,350 m). They used this harvest for the June planting, and this led to the total stoppage of seed potato imports from Australia in 1979 for paddy-field planting. Meanwhile, the information obtained from research indicated that imported certified seed potato could be multiplied up to three times, without any serious decline in yield. All these factors, together with the fact that some farmers retained their own seed, resulted in significant reduction of seed potato imports from 1981 on. Subsequently, to sustain this effort and to further reduce seed potato imports, the government seed potato farms adopted research recommendations for using presprouted seed and the appropriate fertilizer applications at planting, which resulted in a significant increase in seed potato yields. Besides the above approach, the establishment of registered private seed potato organizations and, also, the use of government livestock farms in the highlands for seed potato production, helped to increase local production.

Table 6. Planting times of different regions in Sri Lanka.

District	Planting time	Harvest	Average Temp (C ^o).		Remarks
			Min.	Max.	
N'Eliya	Aug/Sep	Dec	11.2	19.6	Seed and consumption
(2000 m)	Feb/Mar	Jun	10.5	21.0	Mainly seed
Badulla (1359 m)	Jun-Jul (paddy fields)	Sep/Oct	16.9	25.5	Consumption
	Nov/Dec.	Mar-Apr	14.6	23.5	Seed (highlands)
Jaffna & Other low country area (sea level)	Nov/Dec	Feb-Mar	22.8	28.9	Consumption

Recent Advances in Local Seed Potato Production

The following concepts are being given attention, in an attempt to achieve a successful local seed potato production program:

1. Seed certification.
2. Improving the quality of locally-produced seed.
3. Alternate strategies for producing non-conventional planting material.

Since farmers retain about 9,500 tons of seed, a program to improve the quality is relevant and urgent. This will involve the production of disease-free seed and also willingness to accept storage techniques which will obtain the optimum type of planting material. The use of diffused-light stores and the planned expansion of the seed certification scheme to utilize serology and indicator plants could be relevant in achieving these objectives.

Alternate strategies for providing non-conventional planting material are the use of tuberlets, true potato seed, and rapid multiplication techniques. Research investigations of these approaches are currently being pursued, and some of the recommendations for replacing traditional seed potato as planting material are becoming popular. A program of cleaning locally-grown seed from virus diseases through the use of tissue culture and micro-propagation has also been initiated. Relevant to the concept of alternate strategies, is the impact created by the use of diffused light stores (DLS) by the potato farmers in Sri Lanka, where storage periods up to 8 months are possible. DLS is also beneficial for producing the optimum type of planting material, i.e. presprouted seed available at the correct time of planting, with very little investment by the farmers. The impact strategy has been reported as an enhanced 1:7 multiplication rate, compared with the previous 1:3 obtained by planting imported seed, which was supplied late and was sometimes in poor physiological condition. This reflected a 133% increase in yield due to availability of seed at the proper time and planting conditions.

Future Programs for Availability of Planting Material

It is anticipated that in the near future, new areas in the low- and mid-country will come under potato cultivation, creating a further demand for seed potato or non-conventional planting material like TPS, stem cuttings or tuberlets. Land in traditional seed-potato growing areas is limited. Consequently, new areas under the tea replanting scheme in the highland tea estates offer the prospect for increasing seed potato production.

Finally, there are also plans for encouraging and establishing commercial-private sector seed production to grow and sell certified seed independently, with initial high quality seed supplied by the Department of Agriculture. This venture will also have to be supported by storage systems, using forced ventilation or temperature-controlled units in seed production areas.

A short review of the status of seed production in Region VI countries brings out the following facts:

1. Although India has had a well organized certified production system since 1964, other countries are also building up their capabilities to achieve self-sufficiency in their local seed production to eliminate importation of seed potatoes.
2. Unlike India, other countries do not have infrastructural facilities and trained manpower to produce antisera for their own use. Since setting up such a facility involves high financial commitments, it would be advantageous to support the Indian efforts to produce larger quantities of antisera for important viruses to supply the neighboring countries at cheaper rates. Or on the other hand, the capabilities should be strengthened for in vitro maintenance and multiplication of tested stocks of varieties commercially grown in these countries, so that they can produce the required amounts of pre-basic stocks to feed their seed programs. This will also mean the commitment on the part of CIP to supply tested in vitro stocks of the varieties being multiplied in each of these countries.

Acknowledgments

I am grateful to the heads of the National Potato Development Programmes of Bangladesh, Bhutan, India, Nepal, and Sri Lanka for making available the latest information on the status of their seed production.

Methods of Virus Eradication

J. H. Dodds, R. Lizarraga, H. Griffiths, and
S. A. Slack¹

Introduction

The eradication of viruses by excision of meristems was first demonstrated almost 30 years ago (Morel and Martin, 1952). The principle is based on the uneven distribution of virus particles throughout the plant.

In the case of vegetatively-propagated crops such as potato and sweet potato, there is clear evidence that the eradication of virus infection to produce so called "pathogen-tested" plants enhances the yield of the crop (Mori, 1971; Morel and Martin, 1955; Stace-Smith and Mellor, 1970; Pennazio, 1978).

Pathogen-tested materials are the basis of modern "seed schemes" whereby clean material is propagated under conditions of zero or minimal reinfection. In this way, higher quality planting material can be produced for farmers, that will in turn give both higher yields and enhanced tuber quality.

Although the excision of meristems has been a key technique in virus eradication, a few important developments have followed this procedure. First, the quality of a pathogen eradication program is only as good as the detection methods used to show that the pathogen has been eradicated. In this area, great advancements have been made recently in both routine serological testing (Clark and Adams, 1977) and nucleic acid hybridization techniques (Owens and Diener, 1981).

Secondly, a number of additions have been made to virus eradication procedures that enhance the probability of cutting meristems that are virus-free. These additions are normally chemical treatments (chemotherapy), heat treatments (thermotherapy), or a mixture of the two.

The use of thermotherapy on both potato and sweet potato is now routine at CIP. Sometimes chemotherapy can be applied.

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Chemotherapy

In the case of sweet potato, we have found the plants to be highly susceptible to virazole and other antiviral chemicals. This is similar to the situation with cassava*. Thus to date, chemotherapy has not been used on sweet potato.

In potato, antiviral chemicals were sometimes used in the old protocols as a pretreatment of intact plants before placement in the growth chamber for chemotherapy. With the new protocol, the optimal treatment to date is a mixture of in vitro heat treatment with the inclusion of ribavirin in the medium. Table 1 shows the effect of a wide range of antiviral chemicals when used in the in vitro system.

Table 1. The effect of a range of antiviral chemicals when included in a tissue culture medium as a way of enhancing the virus elimination process.

Chemical	Concentration (nM)	Desiree/PVS % Titer reduction	Russet Burbank/ PLRV % Titer reduction
1. Autoclaved chemical			
Adenine arabinoside	0.01,0.1,0.2	0	0
	0.4,0.8	40	0
5-azacytidine	0.1,0.5	17	52
6-azauracil	1.0,2.0	-	-
3-deazauridine	0.05	25	33
	0.1,1.0	-	-
2,3 diaminopyridine	1.0	0	0
5-diazouracil	1.0	27	0
2,4 dimercaptopyrimidine	0.15	65	-
5-fluorouracil	2.0,3.0	-	-
Tubercidin	0.1	0	0
2. Filter sterilized			
Adenine arabinoside	0.01, 0.10	0	0
	1.0,1.5,2.0	0	-
5-azacytidine	0.1	-	-
6-azauracil	1.0	-	-
cordycepin	0.04,0.06,0.08	0	-
5-diazouracil	1.25,1.5	0	-
Dicyandamide	0.6	0	-
	1.0	-	-
	2.0	0	74

*W. Roca, personal communication.

Thermotherapy

Thermotherapy at elevated temperatures (37°C) does not eliminate the potato spindle tuber viroid (PSTVd). PSTVd consists of a single-strand, ring-shaped RNA that is twisted in the form of a supercoil. In this form it is resistant to nucleases. Elevated temperature, far from decreasing the concentration of the viroid, favors its multiplication (Sanger and Ramm, 1975). Therefore, a first test for PSTVd should be carried out at the end of the thermotherapy period.

A method that permits the eradication of PSTVd (Lizarraga et al., 1980) is based on the observation that in plants grown at low temperatures, the viroid concentration is low. In an experiment, plants were grown at 8°C for four months. Then apical domes were excised. Thirty percent of the plantlets regenerated were free of PSTVd, even in the second tuber generation. A clear (negative) relationship between meristematic explant size and eradication success was observed (Lizarraga et al., 1982).

This method, however, is not suitable as a routine technique, since it is time-consuming and costly. It may be useful in specific cases in which a valuable clone is not available without PSTVd infection.

Experiments carried out with different virus host systems have shown that treatment of plants with elevated temperatures (thermotherapy) leads to a reduction in virus concentration (titer) in the plant (Kassanis, 1957; Quak, 1977). Different reasons have been given to explain this phenomenon; most likely, the reduction in virus titer is caused by a combination of several factors. These can include competition for sites of synthesis of nucleic acids and proteins between the fast-dividing host cells and the virus particles, which may lead to a change in the balance between synthesis and degradation of virus particles. Also, the nucleic acid of the virus, the carrier of its genetic information, is usually protected from attacks by degrading enzymes by a coat of many protein subunits. At elevated temperatures, the linkage between these subunits becomes weaker, temporary holes may open and permit the attack of nucleases, leading to virus inactivation and decreased virus concentration.

Thermotherapy has been applied to dormant potato tubers and, as a result, a reduction of virus concentration, mainly of potato leafroll virus (PLRV), has been observed. However, elimination was not achieved except for PLRV.

Thermotherapy applied to the whole plant as well as to sprouted tubers followed by meristem culture has been successfully used as a standard procedure for elimination of many viruses in potato (Stace-Smith and Mellor, 1970; Pennazio and Redolfi, 1973).

In the standard procedure used at CIP, best results have been obtained when the plant is decapitated before introduction into thermotherapy and axillary buds grown while undergoing heat treatment. A daily temperature regime of 36°C for 16 hours and 30°C for eight hours and

continuous light of high intensity (10000 lux) improved elimination rates. Plants are kept under these conditions for four weeks. Meristems are isolated and cultured both from axillary buds and apical buds.

In vitro thermotherapy is an innovative method that is gradually replacing the standard procedure explained above. Nodal cuttings from in vitro plants are placed in plastic boxes with propagation medium using agar as gelling agents. When the plantlets reach 3 to 4 cm and have a good root system, they are ready for thermotherapy treatment in an incubator. After a 1-month treatment, apical meristems are isolated and cultured. Figure 1 shows a low-cost incubator that can be constructed for in vitro thermotherapy. The optimal treatment to date is the application of heat and the inclusion of antiviral chemicals in the culture medium.

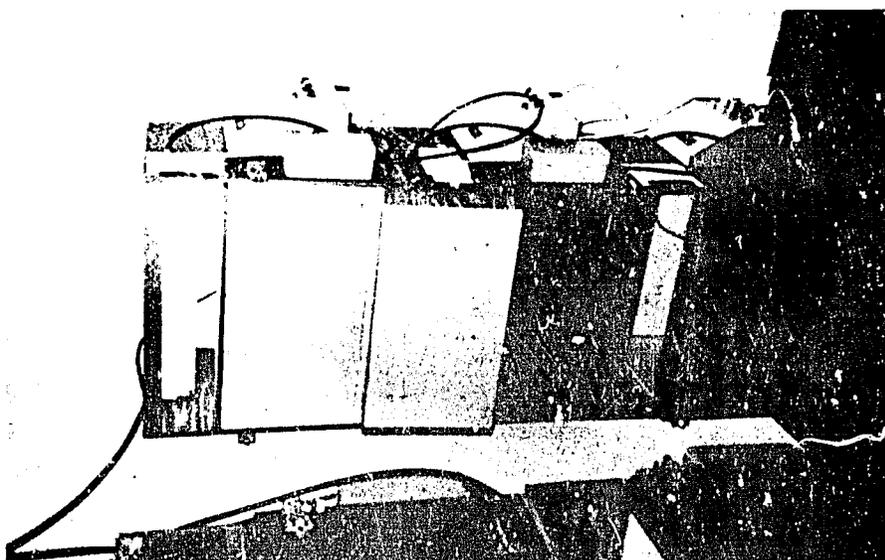


Figure 1. Low cost construction thermotherapy chamber. A wooden box construction, insulated with polystyrene, fitted with a heat tape and fire thermostat. A small fluorescent light is used for illumination.

Meristem Isolation and Culture

The active growing point of the plant shoot is the meristem. It is a small region composed of rapidly dividing (meristematic) cells.

The dome of a shoot apical meristem contains the truly meristematic cells and is surrounded by leaf primordia and primary leaves. Since the differentiated vascular tissues are located far from the meristem (towards the older stem tissue), the vascular elements of the leaf primordia

are still incipient, and have not yet made contact with the main strand of the vascular system in the stem. Therefore, virus particles, which may be present in the vascular system, can reach the meristematic region of the apex only through cell-to-cell movement; a slow process. This is one of the main reasons why in a virus-infected plant, virus concentration decreases acropetally toward the meristem of both the apical and the axillary buds.

The apical portion, called the meristem tip, when isolated under aseptic conditions and cultured on an adequate aseptic nutrient medium, leads to the development of plantlets. This developmental sequence, in principle, simulates normal plant growth.

The aseptic dissection of the meristem is a delicate process requiring many hours of practice.

Stems are cut from the plant that has just undergone thermotherapy into segments which each contain one node with its axillary bud. The leaves are carefully removed. The stem segments are disinfected for 30 seconds in 70% alcohol, followed by 2.5% calcium hypochlorite for 15 minutes. Then the stems are washed four times for five minutes each time with sterile distilled water to remove excess hypochlorite. If in vitro heat treatment is carried out, then the meristems are isolated directly without the need for surface sterilization.

Under a binocular dissecting microscope, the leaflets surrounding the growing point are removed until only the apical dome and a few leaf primordia remain. The dome and two leaf primordia are excised and transferred to the meristem culture medium. The excised apical dome is transferred weekly to a fresh medium. After six to eight weeks, the small plantlets are subcultured for further growth and micropropagation.

After regeneration from the cultured meristems, plants are tested (indexed) to detect any persisting virus infection.

Genetic Stability of Regenerated Plantlets

Once the plants have been through the pathogen elimination process it is important to check that no abnormalities have occurred during the culture/regeneration process. At CIP, the standard tests for this are the analysis of morphological characters and the running of protein electrophoresis patterns from mature tubers. These tests are carried out prior to the international distribution of the pathogen-tested genotype.

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Epidemiological Studies on Virus Diseases

L. Bertschinger¹

Introduction

The aim of this paper is to explain the purpose, utility, and present status of potato virus epidemiology, illustrated with some preliminary results of the studies being conducted in Peru.

"Epidemiology is the science of populations of pathogens in populations of hosts, and the disease resulting therefrom under the influence of the environment and human interferences" (Kranz, 1974).

Profound scientific studies on epidemiology were not initiated until after World War II (Gregory, 1945; Vanderplank, 1946).

Studies on potato-virus epidemics have not been the principle topic in the development of this discipline up to now. In the late 1970s and early 1980s however, the number of publications on the subject has increased.

The epidemiological setting for the study of potato virus diseases.

The analyzed simplified system consists of 3 biotic components (the virus, the plant, and the virus-vector), one partially abiotic component (the field-ecosystem) and their interactions. The field-ecosystem includes, for example, water pressure in soil and microclima, but also field management by man, etc. All the components that are not influenced by the components belonging to the system belong to the environment (Campbell et al., 1988), for example, the macroclima (Fig. 1).

Some epidemiological studies investigate specific interactions between the components of this system or between some components of the system with its environment. Others try to contribute to an understanding of the disease system with a holistic approach. However, for potato viruses this kind of study is still scarce.

How are virus diseases measured? The proportion of infected plants (disease incidence) is most appropriate, as virus diseases are normally systemic (Madden and Campbell, 1986).

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Principal utilities of epidemiological studies of potato virus diseases

Epidemiology offers innovative approaches for disease management practices.

Epidemiological studies are already used in developed countries for potato virus-disease management, and will become more and more important in this field. I will try to explain how this will occur in the case of the potato viruses. Here is an example of a simple epidemiological study:

The moment when aphid-transmitted viruses are actually transmitted in a particular zone may be monitored by periodically-changed, initially healthy stem cuttings planted in seed trays which are positioned in the center of a highly virus-infected potato plot (Fig. 6b).

Such kind of data may be used in seed production for prediction of haulm-killing dates to avoid major tuber infection or to determine the appropriate season for seed production.

More complex studies aim at forecasting the proportion of infected tubers of the produced seed at any moment of the season; this is of great interest to seed growers, to their associations, and to agricultural research institutions in countries where it is usual to buy new quality seed each season, and where degeneration is rapid.

However, in many developing countries, the renovation of seed each season does not occur for several reasons.

In many cases, the farmers do not have access to high quality seed or cannot afford to buy it. In some specific zones seed degeneration may simply be very slow or cause little yield reduction. In these cases forecasting the proportion of infected seed tubers as a function of the number of seasons the same seed is used, is of particular interest.

In Figure 2 one example is given of a degeneration regression based on preliminary results of one experimental season, representative of the degeneration caused by PLRV in the Peruvian coastal valley of Cañete. Experimental data have been fitted by the logit-transformation (Vanderplank, 1963).

This kind of model is very useful in obtaining technical proof of a zone's suitability for seed production from the agronomic point of view.

The Peruvian case and its implications.

In 1983, the Peruvian Institute for Agricultural Research (INIAA), the International Potato Center (CIP), and the Swiss Development Cooperation (DEH) jointly initiated a project to develop and implement new technologies in order to produce high quality potato seed, to assess the agronomic and socio-economic advantages of this seed, and to develop and test strategies for large-scale distribution of the seed (Ezeta and Scheidegger, 1985).

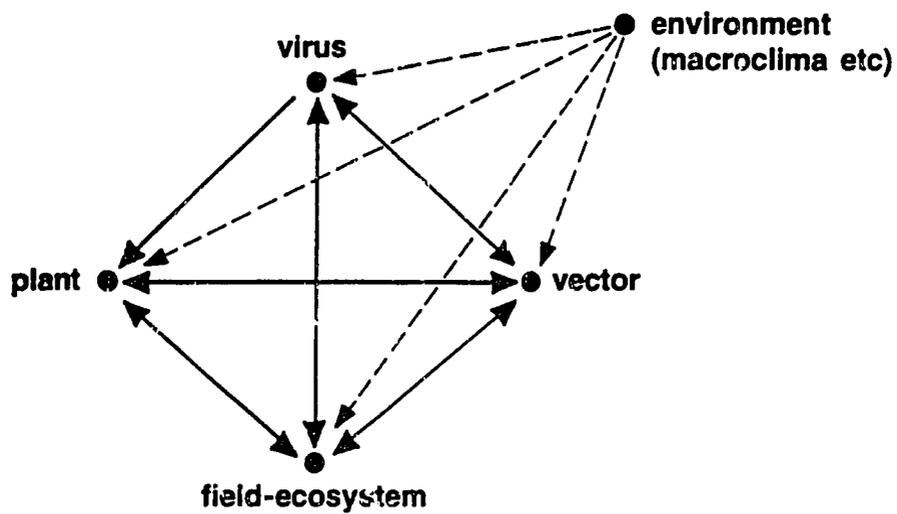


Figure 1. Interactions within the composite disease system and its relation to the environment for epidemiological studies of potato viruses.

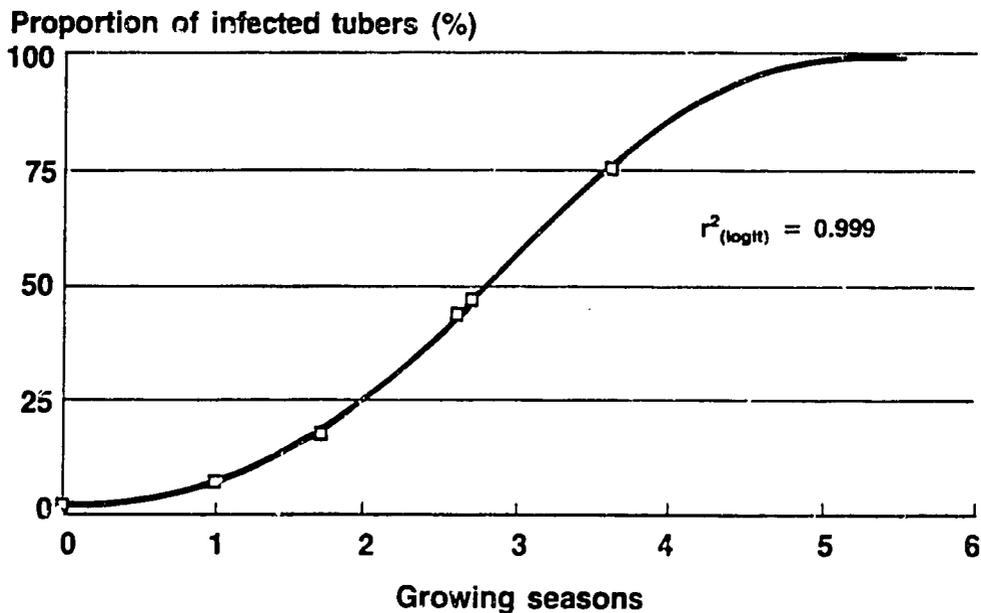


Figure 2. Seed degeneration by PLRV for a modern potato variety in Cañete, Peru (400 m.a.s.l.), 1987.

High quality seed is multiplied in fields in the highlands. The project tried to determine the rate at which high quality seed was degenerating season by season, degeneration being understood as the increase of the proportion of infected tubers.

In Peru, the vast majority of farmers are small peasant producers who grow potatoes above 3500 m.a.s.l. with techniques highly adapted to the environment. They maintain their own seed over several seasons (informal seed system; Prain and Scheidegger, 1988).

The suitability of this prevalent, informal system for distribution of high quality seed has been proven, and client-oriented approaches have been developed to introduce the seed into the informal system.

Based on this situation, it was important to determine how often farmers would need to introduce small quantities of fresh high quality seed in order to multiply it.

Initial degeneration trials in the highlands in 1985/86 were not able to detect significant changes of the infection level of a seed between planting and harvest (INIPA-CIP-COTESU, 1987). It was concluded that degeneration had been very slow because few plants were primarily infected and that experimental methodology needed to be changed.

However, in detailed degeneration experiments in the following three years, other possible reasons for a slow degeneration were found. Evidently, not all daughter tubers of secondarily infected mother plants were infected (autoliberation).

With the new methodology applied in these experiments, it was also possible to explain the proportion of infected tubers in the harvest as a result of the number of secondarily- and primarily-infected plants, the fraction of infected tubers of primarily-infected plants, and the fraction of infected tubers of secondarily-infected plants (Fig. 3b). Disease progress in space can also be monitored with this methodology (Fig. 4). This will be useful for understanding virus diffusion, and for calculating the probabilities of infection of a healthy plant for different distances from an initial inoculum. I have been asked many times in the last three years by INIAA seed production specialists what the probability is that a virus like PVX is being transmitted from an infected plant to the neighboring healthy plant by somebody walking through a field.

The studies under way are being made for PVX, APMV, PVY, and PLRV in 4 agroecological zones in traditional potato growing areas of Central Peru: the eastern-facing Andean valleys opening up to the Amazon jungle; the steeply-rising slopes of the cool humid, inter-Andean valleys, the floors of these inter-Andean valleys, and the hot arid western coastal valleys (Fig. 5).

Experimental data have now been collected and degeneration curves for these 4 zones will be modelled. This will help to compare different zones in terms of velocity of degeneration.

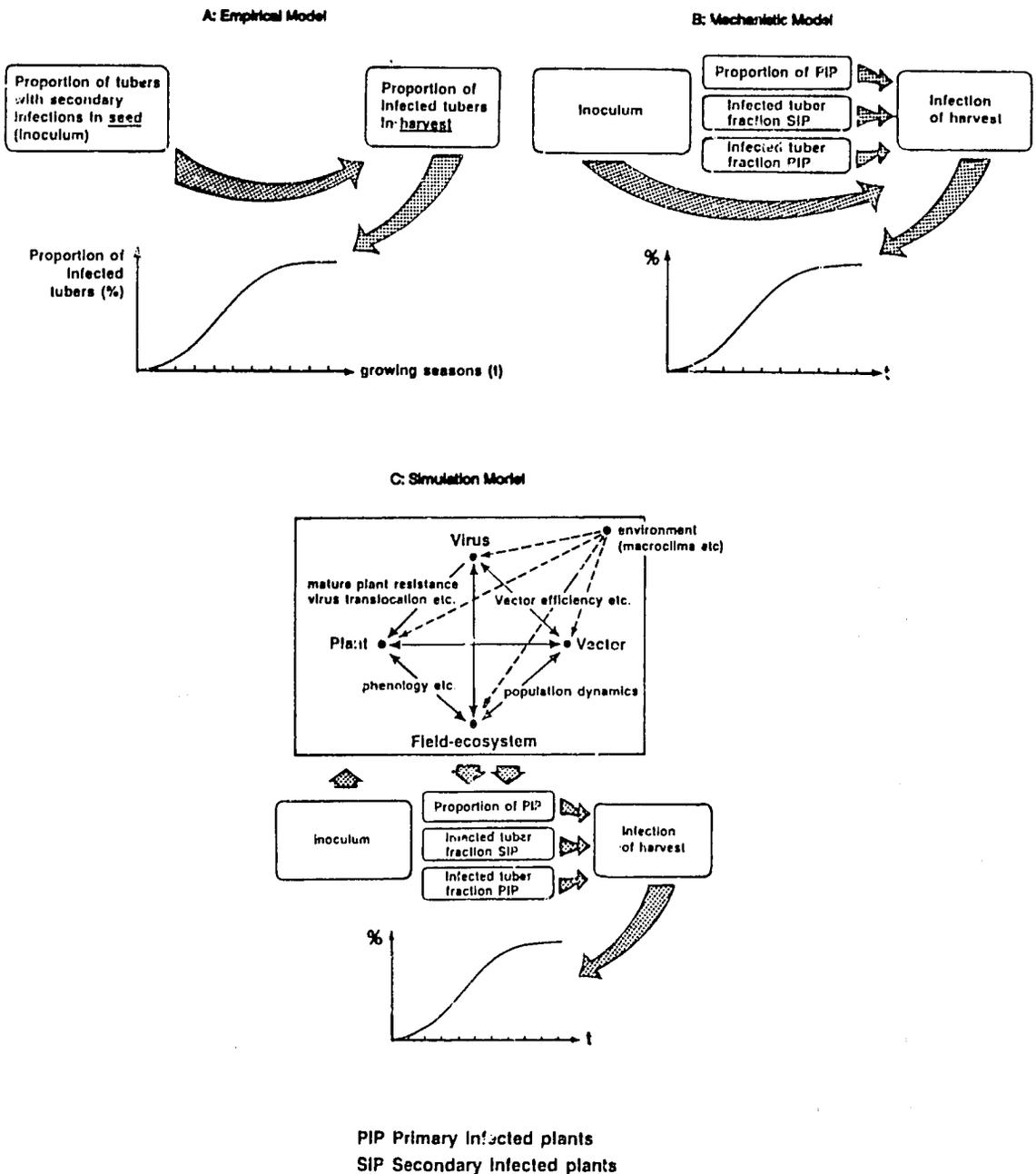


Figure 3. Concepts for an empirical, a mechanistic and a simulation model of potato seed degeneration by viruses.

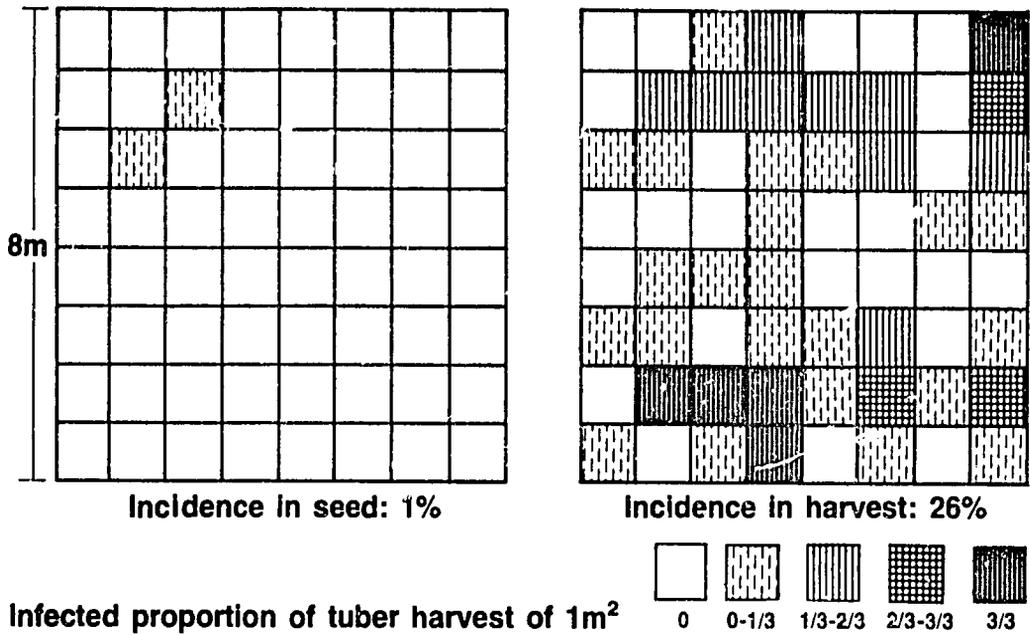


Figure 4. PLRV-progress in space in a field of the native variety "Amarilla del Centro" (*S. goniocalyx*), Huancayo, Peru 1987/88 (3250 m.a.s.l.).

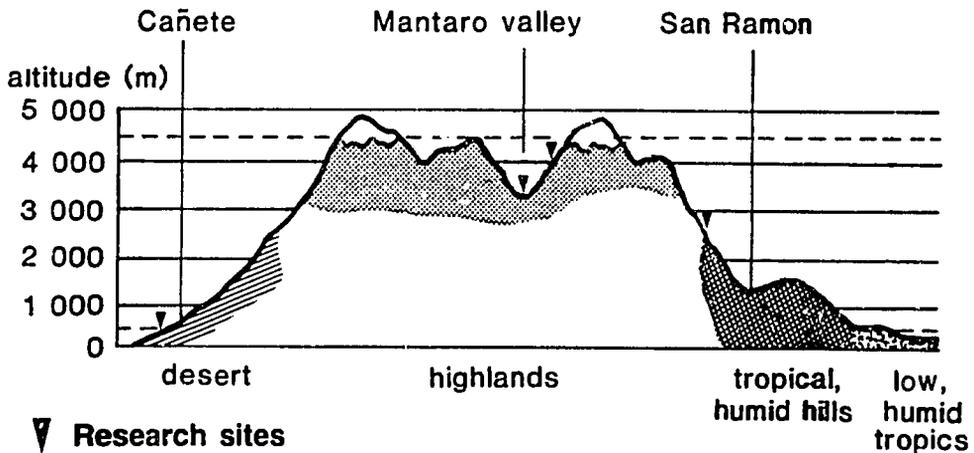


Figure 5. Cross section of the Central Andes: agroecological zones and research sites for epidemiological studies of potato virus diseases in Peru.

A lot of models are very problem-oriented and only valid for one practical situation. The difficulty can be solved by simulation modelling. "Simulation is the art and science of constructing composite models and mimicking one system with another" (Campbell et al., 1988).

The simulation model would consider the most important interactions between components of the studied disease-system and the environment, in order to model with a computer, in multiple data-processing procedures, the parameters which had just been measured by the models presented above.

Data on mature-plant resistance and other interactions in the disease system have been obtained in the last three years in Peru.

It is hoped that the projected simulation will serve as a seed-degeneration forecaster in many situations in distinct geographical locations as data have been evaluated in 4 different zones covering a wide range of climatic conditions.

I have found another quality in epidemiological studies which I consider very useful: they offer an excellent opportunity for a general validation of common research concepts. What do I mean by that?

Let me give you an example: aphid populations are often quite high in the Mantaro Valley (3,250 m.a.s.l.). They are able to transmit PVY, especially in hot, dry seasons. But even in seasons with normal climatic conditions, *M. persicae* is able to transmit PVY quite efficiently. However, PVY and PLRV incidence in farmers' fields planted with common seed has been found to be very low in modern varieties (Scheidegger and Luther, 1987), and plants of varieties known to be susceptible to PVY are very difficult to inoculate mechanically under highland field conditions (Fig. 6). These findings hardly fit into the common concept that high aphid populations correlate strongly with high virus transmission rates.

They point to an alteration of genetic resistance levels through environmental conditions. What is resistance, and under which conditions are resistance genes primed and expressed? These are problems now being studied intensively at many research institutes. This is where epidemiological studies may make valuable contributions to a successful investigation through practical field observations.

Epidemiological studies: The present status and the future for potato viruses

The studies related to potato virus epidemiology that have been published to date emphasize PVY and PLRV. Some interactions of the composite disease system and its environment have been investigated.

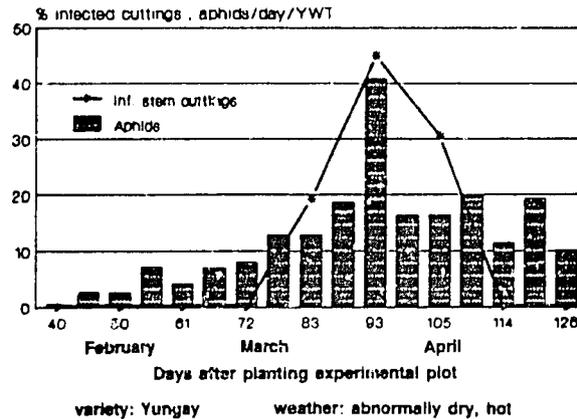
a - PVY transmission efficiency of *M. persicae*

Site	Strain	Condition	Feeding days	% Infected test plants
Sweden ^{a)}	PVY ^O	glasshouse	1.5	26.0
Huancayo ^{b)}	PVY ^N	field	1.5	8.3
Huancayo ^{b)}	PVY ^N	field	7.0	66.7

^{a)} Source: Sigvald, R. 1984

^{b)} Weather: normal (humid, cool).

b - PVY^N transmission to periodically renewed stem cuttings planted in seed trays, Huancayo 1986/87.



Trays in the center of a potato plot (9 x 15 m) with 100% PVY^N infection

c - PVY and PLRV in farmers' fields, common seed, Mantaro Valley 1985/86.

PVY	1.0%
PLRV	0.8%

Source: Scheidegger U. C. and K. Luther, 1987.

5 modern varieties, 6 fields of each tested.

d - Successful mechanical PVY-inoculations of plants of three modern, susceptible varieties under field conditions, Huancayo.

Season	Variety	Strain	Plants	
			inoculated	infected (%)
1986/87	Mariva	PVY ^N	21	4.8
1986/87	Yungay	PVY ^N	22	4.5
1988/89	Yungay	PVY ^N	76	5.3
1988/89	Tomasa C.	PVY ^O	113	2.7

Figure 6. Four fundamental or empirical studies, suggesting an alteration of genetic resistance and of interactions within the potato virus disease-system by environmental conditions.

Some models have been formulated and have been proposed or are actually being used in industrialized countries for the prediction of the haulm-killing date (e.g. 4). The first integral simulation model was published in 1986 for PVY^O disease increase between planting and harvest and is based on Sweden's situation (Sigvald, 1986).

The model we want to construct in the near future should be flexible. To adjust it to some particular condition should require only some simple experiments in the particular zone on key components and interactions of the disease system such as aphid population dynamics, and others.

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Transfer of CIP Germplasm

J. E. Bryan¹

CIP, among the IARC's, has been the leader in attempting to insure that their potato germplasm for international distribution is in the best possible health. This philosophy will continue with sweet potato.

Potato

CIP distributes several types of potato germplasm:

1. Tubers and in vitro plantlets of varieties and clones originating from in vitro mother plants that have gone through thermotherapy, meristem propagation, and accepted testing procedures. This material is listed on our "Potato Pathogen-Tested List" of material available for international distribution, revised annually.
2. Tuber families, derived from true potato seed, segregating for various biotic and abiotic attributes. The seeds planted are from parental stocks tested and found negative to the sexually transmitted Potato Virus T (PVT) and Potato Spindle Tuber Viroid (PSTVd). Samples of the seed also tested negative to PSTVd.
3. Advanced clones derived from tuber families and maintained only in our export quarantine facilities with testing by antisera, indicator hosts and NASH.
4. True potato seed (TPS) with the same purpose of tuber families and also for growing ware potatoes. Both types meet the same criteria as the seed used to produce tuber families.

All in vitro mother stocks are checked once each year for virus and PSTVd on in vitro plantlets. All tubers produced for export are produced in aphid-proof export quarantine screen houses where mother plants are tested at least once during the growing period, using both antisera and the indicator host range for varieties and clones and random testing for PVT and PSTVd on tuber families while growing. All exported material is sent out with the CIP phytosanitary statement (Figure 1), which lists the diseases tested for and the type of test used. The export quarantine houses are aphid-proof, have limited access and are strictly controlled. Disinfected sand and peat or peat mixes are used instead of soil. We have stopped using local "peat-like" material because of phytotoxic effects and now use "Promix" imported from the USA. Pathologists, entomologists and nematologists make visual inspections of growing plants and tubers.

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PHYTOSANITARY STATEMENT

This is to certify that the tubers, true seed, in vitro cultures or cuttings, or representative samples of them, were thoroughly examined on (date of dispatch): _____

by (name): _____

a Senior Scientist of the International Potato Center; and that the consignment is believed to conform to the declaration below.

ADDITIONAL DECLARATION

CHECK APPLICABLE STATEMENT(S) / CROSS OUT NON-APPLICABLE STATEMENTS

- The mother plants were indexed and found to be negative for viruses: A, M, S, T, X, Y, potato leafroll, andean potato latent, andean potato mottle, tomato black ring and tobacco ring spot viruses by symptoms on inoculated indicator hosts, by latex or ELISA serology, electron microscopy and also found free of visual diseases symptoms. Potato spindle tuber viroid (PSTV) was negative by the nucleic acid spot hybridization test (NASH).
- True seed harvested from pathogen tested parents that tested negative for PSTV by the nucleic acid hybridization test (NASH) and to PVT. A statistically valid sample of seed was also tested and found to be negative to PSTV by the NASH test.
- True seed from parents of unknown health status. A statistically valid sample of seed has been tested and found to be negative for PSTV by the nucleic acid spot hybridization (NASH).
- Other:

DISINFECTION TREATMENTS

DATE	TREATMENT

CHEMICAL AND CONCENTRATION

--

Figure 1. CIP phytosanitary declaration for potato germplasm exportation.

Sweet Potato

Sweet Potato will be distributed only as in vitro plantlets and true seed. At this writing, only (five or six) cultivars are available for shipment and distribution of true seed is restricted in accordance with the recommendations of the first Planning Conference on Sweet Potato Clean-up, (1). Associated testing is now being done on about 70 sweet potato cultivars.

Our phytosanitary statement for sweet potato is tentative (Figure 2) and will not be used until we consider the recommendations of this Planning Conference.

- The mother plants were tested and found negative to sweet potato feathery mottle virus (SPFMV), sweet potato mild mottle virus (SPMMV) and sweet potato latent virus (SPLV) by serology and to potato spindle tuber viroid (PSTVd) by nucleic acid spot hybridization test (NASH). The mother plants also tested negative to the above viruses and other agents infecting the indicator hosts *I. nil* and *I. setosa*.
- True seed harvested from pathogen tested mother plants.
- True seed harvested from mother plants of unknown health status. Recipient accepts these at their own risk.
- Other.

Figure 2. Proposed phytosanitary statement for sweet potato

The general procedure for clean-up of sweet potato is found in Figure 3.

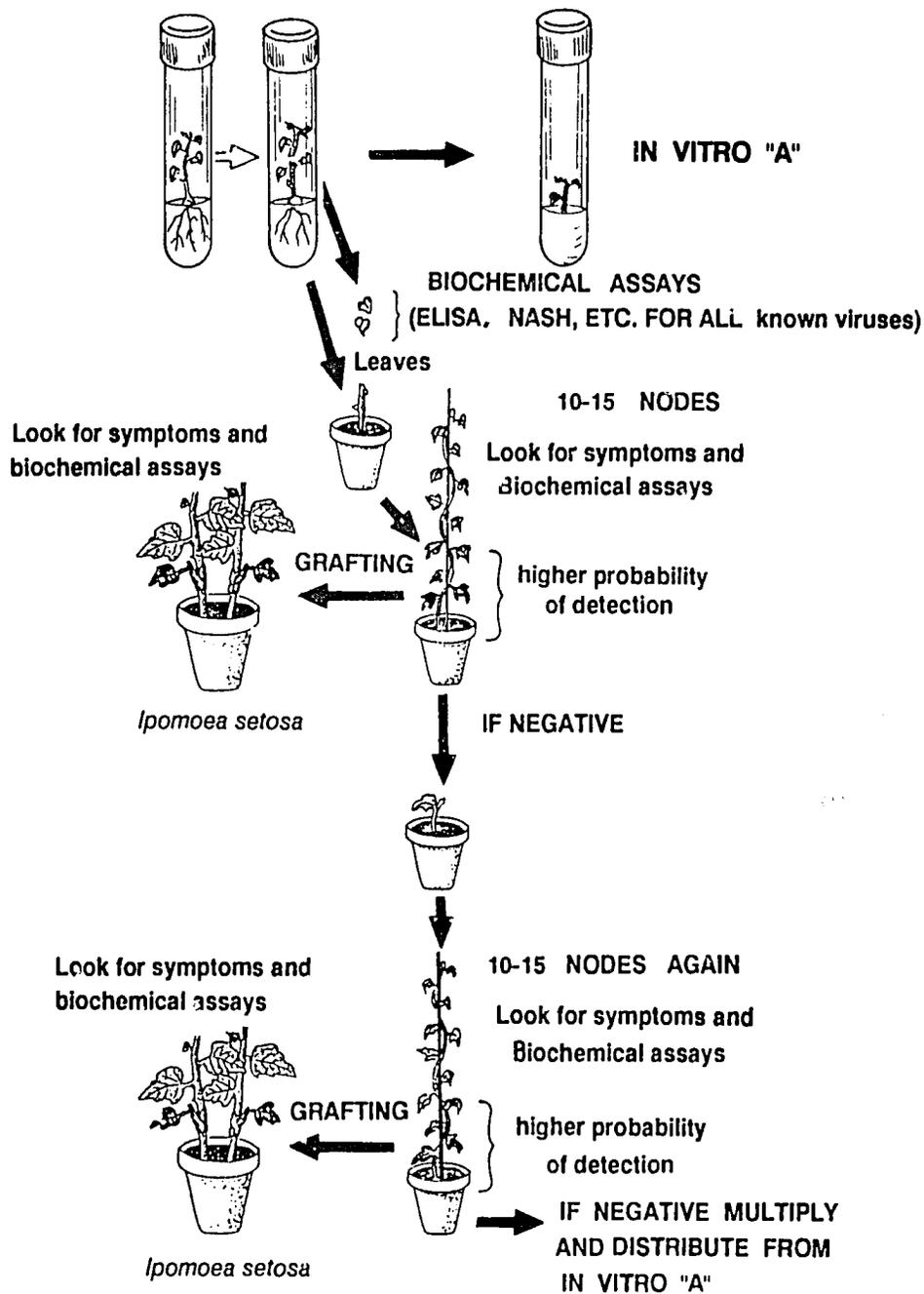


Figure 3. General procedures for clean-up of sweet potato.

Comments on the Recommendations of the last Planning Conference (3)

1. Our designation of quality for all exportable material is "Pathogen-Tested." This designation, together with the phytosanitary statement, conforms to the use of the IBPGR terminology (2): pathogen-tested, pathogen-untested and pathogen-positive.
2. CIP does not export field-grown tubers of potato. Infrequently, a developed country can get untested pot-grown tubers, if they send us a disclaimer clause signed by a high official of the country's Ministry of Agriculture.
3. TPS. 10% of the TPS is assayed for PSTVd. Research has shown that generally, smaller samples are adequate. These are shown in Table 1.

Table 1. Sample size (n) adjusted for different seed-lot sizes at three precision levels.

Size of Seed Lot	Precision level required		
	10%	5%	1%
< 25	Test each individual seedling. ^a		
26-99	Test 20% of the seed lot. ^a		
100	26 ^a	58	97
250	31 ^a	89 ^a	233
500	32	109 ^a	437
750	33	117 ^a	617
1,000	34	121 ^a	775
2,000	35	130 ^a	1,267
5,000	35	135 ^a	2,044
10,000	35	136	2,569 ^a
100,000	35	139	3,342 ^a
200,000	35	139	3,398 ^a
300,000	35	139	3,417 ^a
400,000	35	139	3,327 ^a
500,000	35	139	3,433 ^a
1'000,000	35	139	3,445 ^a
10'000,000	35	139	3,457 ^a

^aRecommended sample size.

Note: For plants, sprouts or tubers, test composite samples of 5. If any positives show, recheck individual units.

4. CIP disagrees with the recommendations to send TPS with no statement of freedom from seed-borne diseases, regardless of whether or not the country accepts the responsibility.
5. We agree with the recommendations on CIP's receiving germplasm. The section on priorities for such testing should have the word "source" added to "available space, facilities and personnel."

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CIP Training Program with Emphasis in Virology

F. N. Ezeta¹

The Training Program at CIP

Agricultural development in Third World countries depends largely on the ability of their scientists to generate appropriate technology to increase crop production and productivity. The international agricultural research centers have a major responsibility for assisting the national agricultural research systems -NARS- to develop and conduct research. The International Potato Center (CIP) was created nearly twenty years ago to develop and disseminate knowledge for greater use of potato and other tuber and root crops as basic foods in the developing world. Until 1986 CIP worked exclusively with potatoes but since then it has included sweet potatoes in its work of active research training and dissemination of information.

In its two decades of existence CIP has created a global network for generation of technology and exchange of information within a worldwide community formed by national research institutions in the developing world, other international research institutions, and research centers and universities of the developed world. Training has played an important role in strengthening the research capacity of NARS and facilitating the exchange of information and dissemination of knowledge among the members of this global network. In the view of the national leaders of the potato programs in developing countries, training is the activity that has been most beneficial to them in their collaboration with CIP. The leaders also recognize that their programs' capacity to carry out their own training activities have been significantly improved (NARS Survey 1987/89).

CIP's Training Program is based on the philosophy that research and extension efforts conceived and executed in collaboration with national programs will be more appropriate for the conditions of the country and have a longer-lasting beneficial effect than those conceived and executed independently by CIP (Piña, 1988).

Therefore, CIP's training activities are directed toward improving the abilities of national programs to:

1. Identify research priorities and needs.
2. Conduct research on priority problems.

¹Head, Training Department, International Potato Center (CIP), Lima, Peru.

3. Use existent technology that has been identified as relevant to the country's needs.
4. Evaluate research results from other sources under home-country production conditions
5. Participate in transferring appropriate technologies within their own country and surrounding countries.
6. Train others to identify existent technology and research needs, to conduct research and evaluate results, and to participate in the transfer process.

Assesment of training needs

CIP reaches the national programs through its regional offices. CIP's regional leaders and regional scientific staff interact with local scientists to determine their training needs. Input is also given by headquarter scientists whose research extends into the regions and who can be very helpful in identifying specific training needs. The Director of Regional Programs and the Associate Director of Transfer of Technology visit the regions once or twice a year to review the research and training program in terms of budget and short- and long-term objectives. Prior to the Annual Internal Review a plan for group and individual training activities is prepared by the regional leader and submitted to the Head of Training for revision. At the Annual Regional Planning Meeting the plan is discussed with regional and headquarters scientists who are then able to make commitments for their participation in training activities for the coming year. Final approval of the training plan is given by the Director of Regional Programs.

CIP also keeps a five-year plan for training which is under constant revision and updating through consultation with national program leaders and CIP regional scientists.

Training in Virology at CIP

The seed potato industry demands efficient procedures to detect plant viruses and viroids. Research institutions and certification services under government or private control and individual seed growers require the testing of large numbers of samples in relatively short periods of time.

Diagnostic procedures for virus detection have experienced a fast evolution in the last twenty years moving from the slow indicator plant techniques to the advanced serological and nucleic acid hybridization analysis. CIP's training efforts have accompanied the evolution of virus and viroid detection methodologies aiming to develop capabilities and self-sufficiency at the NARS. Large numbers of scientists from national programs have received specialized training in virology through individual or group training events. Also, most training courses on seed production have included general virology and detection techniques practicals as an important part of the program. A course on basic seed production may allocate as much as 60% of its total time to virology and virus detection techniques. During the period 1984-88 nearly two hundred scientists from 33 developing countries received specialized training in virology in either individual or group training activities (Fig. 1), and approximately seven hundred were trained in

topics related to seed production. In addition to receiving training for their scientists, many national programs have received serological kits. Between 1984 and 1988, CIP, through its regional offices, filled national programs requests for enough kits and sensitized antisera to process nearly two million samples. The INSE test for PSTVd detection has been distributed free of charge as a service to official seed programs in the developing world.

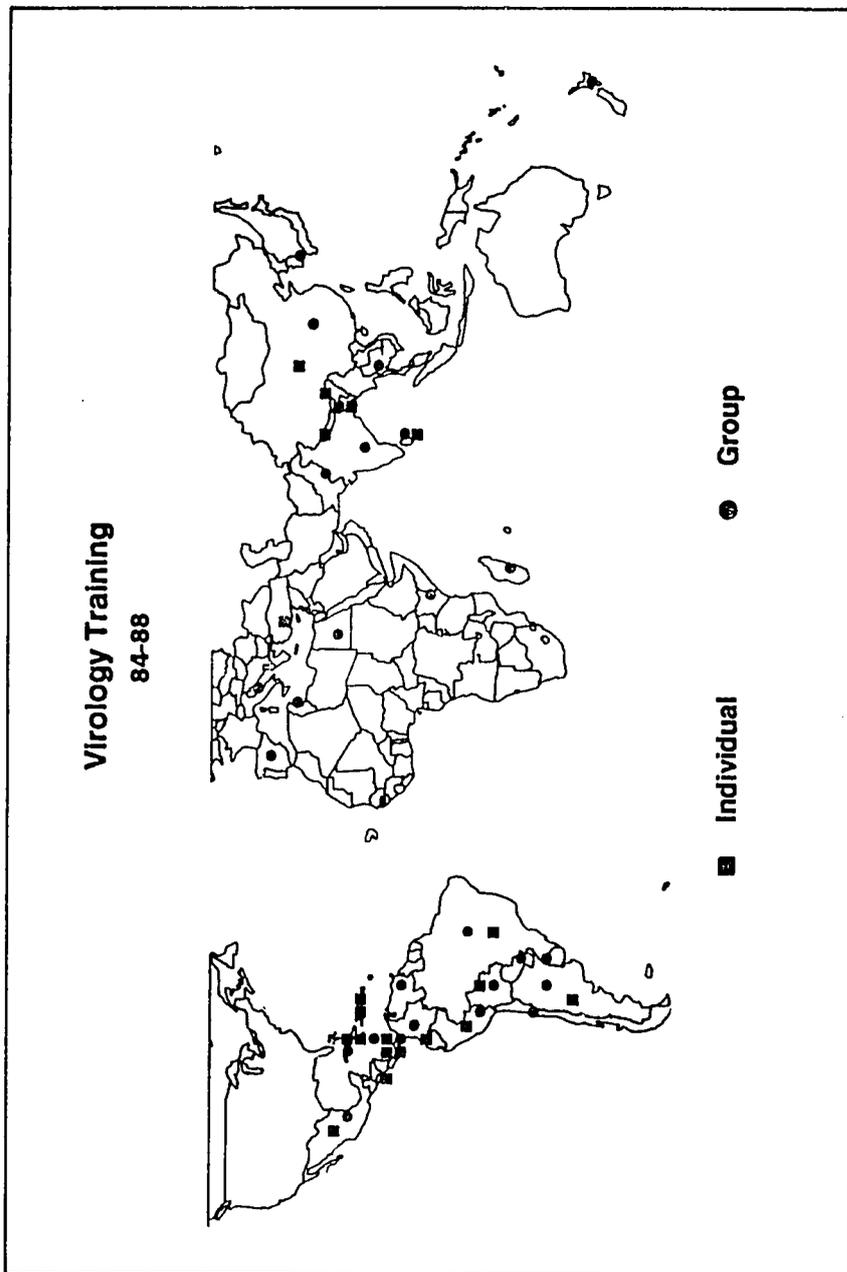


Figure 1. Virology training (1984-1988).

Because the demand for antisera from national programs exceeded CIP's virology capabilities, there was an urgent need to develop collaborative research projects in virology with some selected national programs to produce their own antisera. Brasil, Tunisia, Korea, China, and Thailand were selected in view of their potential to become self-sufficient in antisera production. Training has been an essential component of these projects. The basic idea was that antisera produced in these countries could be used in other countries of the region and that the training given to them could be readily spread to other countries. These projects were developed in two phases.

Phase I

CIP provided the crude antisera for the national program to transform and use it as LATEX, or ELISA. "On the job" training was provided initially on the techniques for antisera transformation. The amounts of antisera were sufficient for one to three years of operation.

Phase II

The national programs started to produce their own antisera under the supervision, guidance, and quality control of CIP. Again "on the job" training was required for virus purification and antisera production.

CIP's survey of regional leaders conducted early this year indicated that in improved pest and disease management, virus control has been the area most benefited as a result of CIP-NARS collaboration. However, most of CIP's regional leaders also believe that very few developing countries have the expertise and laboratory facilities required for production and transformation of antisera. As previously mentioned, CIP's scientists in the regions are responsible for identifying the virology capabilities of national programs and for proposing a training program to meet the needs of the individual countries. Nevertheless, the limited amounts of antisera often required by most developing countries would not justify heavy investments in human resources and physical facilities, in all cases. A more attractive alternative seems to be that of pooling the resources of a group of countries with similar requirements in a collaborative network approach. Networks can be extremely helpful in filling regional needs for technology and know-how in antisera production and transformation in a cost-efficient way. It has been found that CIP's training efforts are greatly enhanced by interacting with organized groups of countries or networks instead of individual countries. The horizontal transfer of technology promoted by the collaborative research networks has already been instrumental in the rapid dissemination of knowledge and technology for several areas of potato production. Scientists from national programs who are given the opportunity to visit successful programs in other developing countries readily adapt new technologies to their own particular set of conditions.

Another approach that can improve cost efficiency of virological training is that of pooling the individual efforts of several international research institutions. Virological techniques are common to the various crops that are in the mandate of some of our sister centers such as CIAT,

IITA or CYMMYT. On the other hand, due to limitations in human and physical resources at the NARS, national leaders will be in favor of a coordinated action from the IRC's on virology training.

Training in Virology at headquarters

Until 1986 most virology training at headquarters was conducted on an individual basis which demanded tremendous effort and time from the scientists in the virology laboratory. Today, training at headquarters has been concentrated in two courses that are offered once a year. An elementary course in virology is offered to beginners for two weeks. This course aims to train technicians in the use of serological tests to detect viruses and viroids and in understanding the basic principles for virus transmission. A more advanced course is available for those students who are going to be involved in antisera production, have access to the equipment required for purification, and are well trained in laboratory techniques. This course has a duration of six weeks and is organized in modules that can be taken as independent units according to the needs and interests of the participant.

Another method of training in virology at headquarters is through cooperating in formal academic degree courses at the Universidad Agraria, La Molina. The subject of virology for either detection techniques or breeding for resistance is very popular among students for the M.S. degree. Research results obtained through thesis work have made major contributions to the knowledge of virological aspects of the potato. It is expected that sweet potatoes will be the subject for research in the coming years.

Regional Virology training

CIP is a de-centralized institution for research and training. Most of CIP's training activities today take place out in the regions under the responsibility of regional scientists with some support from headquarters scientists. Consequently, of 30 to 40 training courses conducted annually, only one or two are carried out in Lima. Most regional courses are conducted in close collaboration with national programs. Sharing the responsibility for training with national institutions allows CIP to reach a wider audience, while at the same time decreasing the training load of CIP's regional and headquarters scientists. In fact, as an ultimate goal CIP should aim at enabling the NARS to conduct training on their own in those areas where sufficient expertise is already in place.

CIP virology training is not exclusively for the official sector of the developing countries. Often we get requests to provide training to scientists and technicians from the private sector and we welcome and incentivize their participation, if our regional representatives judge it convenient, in the development of coordinated activities with the official sector.

A new approach to training in virology has been used in China and Ecuador whereby a survey on sweet potato virus diseases was combined with training on application of virus detection techniques. This is a very interesting approach since trainees are requested to collect and send

tissue samples and whole diseased plants before the course starts. During the course, the trainees use the samples collected for practical work. In this way, the results obtained from several courses serve to build a wide base of information on the virus incidence across the country. We anticipate an increased demand for this training survey approach from the national leaders of developing countries in the immediate future.

Finally I would like to cite Dr. Nyle Brady who in 1977 speaking to the Heads of Training of the IARC, said: "It is very likely that the contributions through training made by international agricultural research centers to the developing world may in the long run exceed those of research."

III CIP Planning Conference on the Control of Virus and Virus-Like Diseases of Potato and Sweet Potato

(November 20-22, 1989)

Day 1 - November 20 1989

Session I: Viruses and Virus-Like Agents Potato and Sweet Potatoes

Chairman: E. R. French

Recorder: R. Cortbaoui

8:00	Welcome	R. L. Sawyer
8:15	Objectives of Planning Conference	P. Gregory
8:30	Overview of Thrust IV. Review of Previous Planning Conference Recommendations.	L. F. Salazar
8:45	Main Virus Diseases of Potato	L. F. Salazar
9:00	Virus and Virus-like Diseases of Sweet Potato	J. Moyer/L. F. Salazar
9:40	Coffe Break	
10:00	The Importance of Potato Virus Diseases in Developing Countries	P. Accatino
10:20	Discussion: Importance of Virus Diseases and CIP's Responsibility	

Session II: Virus and Viroid Detection Techniques

Chairman: J. Dodds

Recorder: M. Upadhya

11:10	Advanced Immunological Techniques for Virus Detection	P. Gugerli
11:30	Hybridization Techniques for Viroid and Virus Detection. Recent Refinements	R. A. Owens
11:50	CIP Efforts at Virus Detection by Serological Means	J. Castillo
12:30	Lunch	
14:00	Development of Molecular Probes for Use in NARS	M. Querci

- 14:20 Virus and Viroid Detection Techniques: Status, Constraints and Needs in NARS of Latin America
(Presented by C. Lizarraga) O. A. Hidalgo
- Needs, Status and Constraints in NARS North Africa and Middle East R. Cortbaoui
- Detection of Potato Viruses and Viroid in India SMP. Khurana
- Virus and Virus-Like Diseases of Potato and Sweet Potato in South East Asia: Status, Constraints and Needs
(Presented by U. Jayasinghe) P. Vander Zaag
- The Advances of Virus Testing in China H. Zhang
- 15:20 **Coffe Break**
- 15:30 Diagnósticos Vegetales - Success in Private-Public Interaction A. M. Escarrá
- 15:50 The Brazilian Approach A. Dusi
- 16:05 Virus and Viroid Detection: Strategies in Cuba L. Lago
- 16:20 Strategies for Collaboration Between the Private Sector and IARC's R. Davis
- 16:35 Discussion: Approaches to Virus Detection in through CIP

Day 2 - November 21

Session III: Control of Viruses by Genetic Resistance

Chairman: P. Accatino

Recorder: P. Gugerli

- 8:00 Approaches to Developing Resistance to Viruses through Breeding A. Mendiburu
- 8:20 Genetic Engineering for Virus Resistance J. Dodds
- 8:40 Sources of Resistance to Viruses in Andean Potato Cultivars Maintained at CIP. Z. Huamán
- 9:00 Resistance to Sweet Potato Viruses J. Moyer/L. F. Salazar
- 9:20 **Coffe Break**
- 10:00 Variability of PVX and PVY and its Relationship to Genetic Resistance E. Fernandez-Northcote
- 10:20 Variability of, and Resistance to Potato Leafroll Virus (PLRV) U. Jayasinghe

- 10:40 **Breeding for Resistance to Potato Viruses Y, X, and Leafroll:**
 Research Strategy, Selection Procedures, and Experimental
 Results H. Mendoza
- 11:00 Discussion: Combination and Balance of Different Methodologies
 for Virus Resistance

12:30 **Lunch**

Session IV: Seed Production and Transfer of Germplasm and Technology

Chairman: J. Moyer

Recorder: U. Jayasinghe

- 14:00 Virus Detection Procedures for Seed Production J. Hammond
- 14:20 Seed Production of Seed Potato in Chile J. Santos Rojas
- 14:35 Status of Seed Production in India and Nearby Countries M. Upadhyia
- 14:50 Methods of Virus Eradication J. Dodds
- 15:10 **Coffee Break**
- 15:30 Epidemiological Studies on Virus Diseases L. Bertschinger
- 15:45 Transfer of CIP germplasm J. Bryan
- 16:15 CIP Training Program with Emphasis in Virology F. Ezeta
- 16:30 Discussion: Are CIP efforts on Germplasm Transfer and Virus
 Control Adequate?

Day 3 - November 22

- 8:00 Development of working groups on germplasm transfer and breeding for virus
 resistance, virus detection and research on sweet potatoes
- 8:30 Working groups discussion
Coffee Break

Session V: Formulation of Recommendations

Chairman: P. Gregory

Recorder: L. F. Salazar

- p.m. 15 minutes report by representative of each working group. General discussion
 at the end of the three reports

Participants at III CIP Planning Conference on the Control of Virus and Virus-Like Diseases of Potato and Sweet Potato

(November 20-22, 1989)

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24. M. Nakano
25. M. Querci
26. L. Salazar
27. R. Sawyer
28. M. Upadhya