ʼRT OF PLANNING CONFERENCE ON

ELOPMENT IN THE CONTROL OF POTATO VIRUS DISEASES

INTERNATIONAL POTATO CENTER

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INTERNATIONAL POTATO CENTER

REPORT OF THE

PLANNING CONFERENCE

ON

DEVELOPMENTS IN THE CONTROL

OF POTATO VIRUSES

Held at CIP - Lima, Peru

November 14 - 18, 1977

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(Published November 1978)

ADDENDUM

Fig. 1, Page 146, see Fig. 1, Page 48.

FOREWCRD

FOURTEENTH PROGRAM PLANNING CONFERENCE

The International Potato Centre (CIP) is a scientific institution, autonomous and non-profit making established by means of an agreement with the Government of Peru with the purpose of developing and disseminating knowledge for the greater utilization of the potato as a basic food.

CIP is one of nine centers and two programs supported by the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is sponsored by the Food and Agricultural Organization of the United Nations, the World Bank and the United Nations Development Programme; and it consists, in addition, of 32 governments, international organizations and private foundations.

Since 1973 CIP has invited 112 experts from 27 countries to attend Planning Conferences to assist in developing five-year plans of research within specific research thrusts or goals. The general Conference strategy has been to utilize invited experts to assist in structuring CIP's research activities.

The Participants at a Conference have a mandate to develop a plan of action, including the identification of priorities. The plan must have a satisfactory balance of research activities between those requiring a long-term effort before breakthroughs can be anticipated and those requiring a shorter term before desired results can be expected. Five year guidelines for CIP activities take into consideration research being done or planned at other institutions.

This Planning Conference on "Developments in the Control of Potato Viruses" is the first Conference devoted to viruses. Prior to this Conference, CIP had designated potato leaf roll virus and potato virus Y as being important constraints to potato production in developing countries. In addition, the identification and characterization of novel viruses, as well as the spindle tuber viroid, are of significance in the international exchange of genetic material.

O. T. Page

November, 1977

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The following members of CIP staff also attended the Planning Conference:

Dr. E. French	Head, Pathology Department
Eng. C. Fribourg	Virologist
Dr. D. Horton	Head, Social Science Unit
Dr. R. Jones	Virologist, Thrust Co-ordinator
Dr. A.M. de Lekeu	Virologist
Dr. H. Mendoza	Head, Breeding & Genetics Dept.
Dr. O. T. Page	Director of Research
Dr. L. Salazar	Virologist
Dr. R. L. Sawyer	Director General

RECOMMENDATIONS

In recognition of the problem in potato growing all measures taken not resulting in extreme resistance are of a temporary nature only. Therefore, breeding for resistance should be of the highest priority in any attempt to control virus diseases of the potato.

Recommendations are based on the order of priorities set out in Table 1.

A BREEDING

PLRV

1.1 Best known PLRV resistance breeding lines and varieties should continue to be collected and put into comparative trials in different and suitable geographic areas. Main breeding should be done via population genetic principles with 4x types.

However, it is felt that 2x and 2x - 4x breeding will yield advantages which need to be explored in the coming five years.

PVY - PVA

2.2 Continue breeding for PVY - PVA resistance should be based on selection of characters for extreme resistance.

TABLE 1

ASSIGNMENT OF PRIORITIES

		Breeding	Testing	Research	Novel Approaches	
1	PLRV	1	1	1	True seed	Cross protection
2	PVY-PVA	2	4	3	True seed	Cross protection
3	PVX	3	5	5	True seed	Cross protection
4	PSTV	4	2	2	True seed	DNA sequences
5	New Viruses	5	3	4	True seed	---

PVX

3.3 Continued breeding for resistance to PVX should be based on selection of characters for extreme resistance.

PSTV

4.4 A screening program for resistance to PSTV should be initiated only after reliable detection methods are developed.

New Viruses

5.5 After evaluating the importance of newly detected potato viruses, searches for resistance should be initiated according to the assigned importance of the respective viruses.

B TESTING

Reliance on the outcome of one testing procedure in biological systems creates unacceptable risk for any potato program. Principally, different and complementary methods like serology, electron microscopy and the study of indicator plants should be utilized and ought to be an integral part of the program.

1.1 In testing for PLRV, present best methods should be used with the highest priority given to the development of reliable new methods (See Research).

Present best methods are considered to be:

1. Infection of sprouts and seedlings by viruleferous aphids.

2. Field exposure tests in suitable sites (high aphid population).

3. Appropriate and applicable indicator host testing.

2.4 & 3.5 PVY - PVA and PVX

Methods used at this time seem to be adequate and should be continued until such time that research will provide more reliable and economical procedures.

4.2 PSTV

One or a combination of the current testing methods for PSTV (Scopolia, tomato, electrophoresis) should be incorporated into a testing program for PSTV until such time that either a better method is available or one of the above is shown to be superior.

5.3 New viruses

Current programs do not call for expansion.

C RESEARCH

In fulfilling the charge of CIP in breeding and dissemination of virus-free material, the greatest research effort should be directed to improvement and application of new developments.

1.1 PLRV

a. Purification of PLRV and antiserum production (ELISA).

b. Searching for better or stronger types of resistance among wild species and primitive cultivars.

c. Test the feasibility of the callose test under Peruvian conditions.

2.3 PVY - PVA

a. Updating and continual development of serological techniques toward more economical and refined methods for detection.

b. Aberrant virus Y strains detected should be tested with regard to their ability to break R_y resistance.

3.5 PVX

a. Testing of aberrant strains against the different genes for resistance.

b. Updating and continual development of serological techniques towards more economical and refined methods for detection.

4.2 PSTV

a. A search for indigenous isolates of PSTV in the Andean highlands should be initiated.

b. Finding a reliable method for detection of PSTV is of main importance.

c. Development of methods to free important infected clones from PSTV.

d. A screening program should be initiated to pinpoint possible resistance in the present available germplasm.

5.4 New viruses

a. Characterization

D NOVEL APPROACHES

a. We would like to restate the recommendation of the Planning Conference on the "Utilization of Genetic Resources" that:

The main solution to virus problems in potatoes may well be the utilization of botanical seed to produce crops on a farm scale. The short range approach would be to find heterozygous parents giving a rather uniform progeny. The long term approach would be the usage of homozygous parents created via monohaploids.

b. A serious attempt in the form of a pilot trial should be made to evaluate the use of cross protection as a short term temporary solution to PLRV and PVX-PVY infection related problems.

c. Encouragement of the study of PSTV through the establishment of innovative pilot projects regarding for instance, the possible importance of DNA sequences in solanum species (or their absence) complimentary to PSTV-RNA.

d. An evaluation should be initiated to elucidate the possibilities of hybridization of S. etuberosum in order to make use of PLRV immunity present in this species.

GENERAL RECOMMENDATIONS

1. The pilot projects referred to under "Novel Approaches" do not necessarily have to be carried out at CIP but should be encouraged elsewhere.

2. It is the considered opinion of the participants of the Planning Conference that additional greenhouse facilities with environmental control possibilities be provided for the indicator testing program to alleviate light, temperature, dust and virus isolation problems.

3. The members of the Planning Conference feel that vector research is recognized as an important element of the virus program.

4. We endorse the suggestion in the five year review concerning the tentative status of the serologist. It is suggested here that a courtesy appointment be extended to C. E. Fribourg to consolidate the excellent relationship now existing.

5. The participants of the Planning Conference feel that the work outlined as having high priority can be accomplished only with a group of approximately 5 competent research scientists.

   Provisions should be made to maintain this competence in the future.

Centro Internacional de la Papa

PLANNING CONFERENCE ON DEVELOPMENTS IN THE CONTROL OF POTATO VIRUSES

AGENDA

Monday, November 14

	Chairman morning session. Dr. O. Page
08:30	Welcome and opening remarks. Dr. R. Sawyer
09:00	Tour of CIP's facilities for virological work. Dr. E. French
10:15	Progress in PLRV resistance work. Dr. R. Jones
10:50	Progress in PVY resistance work. Dr. L. Salazar
11:25	Progress in antiserum production and present status of tests for viruses in CIP's seed program. Eng. C. Fribourg

12:00	Lunch

Monday afternoon

	Chairman afternoon session. Dr. J. de Bokx
	Progress in characterization of new and little known viruses and virus strains from the Andean region.
13:30	PLRV, APLV, WPMV. Dr. R. Jones
14:00	PVX, TRSV, APMV. Eng. C. Fribourg
14:30	Coffee
14:50	PYVV, PVT, 14R. Dr. L. Salazar
15:20	Quinquennial Review Mission Report on Viruses. Dr. O. Page

STRATEGY IN BREEDING FOR RESISTANCE TO PLRV, PVY AND PVX

Tuesday, November 15

	Chairman morning session. Dr. H. Ross

08:30	Potential importance of virus resistance to small farmers in developing countries. Dr. D. Horton
09:20	Sources of resistance. Dr. W. Hooker
10:10	Coffee
10:30	Population breeding for accumulation of virus resistance genes. Dr. H. Mendoza
11:20	Discussion
12:00	Lunch

Tuesday afternoon

	Chairman afternoon session. Dr. E. French
13:30	Field trials for virus resistance. Dr. T. Davidson
14:40	Rapid and efficient methods for screening for resistance in seedlings and clones. Dr. H. Ross
15:30	Discussion

IMPROVED TECHNIQUES FOR DETECTION OF POTATO VIRUSES

Wednesday, November 16

	Chairman morning session. Dr. R. Gamez
08:30	Detached leaf methods and new indicator species. Dr. J. de Bokx
09:15	Histological staining methods. Dr. D. Peters
10:00	Coffee
10:20	Electrophoresis. Dr. R. Singh
11:10	Electron microscopy. Dr. G. de Zoeten

Wednesday afternoon

	Chairman afternoon session Dr. G. de Zoeten
13:30	New ultrasensitive serological techniques. Dr. R. Koenig

14:30	Coffee
14:50	A simplified radial-diffusion technique. Dr. A. M. de Lekeu
15:20	Discussion

FORMULATION OF RECOMMENDATIONS

Thursday, November 17

A committee of three will formulate recommendations arising from presentations and discussions during the first three days of the conference.

Participants not involved in developing recommendations will have the opportunity to visit CIP facilities in Huancayo.

APPROVAL OF RECOMMENDATIONS

Friday, November 18

AM-PM	Final discussions and approval of recommendations.
	All participants.

WELCOME AND OPENING REMARKS

Dr. Richard L. Sawyer

I am very sorry that I am unable to participate in the opening of this International Planning Conference on virus work at CIP. However, since the International Planning Conference have played such a vital role in CIP's program, I have written a few words to indicate what I feel is your general mandate.

One of the major tools that CIP has utilized to get its roots into as many institutions around the world as possible has been International Planning Conferences. This constant exposure of CIP's program to senior exterior experts has been commended by the Consultative Group on International Agricultural Research. The value of these International Planning Conferences was recently recognized by the CGIAR-TAC Quinquennial Review of CIP by a panel of exterior experts.

I personally appreciate the help that all of you attending are giving us. I particularly hope that you will find a selfish reason for associating your own scientific programs and your institutions with that of the International Potato Center.

We have recently finished with a 5 year exterior over-all review which has implications on your deliberations here. There can be no major change or shift in the general program we are presently conducting without an adequate exposure of the suggested changes to the Technical Advisory Committee of the Consultative Group on International Agricultural Research and a recommendation and submission to the CGIAR for funding. Thus, I suggest that your considerations for the coming week should start with an exposure to the comments on Thrust V in the Quinquennial Review. The complete comments have been made available to you and I strongly urge each of you to read them.

There is still considerable room for consolidation and improvement of the present program. That is your basic reason for being here. The period of institutional growth that takes place by increasing numbers in both staff and budget, is now over at CIP. The growth which takes place through refinement and improvement of the quality of program through identification of, and concentration on priorities is now here.

I thank each of you for taking the time from your busy schedules to come and help us. I hope that in the process of helping us you will feel you are helping yourselves and your institutions.

Once again, I am sorry that I will not be a part of your deliberations. I will be back to participate in the Thursday - Friday sessions and meet with you and renew acquaintances with many who have helped us in past years.

PROGRESS IN LEAF ROLL VIRUS RESISTANCE WORK AT CIP

R. A. C. Jones

Potato leaf roll virus (PLRV) is the most important virus disease affecting potato crops in developing countries. It is particularly troublesome in tropical and sub-tropical areas where aphid populations tend to be extremely high throughout the year. A major aim of CIP work is toward improving the yield of small peasant farmers in warmer areas whose potato crops invariably suffer from this virus and who cannot afford to plant healthy seed. Resistance is considered to be the most appropriate means of control in the long term, but no source of immunity to PLRV is known, only infection resistance (relative insusceptibility to transmission by aphids) which is polygenically inherited. A form of hypersensitivity is also available (6, 7). The virus is difficult to work with because it occurs at low concentration in the plant, is not mechanically transmissible and is not amenable to serology. Test methods are unreliable (histological staining) or laborious (aphid transmission or grafting) (5).

At present, the main aims of CIP's PLRV resistance work are:

1. To create potato populations with high levels of infection resistance.

2. Improvement of techniques for screening clonal and seedling material on a large scale for resistance to PLRV emphasizing labor saving and decreased virus exposure and detection times.

3. Detection of new sources of infection resistance in the CIP germ plasm collection and search for possible sources of immunity to the virus in wild Solanum species.

4. To establish cooperative resistance evaluation and breeding programs with national potato programs in developing countries.

The work reported here which was started in 1974 was done partly in cooperation with J. Franckowiak and partly with C. Chuquillanqui.

Breeding

Two potato populations were established for developing of leaf roll infection resistance through phenotypic recurrent selection, one tetraploid and one diploid. Each has so far been taken through 1 cycle of selection and crossing. The material with PLRV resistance incorporated into these populations was from two sources (Table 1): (1) True seed of crosses from breeding programs in Germany diploid and tetraploid material), Britain, Holland and Canada (tetraploid material). (2) Some crosses made from clones in a very complete collection assembled at CIP of breeding lines and cultivars with high levels of resistance from breeding programs in eight different countries in South America, North America and Europe. However, much less use of this collection could be made than was originally hoped because of poor flowering of Tuberosum types under our short day conditions. The other major source of crosses for the two populations was the CIP germ plasm collection. This material was included mainly to increase genetic diversity and had not been previously tested for PLRV resistance. Crosses with Neo-tuberosum material (originally from Cornell University, USA) with single gene resistance to PVY + PVA and to PVX were also incorporated into the tetraploid population.

Resistance screening techniques

In 1974 and 1975, large scale field exposure trials were done on the coast at La Molina using germ plasm clones but in both years aphid populations were too low to permit a reasonable test for PLRV resistance. Similarly, in the Andean highlands at Huancayo, the aphid population is too low. The only CIP experimental site in Peru that has sufficient aphid population is in the high jungle at San Ramon.

TABLE 1

SOURCES OF LEAF ROLL RESISTANCE OBTAINED BY CIP

COUNTRY *	TRUE SEED	TUBERS
ARGENTINA		+
BRAZIL		+
BRITAIN	+	+
CANADA	+	+
COLOMBIA		
GERMANY	+	+
HOLLAND	+	
PERU (CIP GERM PLASM)	+	+
U S A		+

* Material sent by breeding programs in these countries.

However, although the traditional field exposure trial method (2) at this site may be suitable for testing small amounts of selected material, it is not suitable for development of large populations by recurrent selection. It is very laborious, over a year is needed to get results and only few clones can be tested at any one time. A study of alternative procedures which permit efficient screening of large amounts of material and emphasize both labor saving and decreased virus exposure and detection times was, therefore, initiated.

When potato seedlings (5 - 10 cm high) growing in jiffy pellets were exposed to PLRV - carrying aphids (Myzus persicae) for 2 - 3 weeks in a large cage, treated with insecticide and transplanted to the field, many developed typical secondary symptoms of PLRV. The following routine was, therefore, used for screening the two CIP PLRV-resistant populations in which up to 6,000 seedlings were tested at the same time in a large screenhouse compartment. Seedlings were transplanted to jiffy strips, the different families arranged in rows in replicated blocks and cut leaves taken from PLRV - infected potato source plants heavily colonized by aphids placed on top of them in a regular fashion. After 2 - 3 weeks exposure, insecticide was applied, the seedlings transplanted to the field, readings for PLRV symptoms made and relative levels of infection resistance in the different families estimated. Secondary rather than primary PLRV symptoms again developed. However, losses of seedlings in the field were high and this hindered symptom evaluation. These losses seemed not simply due to the fungus Rhizoctonia solani which is prevalent in soil in Peru because soil treatment with Pentachloro nitrobenzene (PCNB) when seedlings were planted had little effect.

By contrast, survival in the field was high when clonal material was tested by substituting rooted cuttings growing in jiffy pots for the seedling families and using exactly the same procedure. Again, secondary symptoms developed directly. Table 2 gives the results of trial in which six Peruvian cultivars were tested in this way. Percentages of plants with secondary symptoms ranged from 95 in the highly susceptible cultivars Merpata to 24 in the relatively resistant cultivar Mariva. Exposure of cuttings, therefore, seems feasible as a rapid method for screening clones for PLRV infection resist-

TABLE 2

SYMPTOM EXPRESSION (%) IN ROOTED CUTTINGS EXPOSED TO PLRV BEFORE PLANTING IN THE FIELD*

CULTIVAR	% WITH SYMPTOMS
MARIVA	24
CCOMPIS	25
REVOLUCION	64
MI PERU	66
RENACIMIENTO	92
MERPATA	95

* Cuttings growing in jiffy pots arranged in cultivar rows in four replicates, exposed to infective aphids for 2 weeks and treated with insecticide before going to the field; between 75 and 95 plants of each cultivar survived out of the original 100 cuttings of each.

ance. However, when a tuber was harvested from each of the normal looking plants of Ccompis in the trial in Table 2 and the tubers replanted, 23% of plants showed typical secondary symptoms. It may, therefore, be necessary also to harvest a tuber from each of the cuttings which do not show symptoms, plant these and record any secondary symptoms which develop, because some cuttings may be symptomslessly infected.

Because loss of plants after transfer to the field was a drawback when the method described above was used with seedlings, the possibility of exposure to infective aphids at an earlier stage of growth was explored to try to completely avoid going to the field. In a preliminary test, tiny seedlings from open-pollinated seed of two cultivars were exposed directly in seedling trays, treated with insecticide and transplanted to jiffy pots. Distinct symptoms consisting of an upright habit, chlorosis of upper leaves, rolling of lower leaves and/or purpling of leaf undersides (i.e. typical secondary symptoms) developed in 30/33 and 7/44 seedlings of Renacimiento (susceptible) and Ccompis (resistant) respectively.

Larger scale tests were, therefore, done in which open-pollinated seed of cultivars was sown in rows in large trays in four replicated blocks. Seedlings (1-5 cm high) were exposed individually by placing infective aphids directly onto each seedling, treated with insecticide after 2 weeks, transplanted to jiffy pellets and readings made periodically for symptoms. Table 3 gives the results of two trials of this kind. Distinct symptoms developed in both and there was good agreement between the two in percentages of seedlings with symptoms obtained with the different cultivars. Renacimiento had the highest percentage of seedlings with symptoms and Hualash the lowest in both. Also, the behaviour of the cultivars Ccompis, Merpata and Renacimiento was comparable when tested either by cuttings (Table 2) or using true seed (Table 3).

Exposure of tiny seedlings before transplanting, therefore, looks very promising as a method of screening for resistance. It gives great savings in time and labor and avoids loss of plants through transfer to the field. The possibility that some seedlings may

TABLE 3

SYMPTOM EXPRESSION (%) IN POTATO SEEDLINGS EXPOSED TO PLRV BEFORE TRANSPLANTING TO JIFFY PLOTS[a]

CULTIVAR	EXPT. 1	EXPT. 2
HUALASH	6 (12)[b]	11 (14)
YANA IMILLIA	13 (26)	16 (20)
CCOMPIS	12 (23)	17 (21)
HUAGALINA	-	21 (26)
CASA BLANCA	-	22 (27)
MI PERU	39 (75)	66 (82)
MERPATA	43 (83)	78 (96)
RENACIMIENTO	52 (100)	81 (100)

a. Seed sown in cultivar rows in replicated blocks, three replications with Expt. 1 and four with Expt. 2. Seedlings were exposed to infective aphids for 2 weeks and then treated with insecticide before transplanting to jiffy pellets; numbers of seedlings per cultivar ranged from 133-162 with Expt. 1 and 82-234 with Expt. 2.

b. Numbers are percentages of plants with symptoms. Percentages calculated taking Renacimiento figure as 100% in parentheses.

become infected symptomlessly remains, but this is also the case with all other methods of testing for resistance to leaf roll and individual back-testing of seedlings using aphids and Physalis floridana is far too laborious to be practical when large numbers are involved (4).

Preliminary tests in which the sprouts on sprouted tubers were directly exposed to infective aphids resulted only in low levels of infection. However, further tests are needed to investigate this as a possible alternative rapid method to use for screening clonal material for resistance. Small scale trials using the resorcinol method for direct detection of PLRV in tubers (1) showed that this technique is not a useful alternative to taking symptom readings in plants because healthy tubers of some clones developed abundant callose in the phloem.

New Sources of Resistance

Evaluation of some Peruvian cultivars belonging to the CIP germ plasm collection by the methods described above has indicated that there may be considerable infection resistance in native Andigena cultivars. For example in Table 3, the five native cultivars examined (Hualash, Yana Imillia, Ccompis, Huagalina, Costa Blanca) seemed much more resistant than the three produced by the Peruvian breeding program (Mi Peru, Merpata, Renacimiento). Infection resistance to PLRV may have played an important role in their survival as cultivars. This potential source of infection resistance genes may prove valuable for breeders when combined with the Tuberosum sources normally used in breeding for resistance to this virus.

Solanum brevidens never developed virus symptoms when kept over a period of many years under optimal conditions for virus spread in collections of potato germ plasm in England. It was, therefore, tested at CIP for immunity to PLRV (3). When four plants of S. brevidens (unknown seed source) were grafter with PLRV - infected potato scions, the grafts left for at least a month and then

cut off, no PLRV was detected in them by aphid transmission to P. floridana. Similar results were obtained in further tests with two plants of S. brevidens (PI 218228) and five of the related species, S. etuberosum (PI 245924). However, in similar tests with S. fernandezianum (PI 320270) the virus was recovered from one out of six plants. These results suggest that these species may be a source of immunity to PLRV and further tests are in progress to provide more evidence of this. No PLRV immunity genes are available for potato breeders so these three species might prove particularly valuable if suitable crosses can be made.

International Trial

Table 4 lists the developing countries to which leaf roll resistant material has been sent for cooperative trials between their national potato programs and CIP and for their use in breeding cultivars resistant to this virus and suited to local conditions. The material sent was in the form of true seed (crosses), tubers or multimeristem cultures (breeding lines and/or cultivars).

True seed is sent out as part of an international program of field exposure trials for testing segregating populations from crosses with PLRV resistant parents. Seed of twenty five different crosses is sent accompanied by data sheets and a field book giving detailed instructions on the different steps to follow and on the experimental design (a balanced lattice square with six replications). The site must be one with a long history of high leaf roll incidence. If available, leaf roll infector source plants are interplanted with the plots. So far this trial has gone out to Argentina, Brazil, Chile, Egypt, South Korea, Pakistan and Turkey. The seed has included mainly crosses from the CIP breeding program and crosses received from breeding programs in Europe and North America. The advantages of this international trial are that it: (1) enables evaluation of a uniform group of genetic material at testing sites with widely differing conditions, (2) serves as a vehicle for moving materials to national programs and (3) establishes a basis for further cooperation between CIP and national program investigators in breeding for virus resistance. It is hoped that from the information obtained CIP will be in a position to send out an improved set of PLRV resistant crosses each year.

Initial results have shown that provided infection occurs soon after transplanting to the field secondary symptoms developed directly and harvest of tubers for evaluation of secondary symptoms may not be necessary. The main problems encountered were poor seed germination, infection with PVY and loss of seedlings in the field due to damping off and insect damage. Gibberellic acid is now sent out with the seed to help with germination and it is hoped that planting in the field in polythene bags will help against loss of seedlings through damping off. An alternative that has been suggested to avoid loss is that CIP send out tuber families (i.e. seedling tubers, one per seedling) instead of true seed.

So far requests for resistant clones and cultivars for breeding purposes have been met with twelve different developing countries (Table 4). Material sent has been from three main sources; the CIP seed program (which now has eight PLRV resistant clones available), the Scottish Plant Breeding Station (in cooperation with Dr. T. Davidson) and the Max Planck Institute, Germany (in cooperation with Dr. H. Ross). The material is being propagated in these twelve countries and a field book is in preparation at CIP, so that these clones can be also used in some of them for cooperative international field exposure trials for PLRV resistance with clonal material, like the ones already in operation for testing seedlings.

TABLE 4

DEVELOPING COUNTRIES TO WHICH CIP HAS SENT LEAF ROLL RESISTANT MATERIAL

Country	True Seed International Trial	Breeding Lines and/or Cultivars
ARGENTINA	+	+
BRAZIL	+	+
CHILE	+	+
COLOMBIA		+
COSTA RICA		+
EGYPT	+	+
KENYA		+
KOREA	+	+
LEBANON		+
MEXICO		+
PAKISTAN	+	
SRI LANKA		+
TURKEY	+	+

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SOURCES OF RESISTANCE TO PVY

L. Salazar

The search for resistance to PVY among clones in the CIP germ plasm began in 1972. The initial steps were directed toward the identification of PVY-free clones assuming that they had greater probabilities to contain high levels of resistance. The reason for this assumption were:

a) Most of the clones had originally been collected in the field or propagated vegetatively for many years. This fact increased their chances to become infected with PVY. Therefore, the PVY-free clones either had a resistance to the virus or had escaped infection. The first assumption seemed more plausible in view of the rapid spread of PVY in vegetatively propagated material.

b) In the Andean Region potato virus Y occurs as many variants differing in virulence and other properties. Evidence for this point was obtained from studies on the variability of the virus. From about 184 field isolates obtained in Peru 5 groups were defined by their reactions in some selected indicator hosts, serology and symptoms in some Peruvian potato varieties. The tobacco veinal necrotic strain (PVYN) was very common in the south of the country - whereas the common strain (PVY °) occurred in almost all the areas surveyed. Three other groups were found scattered in the country and in some clones in the CIP germ plasm, but were less studied than the common and necrotic strains. Furthermore, evidence for the existence of some odd isolates of PVY in some germ plasm clones was obtained, but they were not further studied.

By the use of serology (microprecipitation test) in some instances indicator hosts, 800 clones were found free of PVY. These clones were exposed to infection in 1973 in Arequipa (Southern Peru) where high levels of aphid populations had been recorded in potatoes. Clones were interplanted with previously planted PVY infected tubers in alternate rows. Symptoms were not recorded because most of the clones were infected with other viruses. At harvest tubers were obtained from 573 clones (the others did not tuberize under the climatic conditions of Arequipa) to be tested for PVY infection.

Continuation of the project

This project was conducted by Dr. A.M. Hinostroza de Lekeu from 1974. She found that only 41 clones (List 1) from the trial in Arequipa were free of PVY. Since in a large number of clones infection was only assessed by symptomatology, there remains the possibility of finding more clones without PVY infection.

Some of the 41 clones were used as initial breeding material and clonal families were developed (list 2).

List 1

CLONES WITH HIGH PROBABILITY OF RESISTANCE TO PVY

1)	700005	adg.	21)	701200	Eliminated
2)	700024	adg.	22)*	701209	adg.
3)	700075	adg.	23)	701213	adg.
4)	700138	adg.	24)	701225	adg. (Res. x)
5)	700455	adg. (Res. x)	25)*	701299	adg. (Res. x)
6)	700528	adg.	26)*	701374	adg.
7)*	700530	adg.	27)*	701400	adg.
8)	700575	adg. (Res. x)	28)	701442	adg.
9)	700682	adg.	29)	701535	adg. (Res. x)
10)	700686	Eliminated [1]	30)	701752	Eliminated
11)	700729	adg.	31)*	701758	adg.
12)*	700754	adg.	32)	701761	adg.
13)	700775	adg. (Res. x)	33)	701860	adg.
14)	700787	adg.	34)	701940	adg.
15)	700854	Eliminated	35)	702072	adg.
16)	700862	adg.	36)	702081	adg.
17)*	700980	adg.	37)*	702089	adg.
18)	701026	adg.	38)	702101	adg.
19)	701122	adg.	39)	702114	Eliminated
20)*	701171	adg.	40)	702174	adg.
			41)*	702245	adg.

* Clones selected as starting breeding material.

[1] Eliminated from CIP germ plasm.

List 2

	Clones	Tubers
1.	701209.1	5
2.	701299.1	5
3.	701299.2	5
4.	701299.3	4
5.	701374.1	6
6.	701374.2	4
7.	701374.3	3
8.	701374.4	2
9.	701400.1	7
10.	701400.2	15
11.	701400.3	6
12.	700530.1	8
13.	700754.1	5
14.	701171.1	6
15.	702245.1	5
16.	702245.2	3
17.	702089.1	4
18.	701758.1	5
19.	701758.2	5
20.	701758.3	9
21.	701758.4	6
22.	701758.5	6
23.	701758.6	7
24.	701758.7	4
25.	701758.8	5
26.	701758.9	3
27.	701758.10	8
28.	701758.11	5
29.	701758.12	7
30.	701758.13	8

	Familia 32	Tubers
31.	700754 x 700980	6
32.	700754 x 700980	2
33.	700754 x 700980	4
34.	700754 x 700980	1
35.	700754 x 700980	2

	Familia 18	
36.	T 157 x PVY bulk	1
37.	" "	2
38.	" "	2
39.	" "	1

	Familia 21	
40.	T 199 x PVY bulk	9
41.	" "	9
42.	" "	3
43.	" "	1

Familia 8		Tubers
44.	700754 x Neo Tub. Bulk	2
45.	" "	3
46.	" "	3
47.	" "	3
48.	" "	1
49.	" "	2
50.	" "	2

ANTISERUM PRODUCTION AND PRESENT STATUS OF TESTS FOR VIRUSES IN THE CIP SEED PROGRAM

C. E. Fribourg

Antiserum project

This project has the following objectives:

1. To produce antisera to the commoner potato viruses in sufficient quantities for the following uses: (a) distribution to national potato programs in developing countries, (b) for the routine virus checks in the CIP seed program and in its virus thrust work, (c) for studies on improved serological techniques for efficient detection of viruses on a large scale in seed programs.

2. To produce antisera to the new or little known viruses affecting potato.

Up to the present antisera have been prepared against isolated from the Andes of the following viruses:

- Potato virus X, strains C and HB
- Potato virus Y
- Potato virus S
- Andean potato latent, strain Hu
- Andean potato mottle, strain type
- Tobacco ringspot, strain Calico
- Wild potato mosaic

In most instances the purification procedure used involved use of Triton (5) in combination with polyethylene glycol precipitation (3) and 2 cycles of differential centrifugation. Immunization of rabbits was by intravenous followed by intramuscular injections.

These sera have been used in large amounts by the CIP seed program, the virus thrust and for teaching trainees from several different countries who come to CIP to learn virus identification techniques. They have also been used to standardize the microprecipitation technique for our seed program and more recently to develop the latex test to suit our conditions.

Large quantities of sera have been distributed to national potato programs in developing countries. During the period 1975-1977, they were sent to 17 different national programs, and in some cases repeat sendings were requested (Tables 1 and 2). PVX, PVY and PVS sera were the ones most frequently sent but some requests were also received for Andean potato viruses especially APLV.

Table 1 Number of instances that antisera have been distributed by CIP to countries in Latin America (1975-1977).

Country	PVX	PVY	PVS	TRSV	APLV	APMV
Bolivia	1	1	1	1	1	1
Brazil	2	1	2	1		
Chile	1	1	1	1	1	
Colombia	2	2	2		1	
Mexico	3	2	4	1	2	
Paraguay	1					
Peru	3	3	3	1	3	
Venezuela	2	2	2		1	
Total	15	12	15	5	9	1

Table 2 Number of instances that antisera have been distributed by CIP within Latin America and to developing countries in other continents (1975-1977).

Country	PVX	PVY	PVS	TRSV	APLV	APMV
Latin America	15	12	15	5	9	1
India					1	
Korea	2	2	2		2	1
Nepal	1	1	1			
Philippines	1	1	1			
Thailand		1				
Tunisia	1	1				
Turkey	2	2	2			
Zambia	1	1				
World Total	23	21	21	5	13	2

The sera are sent in concentrated form mixed with glycerine and sodium aside as preservative. Normally sufficient quantity for 1,500 - 2,500 tests with each serum are sent accompanied by information sheets detailing the methodology used for microprecipitation and gel diffusion testing. Requests have also been supplied from some developed countries: for APLV (Canada, USA, Hungary) and for PVX, PVY, PVS, TRSV and APLV from Britain and Holland.

Seed Program Virus Checks

The CIP seed program now contains about 80 clones that have either been cleaned up using meristem culture or have been found healthy. These are distributed to developing countries all over the world in

the form of multimeristem cultures, rooted cuttings or tubers. Very high standards of virus testing are therefore essential to avoid export of virus infected material. It is especially important not to spread Andean strains of well known potato viruses or Andean potato viruses, at present restricted to this region, to other regions of the world. With this in mind a schedule of virus checks was designed in cooperation with R.A.C. Jones. It has been used now for three years since the seed program started in 1975.

The program has three main steps: Gen O constitutes the initial phase where clones and cultivars are received into the program. Those found to be healthy pass directly to Gen 1, but those infected first go to meristem culture for cleaning up and then into Gen 1. Generation 1 is where the nuclear material is kept, normally only two pots of each clone in the program are present at any one time. From Gen 1 material is fed into Generation II where large scale propagation by the stem cutting method is practiced.

Fig. 1 shows the checks done in the different steps of the program. In Gen O each plant found to be healthy either by the direct or meristem culture routes is individually tested twice with a full range of indicator hosts and three times by serology with sera to PVX, PVY, PVS, APLV and APMV.

The different checks are always done at least one month apart. In Generations I and II the plants each receive similar checks, two with a full range of indicator plants and two with serology; in Generation I samples are combined from up to two plants within a clone and in Generation II up to eight/clone.

The range of indicator hosts used routinely is for each sample under test shown in Table 3. It is designed to detect as many potato viruses as possible. For PVY and PVA we use Nicotiana glutinosa and N. tabacum. Clon A6 is not used routinely but for confirmation. These Nicotianas will also detect a PVX strain recently detected in Bolivian potatoes that does not induce local lesions in Gomphrena globosa. Nicotiana debneyi will detect the majority of PVS strains and some

Fig. 1. Testing of material being produced for multimeristem cultures, rooted cuttings and/or tubers for export.

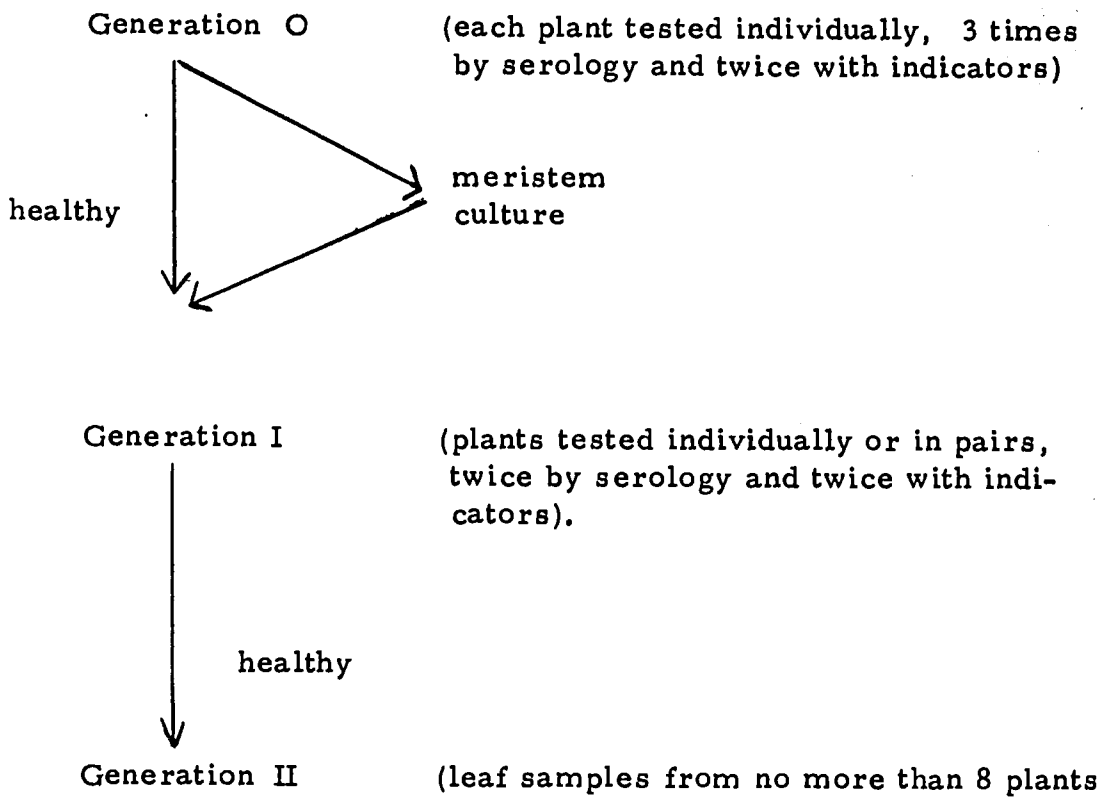

TABLE 3 Range of indicators plants used for detecting potato viruses in the CIP seed program.

Species	Viruses
Chenopodium quinoa-*C. amaranticolor*	PVS, TRSV, PVT, TRV, PMTV (PVM)
Gomphrena globosa	PVX, (PVS, PVM)
Nicotiana glutinosa - *N. tabacum*	PVA, PVY, PAMV (PVX)
N. debneyi	(PVM, PVS)
N. bigelovii - *N. clevelandii*	APLV, APMV

strains of PVM. Chenopodium quinoa and C. amaranticolor will detect PVS, TRSV, PVY, TRV, PMTV and some strains of PVM. Gomphrena globosa will detect the majority of PVX strains and also some strains of PVS and PVM. Nicotiana bigelovii and N. clevelandii are used to detect APLV and APMV. Neither of these two viruses can be detected reliably with any of the other indicators.

Originally for the serological tests we used microprecipitation (1) for PVX, PVY and PVS and gel diffusion for APLV and APMV. The microprecipitation, is done by grinding tissue discs (Cork borer N° 8) in 3% 2-mercapto ethanol in the proportion of 0.1 ml/disc followed by a low speed centrifugation (6,000 rpm/15 min). Drops of sap and antiserum are placed on plastic petri plates and mixed using a mechanical shaker.

In August of 1977 we changed over to the latex method (2,4) which is now used for all five viruses replacing both microprecipitation and gel diffusion (Fig. 2). It is similar to microprecipitation, but we have to add a higher amount of water for grinding the tissue discs (0.2 ml/disc). An additional dilution of 1/10 of this is also included for testing, for greater reliability. After low speed centrifugation drops of sap and sensitized latex are placed on plastic plates, and mixed in the shaker. To facilitate the mixing we add 3% trition or 0.05% tween 20 to the grinding medium. This test has the advantages of being quicker and easier to read. It uses less amount of antisera and it is about 20 to 100 times more sensitive than micropercipitation.

We feel that these tests are extremely through and fulfill the needs for preventing spread of andean viruses outside the region. The only known pathogens not included are spindle tuber viroid and leaf roll virus for which tests are very time consuming. During the 3 year period the viruses detected in Gen 0 have been PVX, PVY, PVS, PLRV, APLV and APMV. PVM, PVY and PVA have not been found. Contaminations in Generations I and II have been very rare, in 1975 one case of APLV, in 1976 one of PVS and in 1977 some cases of PVX. Contaminated plants were inmediately eliminated.

Fig. 2. Serological techniques used for virus testing in the CIP seed program.

Microprecipitation	Latex
Tissue discs ground in 3% 2-mercaptoethanol (0.1 ml/disc).	Tissue discs ground in 3% 2-mercaptoethanol + 3% triton X-100 or 0.05% Tween 20 (0.2 ml/disc)
↓	↓
Centrifugation at 6,000 rpm/15 min.	Centrifugation at 6,000 rpm/1r min.
↓	↓
1 drop of supernatant + 1 drop of A/S in plastic petri plates.	1 drop of supernatant + 1 drop of sensitized latex in plastic petri plates.
↓	↓
Shaker 15'	Shaker 15'
↓	↓
Observation under stereoscopic microscope after 2 hours.	Observation immediately, visual or under stereoscopic microscope.

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POTATO LEAF ROLL VIRUS (PLRV)

R.A.C. Jones

Although McKee (2) showed that infection with PLRV may be common in potatoes in Andean countries, the rolling of lower leaf symptom typical of secondary infection with this virus in plants of Tuberosum potatoes has not been recorded in plants of Andigena potatoes growing in the region. By contrast, this symptom is often present in plantings of Tuberosum x Andigena cultivars.

When fields of the Andigena cultivar Renacimiento were examined in 1974 in the Peruvian coastal valley of Cañete, no typical secondary PLRV symptoms were seen. However, a proportion of plants in several fields showed symptoms consisting of marginal and interveinal chlorosis of leaflets, a marked upright habit of growth and stunting. Although not previously recorded in Peru, these symptoms are widespread in potatoes in Colombia where they have been known for over 25 years as "enanismo amarillo", a serious disease of unknown cause which is transmitted through seed potato tubers (1, 3). Preliminary tests on plants of Renacimiento with "enanismo amarillo" revealed infection with PLRV. A thesis project was, therefore, initiated with A. Rodríguez to (i) determine whether "enanismo amarillo" disease might constitute the typical response of Andigena to secondary infection with this virus or (ii) whether the disease might be caused by an unusual strain of PLRV from the Andean region. Information on the incidence of "enanismo amarillo" in Peru was also sought (4, 5).

Incidence

In 1975, a survey was made of the incidence of "enanismo amarillo"

in fields of the cultivar Renacimiento in the three principal seed potato growing regions of the highlands of central Peru and the two most important coastal valleys for potato production (Table 1). Fields of the Tuberosum x Andigena cultivar Ticahuasi were also examined and the incidence of typical secondary leaf roll symptoms recorded. This cultivar is very susceptible to PLRV and plants with secondary infection show distinct rolling of lower leaves.

' Enanismo amarillo" disease was present in Renacimiento crops in each of the regions. Incidences of the disease were highest in the Huanuco valley and Chaglla and on the coast, reaching a maximum of 46% in one field. Parrallel results were obtained for PLRV symptoms in crops of Ticahuasi but percentages of affected plants tended to be somewhat greater.

Association with PLRV

PLRV was detected in samples from "enanismo amarillo" diseased Renacimiento plants in each of the regions listed in Table 1, by aphid transmission to Physalis floridana seedlings. It was also found in samples from diseased plants of each of the Colombian cultivars Argentina, Beleña, Chava Blanca, Parda Pastusa, Tocana Rosada and Tuquerreña. Tests using serology and inoculation to a range of indicator hosts failed to reveal the presence of any virus in addition to PLRV that could be isolated consistently from samples of plants of different cultivars with these symptoms.

Effects of Andean PLRV isolates

PLRV isolates from plants of cvs. Renacimiento and Parda Postusa with "enanismo amarillo" were inoculated using aphids to the highly PLRV susceptible Tuberosum cultivar Arran Pilot. Both the primary and secondary symptoms induced were typical of those produced in Tuberosum. In contrast, when a PLRV isolate from a Ticahuasi source plant with rolled lower leaves was inoculated to Renacimiento plants using aphids, no primary symptoms were induced but secondary symptoms were typical of "enanismo amarillo" disease.

TABLE 1 Incidence of "enanismo amarillo" and typical secondary leaf roll in crops of the potato cultivars Renacimiento and Ticahuasi respectively in different localities in Peru, in 1975.

Locality [a]	Cultivar [b]	N° of fields with infected plants/ Total N° of fields	N° of fields with incidence over 2.5%	Range of Incidence
Huánuco valley and Chaglla (1,900-3,000)	R	26/28	9	Trace - 46%
	T	6/6	2	Trace - 65%
Huasi - huasi (2,900-3,600)	R	7/16	2	Trace - 3%
	T	15/20	3	Trace - 47%
Mantaro valley and Comas (3,200-3,800)	R	9/31	2	Trace - 15%
	T	2/5	1	Trace - 40%
Cañete valley and Barranca (10 - 250)	R	17/21	2	Trace - 15%
	T	29/37	15	Trace - 39%

[a] Figures in parenthesis show range of altitude of fields examined (in meters)

[b] R = Renacimiento, T = Ticahuasi

Previous failure to realize that "enanismo amarillo" disease is caused by PLRV was because affected plants of most Andigena cultivars often do not develop the symptom from which the virus gets its name, the typical rolling of lower leaves. Lack of knowledge of the symptomatology of PLRV in Andigena which is the most widely grown type of potato in the Andean region, has meant that the importance of the virus to potato cultivation in the Andes has been greatly understimated.

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ANDEAN POTATO LATENT VIRUS (APLV)

APLV was first described in 1966 by Gibbs and coworkers (3) who characterized an isolate from Colombian potatoes (Col); the name 'latent' was given to the virus because APLV - Col caused slight or no symptoms in infected British potato cultivars growing under greenhouse conditions in England. APLV is adapted to multiply under cool conditions such as those encountered in the Andean highlands, where potato cultivation normally occurs between 2,000 and 4,000 m above sea level. The virus is now considered to be a strain of Eggplant mosaic virus (EMV) (4). The type strain of EMV is known to be transmitted at low efficiency by the flea beetle Epitrix sp. (1) and APLV - Col through the seed of Nicotiana clevlandii (3).

At CIP, three APLV isolates (Caj, Hu and Ay) from different locations in Peru were used to provide more information on its host range, symptomatology in potatoes and indicator plants, transmission, physical properties and serology. Further information was also obtained on the distribution of APLV in the Andes (2, 5). This work was done in cooperation with C. Fribourg and Dr. R. Koenig.

Symptomatology and Host Range

Isolates Caj, Hu and Ay each infected 27 species of plants in the families Amaranthaceae, Chenopodiaceae, Cucurbitaceae and Solanaceae, but differed somewhat in symptoms. The most characteristic type induced by the three isolates was systemic chlorotic or necrotic netting of minor leaf veins. Nicotiana bigelovii and N. clevlandii proved the most useful diagnostic hosts (2).

In our greenhouse tests with potato cultivars, secondary infection with APLV isolates Caj, Hu and Ay frequently caused distinct symptoms and all three isolates induced primary reactions in at least

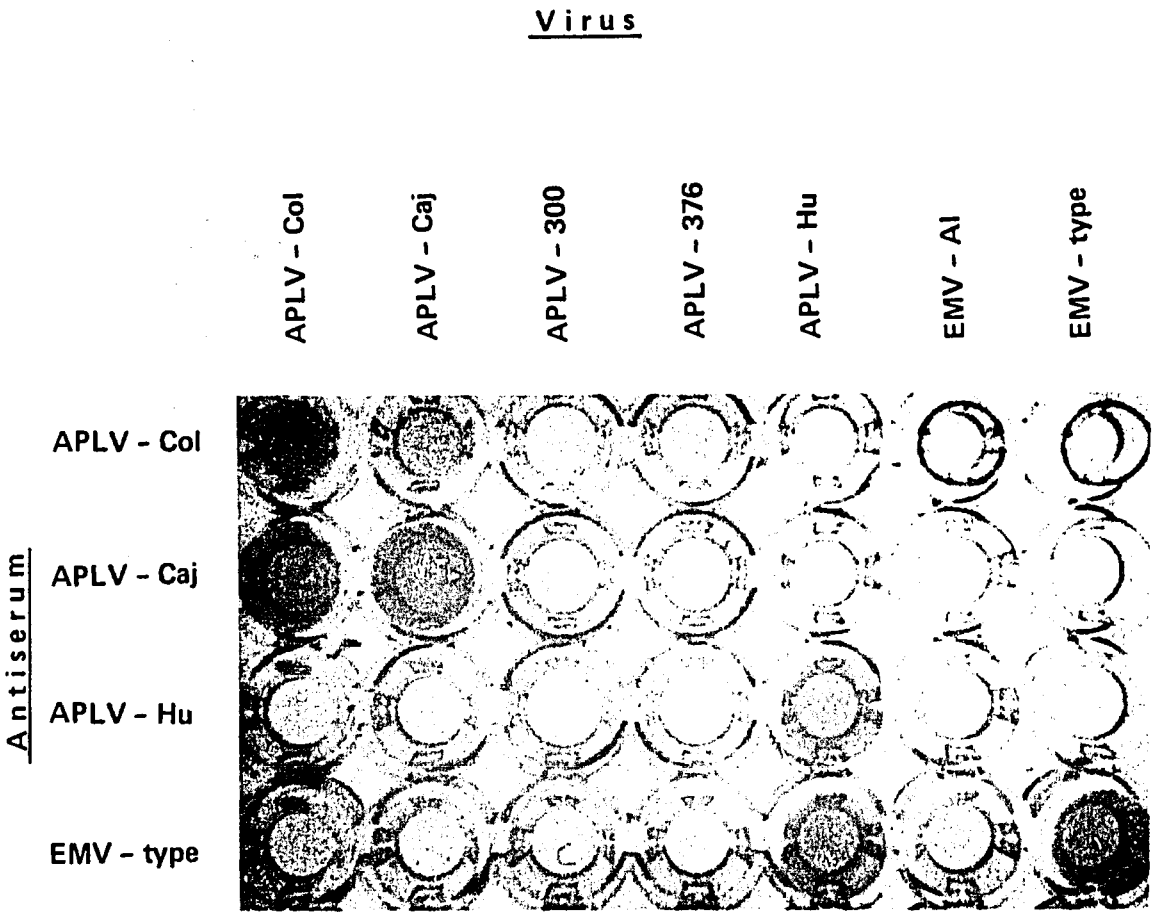

Fig. 1. Differences in the reactivity of different strains of APLV-EMV with homologous and heterologous antisera in the ELISA-test.

one cultivar. Moreover, with wild potato species, symptoms were frequent with primary infection and were always present with secondary infection. Caj caused mosaics and/or netting of minor leaf veins, whereas with Hu and Ay the predominant symptom was mosaic; leaf deformation was a frequent additional reaction with Ay in wild species.

With plants growing in the field which were secondarily infected a further symptom rugosity, sometimes developed. Also, symptom severity was greater at high altitude where the conditions are cool than in plants growing on the coast near sea level, where conditions are warmer and the daily fluctuations in temperature much less marked. Spread of the virus to tubers of secondarily infected plants was erratic.

Transmission

A species of *Epitrix* which occurs in fields near CIP, Lima was used in transmission tests with *Datura stramonium* and isolates Caj, Hu and Ay. In all tests, these isolates were transmitted at low efficiency and only in one trial in which over 100 beetles were transferred from the infected plants did all the healthy ones become infected (Table 1).

Increasing the feeding time on source plants from 1 to 5 days and the inoculation time on healthy plants from 2 - 7 days did not seem to influence the extent of transmission. In further tests, this beetle also transmitted APLV at low efficiency from *D. stramonium* to *Nicandra physaloides* and potato, and from *N. physaloides* to *N. physaloides* and *D. stramonium*. In contrast, the three APLV isolates were very readily transmitted in tests in which infected plants of *D. stramonium*, *N. physaloides*, *N. bigelovii* and potato were brushed against healthy plants of one or more of these species. Transmission through potato true seed was about 1% in an *Andigena* clone (5).

TABLE 1

TRANSMISSION OF THREE ANDEAN POTATO LATENT VIRUS (APLV) ISOLATES FROM INFECTED TO HEALTHY DATURA STRAMONIUM PLANTS BY EPITRIX SP.

Expt. N°	APLV isolate *	Beetle feeding (days) Acquisition	Inoculation	N° of beetles transferred	Transmission +
1	Ay	1	2	76	4/10
2	Ay	2	4	111	8/8
	Control	2	4	113	0/8
3	Hu	2	4	18	3/10
	Caj	2	4	20	2/10
	Control	2	4	20	0/10
4	Hu	3	4	20	3/10
	Control	3	4	16	0/10

* Five source plants/treatment.
\+ N° of plants infected/N° inoculated.

Physical properties

All three isolates proved to be very stable in sap and reached extremely high concentrations in plants. They were still infectious when sap was stored for at least 3 wk at room temperature and when diluted to 10^{-6} in distilled water. Infectivity of Hu and Ay was destroyed by heating for 10 min at 70° but not at 65° C but with Caj some remained after heating at 75° but not at 80° C (2).

The three isolates had spherical particles about 28 nm in diameter and the molecular weights of their protein subunits determined by SDS-polyacrylamide electrophoresis were in the range 19,600 to 20,700 daltons. This data and that from light absorption (A 260/ A 280 = 1.51) are similar to those reported for other tymoviruses (2).

Serology

Antisera with titres of at least 1:1024 were readily obtained. The three APLV isolates proved to be closely related but not identical to APLV - Col and the type and abelia latent strains of EMV. Isolates Hu and Ay were serologically identical but both formed spurs in gel diffusion tests in reciprocal reaction with Caj. Caj was more closely related to Col than were Hu or Ay (2). APLV - Hu type isolates appear to be restricted to the southern part of the Andes, from Central Peru south to Bolivia, whereas APLV Col/Caj types seem to occur throughout the region.

Distribution

Serological tests on new germplasm collections from Colombia, Ecuador, Peru and Bolivia showed that APLV occurs in potatoes in these countries (Table 2). Tests on collections from the Andean region of Argentina and from the Isle of Chiloe, Chile, were negative but on insufficient scale to conclude that the virus is not present. About 15% of clones of the CIP germplasm collection were found infected (2).

TABLE 2

INCIDENCE OF ANDEAN POTATO LATENT

VIRUS IN GERM PLASM

COUNTRY	SAMPLES / INFECTED TOTAL TESTED
Colombia	7/146
Ecuador	3/122
Perú*	9/6
Bolivia	17/225
Argentina (Andes)	0/12
Chile (Isle of Chiloe)	0/195

* CIP germ plasm collection

Importance

The potential importance of APLV to potato cultivation in the Andean region should not be understimated. The virus has a misleading name. It can cause symptoms and these are severe in sensitive cultivars growing in the highlands. Moreover, it is highly contagious, being very readily spread by simple contact and seems common throughout the region, from Colombia to Bolivia.

APLV was the first potato virus to be shown to be transmitted through potato true seed and to have a beetle vector. *Chenopodium quinoa* and *Amaranthus edulis* are other crop plants in the Andes which are hosts. These and weed hosts such as *N. physaloides*, *Amaranthus caudatus* and *D. stramonium* are potential reservoirs of infection for the potato crop.

Although APLV must have been taken from the Andes to Europe and North America many times in potato tubers or true seed, it seems not to have become established there. Possible reasons for failure to spread widely are the erratic transmission of virus from infected plants to tubers, absence of an *Epitrix* species capable of acting as a vector for the virus and lack of the solanaceous weeds abundant in the Andes. Alternatively, perhaps the virus does occur in potatoes outside the region having remained undetected because its symptoms have been confused with those of other viruses and because suitable indicator hosts have not been used in routine tests in seed program. In the absence of evidence of its occurrence, careful quarantine measures should be enforced to avoid spread to commercial plantings outside the Andes.

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WILD POTATO MOSAIC VIRUS (WPMV)

In the winter of 1974, symptoms of mosaic and leaf deformation were found in many plants of Solanum chancayense growing in the lomas vegetation in the peruvian coastal desert at Lachay, near Lima. The lomas are hilly areas of the desert where low lying cloud during the winter gives sufficient moisture to support growth of shortlived plants adapted to this habitat, including several wild potato species. An isolate obtained from diseased S. chancayense was studied in cooperation with C. Fribourg and examination of infective sap in the electron microscope revealed particles typical of the potato virus Y (PVY) group. The virus differed, however, from other members of this group in some of it's properties indicating that it may be a new virus. The name wild potato mosaic virus (WPMV) is proposed.

Host Range and Symptomatology

WPMV has a narrow host range infecting only some species in the Solanaceae; species in the Amaranthaceae, Chenopodiaceae, Cruciferae, Cucurbitaceae, Leguminoseae and Umbelliferae were not infected. Hosts in the Solanaceae, included Tomato, Nicandra Physaloides, Physalis floridana and several Nicotiana and wild potato species. The most useful hosts for maintaining cultures of the virus and for detection in samples were Nicotiana bigelovii, N. clevelandii, N. occidentalis and N. rustica which reacted with distinct systemic mosaics. Solanaceous indicator species which were non hosts included Datura stramonium, Lycopersicon pimpinelifolium, Solanum demissum Y, S. demissum A and clone A6.

Mosaic and leaf deformation symptoms were reproduced in S. chancayense - plants inoculated with WPMV, and the virus also infected the following other wild potato species: S. berthaultii, S. brachycarpum,

S. chacoense, S. megistacrolobum, S. microdontum, S. mochicense, S. polyadenium and S. raphanifolium. However, S. curtilobum, S. cardiophyllum, S. stenotonum, S. stoloniferum and several Andean and European potato cultivars were non hosts indicating a restricted host range even within the genus Solanum.

Stability in Sap

Infectivity of infective sap was lost by diluting to 10^{-4} but no to 10^{-3} in distilled water, by heating for 10 min. at 65° C, but not at 60° C, and by storage at about 20° C for 6 days but not 5 days. These properties resemble those of other members of the PVY group.

Aphid Transmission

WPMV was readily transmitted non persistently by the aphid Myzus persicae, from infected N. bigelovii to healthy N. bigelovii and S. mochicense, and from infected to healthy S. chancayense. However, attempts to transmit the virus using this aphid to the potato cultivars Chata Blanca, Mi Peru and Mariva were unsuccessful.

Electron Microscopy and Serology

In infective N. occidentalis sap examined in the electron microscope, particles ranged from 685 to 800 nm in length with a mean of 735 nm.

WPMV has been purified by differential ultracentrifugation and an antiserum prepared for use in determining detailed serological relationships with other members of the PVY group. This part of the work has yet to be completed.

Importance

WPMV is not of economic importance to the potato crop because it does not infect *S. tuberosum*. It does, however, infect tomatoes and some weeds in Peru e.g. *N. physaloides*. The virus is of considerable academic interest because it appears to be the first studied from naturally infected wild potatoes and it shows adaptation to the highly specialized desert habitat in which it was found; whether it occurs outside the lomas is unknown. Moreover, it represents a third PVY - type potato virus in addition to PVY and PVA.

PROPERTIES OF SOME VIRUSES FROM ANDEAN POTATOES

Dr. L. Salazar

Potato Virus T (PVT)

A virus producing systemic necrosis in *Phaseoulus vulgaris* cv. Pinto was detected in about 14% of the potato plants tested for basic seed production in Peru. Further studies revealed that it was an undescribed virus in potato and was named potato virus T. The isolate studied was originally obtained from a plant of tbr x adg. cv. Antarqui. PVT has a wide host range, infecting 38 out of 50 species tested. Hosts were found in seven plant families but those exhibiting the most obvious symptoms were mostly in the Leguminosae and Chenopodiaceae. In the Solanaceae, most of the hosts developed relatively mild symptoms or were infected symptomlessly. *Phaseolus vulgaris* cv. Pinto and cv. The Prince were good local lesion assay hosts when heavily shaded after inoculation. *Chenopodium quinoa* was a good host for multiplication whereas *C. amaranticolor* was the best diagnostic host.

PVT was transmitted to 6 British potato cultivars (Cara, Arran Pilot, Maris Bard, Arran Victory, King Edward and Orion) *S. demissum* 'A', *S. demissum* 'Y' and two clones of ssp. *andigena* (CPC 2783 and CPC 1807), *S. stenotonum* CPC 4301, *S. chacoense* CPC 3527, *S. spegazzinii* CPC 3780, *S. vernei* CPC 2733 and *S. acaule* CPC 3741, but failed to infect 2 other *andigena* clones (CPC 1864 and CPC 1801). The virus was usually latent on potato but some plants of cv. King Edward showed slight vein necrosis and chlorotic spots. In the *andigena* clone CPC 2783 and cv. Cara top

necrosis developed about 12 days after graft-inoculation but new symptomless infected shoots developed later. A mosaic was observed in one shoot of one plant of cv. Cara two (2) months after inoculation. Plants developed from infected tubers were also symptomless but Arran Pilot and Maris Bard showed some necrosis towards the tips of some leaflets about one month after planting.

PVT was not transmitted by the aphids Myzus persicae and Macrosiphum euphorbiae. The virus was seed transmitted in Nicandra physalodes (28%), Datura stramonium (72 and 5%), Solanum demissum 'A' (39%), S. demissum 'Y' (10%) and Solanum villosum (2 out of 19 groups of 5 seedlings). It was pollen transmitted in S. demissum 'A' but not in N. physalodes. PVT was inactivated when sap was diluted between 10^{-4} to 10^{-6}, by heating the sap at 60° C for min. or on storage of infective sap at room temperature for more than 2 days.

Purified preparations showed that particles of PVT are flexuous filaments c. 640 x 12 nm, helically constructed with a pitch c. 34 A°. They contain about 5% single stranded RNA with a mol. wt. c. 2.2 x 10^6 daltons. Particles of PVY showed substructural detail not observed with other plant viruses. They have a sedimentation coefficient ($S^{\circ}_{20,w}$) of about 100 S.

PVT is serologically related to apple stem groving virus (ASGV) but not to apple chlorotic leafspot virus, PVX, PVS, PVY or PVM. In spite of their serological relationship PVT and ASGV do not protect C. quinoa from infection with the other. Simultaneous inoculation with both viruses produced additive effects. Furthermore, ASGV does not infect potatoes and shows different kinds of symptoms in common hosts.

Further studies on the distribution, natural mode of transmission and effects on yields of potatoes are obviously needed. Its host range, mostly in Leguminosae, Solanaceae, and Chenopodiaceae and its ability to be seed transmitted favour its survival and spread.
(L.F. Salazar and B.D. Harrison*).

Potato '14 R' virus

This virus was found in plants of tbr x adg. cv. 'Aleli'. Its host range is rather wide but all species tested including potatoes, were only locally infected. The exceptions were *Lycopersicon esculentum*, *Petunia hybrida*, *Chenopodium amaranticolor*, *C. quinoa* and *C. ambrosoides* which were erratically systemically infected. This behaviour was puzzling and further isolation of the virus from naturally infected plants is needed to find out whether this is a general property of the virus.

Purified preparations of '14 R' contain rod-shaped particles c. 290 x 18 nm, with a sedimentation coefficient ($S^{\circ}_{20,w}$) of about 180 S. They contain single stranded RNA and a single polypeptide species of a mol. wt. similar to tobacco mosaic virus (TMV). The virus is distantly serologically related to TMV but not to potato mop-top and other tobamoviruses.

'14 R' somewhat resembles an incompletely characterized virus reported by Accatino (1966) in Chile. (L. F. Salazar and B. D. Harrison*).

Potato yellow vein virus

An attempt was made to identify the causal agent of the 'vein yellowing' disease from Ecuador and Colombia. Tubers from cv. Santa Catalina ((tbr x adg) x adg) from a plant showing typical vein yellowing were sprouted and their viruses content determined at 2, 3, 6 and 9 months after harvesting. In all tests only PVY was transmitted to a selected group of indicator species by either mechanical or graft-inoculations. At 9 months, however, a virus was detected in some graft-inoculated *Datura stramonium* plants. In some plants PVY was also detected by electron microscopy and infectivity to *Solanum demissum* 'Y'.

The virus isolated from *D. stramonium* seemed to occur in very low concentration. It was also transmitted to *D. stramonium*

inoculated with infective sap. Efficiency of transmission was increased when 0.06 M phosphate buffer, pH 7.0 containing 0.2 M sodium bisulphite was used to grind the tissue. Partially purified preparations contained isometric particles c. 26 nm in diameter, a proportion of them appearing as empty shells. The virus was graft-inoculated to the British potato cultivars King Edward and Arran Victory, but no symptoms developed in the inoculated plants. Plants from progeny tubers showed a general chlorosis and stunting. These symptoms resemble those observed in older infected plants in Ecuador and Colombia. Results from back tests were not very clear but some particles (empty shells) were observed by electron microscopy and no other potato viruses were detected.

Stems and leaves with symptoms from potato plants collected in Ecuador, did not reveal significant changes in the cells, but 'pinwheel' inclusions were abundant.

The symptoms caused in D. stramonium appeared to be greatly dependant on light intensity. Mechanical inoculated 'cotyledonary' leaves showed necrotic lesions and under reduced light intensities a strong mosaic developed. (L. F. Salazar, B. D. Harrison* and I. M. Roberts*).

Properties and strains of Andean Potato Mottle Virus

Purified preparations of Andean Potato Mottle Virus (APMV) contain isometric particles c. 28 nm in diameter with sedimentation coefficients ($s^{\circ}_{20,w}$) of about 53, and 93 and 112 S. The two nucleoprotein components, middle (M) and botton (B) were serologically indistinguishable and each contained similar relative amounts of two polypeptide species, of weight 22, 100 and 41,800 daltons. M particles had a density in Cs Cl of 1.41 g/ml and contained a single RNA species of ml.wt. 1.4×10^6 daltons. Preparations of M and B particles were much less infective when separated than when mixed. These properties are typical of comoviruses. The present crytogram of APMV is R/1 : 1.4 (27) + 2.0/ (34) : S/S:S/* comovirus group.

The two isolates studied, C and H, apparently differed from the type strain of APMV in being able to infect Gomphrena globosa systemically. Isolate C was antigenically indistinguishable from the type strain and produced similar symptoms in several solanaceaous species. Isolate H differed antigenically from C and the type strain and also differed somewhat in symptomatology and host range.
(L. F. Salazar and B. D. Harrison*).

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RESISTANCE TO CERTAIN VIRUSES IN POTATO

W. J. Hooker

Potato Leaf-roll

Breeding for resistance to the potato leaf-roll virus (PLRV) is complicated by variation within plant genotypes, strain differences within the leaf-roll virus, and seasonal environmental differences which frequently cause symptoms to be absent or very mild and difficult to determine with accuracy. Severity of symptoms ranges from acceptance of the virus without symptoms, through almost imperceptible leaf rolling, to severe symptoms involving leaf rolling, stunting, severe phloem necrosis, severely reduced yields, and early death of the plant.

Resistance to infection is inherited polygenically in which a number of genes as yet unidentified apparently act to increase resistance to virus infection. The mechanism by which infection is inhibited or prevented is not know. Such resistance is overcome by inoculation with heavy aphid populations or by grafting. Plants may remain free for 1 or more years after inoculation and later develop symptoms. Such resistance is consistent, for example, Katahdin resistance has been evident in a number of tests by several individuals.

As early as 1939, Folsom and Stevenson, began selecting for this type of resistance in Katahdin, and confirmed reports of resistance in Imperia, (previously reported resistant in Sweden and France) Kepplestone Kidney (resistant in Ireland), and Flourball (reported resistant in Ireland and England). After 5 years of field exposure (Stevenson et al 1943), Triumf remained free from PLRV, Bevelander and Shamrock showed 32% PLRV, Katahdin, 41%, Friso, West Brabander, Noordeling and Albion showed fewer symptoms

than susceptible U. S. varieties. One variety, Penobscot (Simpson and Akeley, 1946) of this origin has recently been released and shows prospects for being of practical resistance. Field infection over a 3 year average following inoculation of young plants with virulferous aphids was for Chippewa 100%, for Katahdin 97%, and for Penobscot 31%.

Loughnane in 1941 (Cockerham, 1943) reported that resistance in Shamrock was transferred by hybrid progenies with leaf-roll susceptible parents to such a degree that progenies survived field exposure 4 to 5 years before becoming infected. Seedlings segregating from a cross of Shamrock x Skerry Champion (Mc Kay and Clinch, 1951) varied in field reaction over 3 years from highly resistant with no reduction in vigor to very few rolled leaves or only rolling of lower leaves to severe reduction in plant size and severe rolling. Cockerham (1943) reports some resistance in Epicure.

Mc Kinnon and Davies carried out PLRV resistance trials (1967) relying on natural infection from a near by field of PLRV infected plants. They tested advanced seedling selections and newly released Canadian varieties in successive trials over 4 years. In most selections visible symptoms developed from 65 to 100% of the plants within 3 to 4 years. Only 2 selections failed to show symptoms over a 4 year period, F 4896 (Southesk x Katahdin) and F 3411 (Katahdin selfed). Although neither resistant selection showed symptoms early, almost complete infection was obtained by the third year.

Ross and Baerecke (1950) also reported that varieties Paraguay and Bukasov carried PLRV symptomslessly. Tolerance of this type might well be useful in avoiding leaf-roll losses until resistance of a higher type becomes available.

Greenhouse infection trials (Mc Kinnon, 1967) involving 30 plants of each variety were supported by recovery tests to P. floridana. Netted Gem became 90% infected, Sebago Katahdin, and a selection of Katahdin selfed (F 3411) were 20% infected and Penobscot was infected to only 3%.

Baerecke (1955) demonstrated that selfing S. tuberosum yielded no individuals escaping infection. When 2 resistant selections were selfed approximately 4% or each progeny escaped leaf roll infection following aphid inoculation. When these 2 individuals were crossed 22% of the progeny escaped infection. In crosses of the same 2 parents with S. tuberosum containing no resistance only 1.6 and 2.3 percent of the progenies were resistant. Baerecke (1956) again demonstrated similar results with additional crosses and involving different parents. Thus, increased resistance was obtained through crossing different genotypes after maximum levels of resistance had been reached through inbreeding.

Hypersensitivity to the virus has been described as resistance in which infection causes extremely violent reactions accompanied by severe phloem necrosis and obvious rolling of the leaves.

Hypersensitivity to PLRV was identified in Bismark and in 2 hybrid selections (Bals and Hutton, 1950), (Hutton and Brock, 1953) in which phloem was very necrotic and leaf roll symptoms were severe. Severe phloem necrosis usually accompanied severe vine symptoms. Masking of leaf roll was generally, but not always associated with low levels of phloem necrosis. Tubers taken from infected plants of these selections produced healthy plants in relatively high percentages suggesting that virus inactivating mechanism might be operative.

Dr. Baerecke has extensively investigated hypersensitivity and leaf roll resistance. S. berthaultii (Ross and Baerecke, 1951) was the most intolerant species tested producing both severe leaf roll and phloem necrosis. S. raphanifolium also in strongly intolerant (Ross, 1966).

According to Dr. Hans Ross (verbal communication) necrosis of eyes and sprouts and of entire plants follows PLRV infection and results in stand loss in the field. If infection should be light, loss of individual plants could be compensated by increased growth of neighbouring plants. If infection were heavy and stands were greatly reduced severe losses could be expected. PLRV infection in S. fendleri suggested hypersensitivity (Natti et al, 1953).

High resistance sufficient to prevent virus infection in tissues exposed to graft inoculation is being explored by R.A.C. Jones (CIP) in S. brevidens, S. etuberosum and S. fernandezianum. Although this type of resistance is the type which is most desirable, it still remains necessary to demonstrate that resistance is maintained over a wide range of virus collections and that suitable food types may eventually be obtained from resistant selections. Incorporation of resistance of this type into useful cultivars may take a number of years.

Aphid Relations

Adams (1946) studied aphid populations and plant injury in closed cages containing more than 1 potato variety. S. polyadenium was almost immune to aphid attack and supported extremely low populations of aphids. Other species supporting low populations of aphids were S. jamesii, S. commersonii, S. chacoense, and S. demissum. All were either resistant or tolerant except for S. demissum which was susceptible to aphid injury. Outstanding within S. tuberosum, was Sequoia which although susceptible to aphid attack supported essentially no aphid populations. Susceptible varieties generally supported relatively large populations and tolerant varieties intermediate populations.

Other Tuber Bearing Solanum Species

Salaman in 1921, reported resistance in S. edinense (Cockerham, 1943). Infections could not be established (Natti et al, 1953) in 1 collection of S. cardiophyllum nor in 6 collections of S. stoloniferum.

Varieties Apta, Aquila, and Augusta were derived from S. demissum and have useful resistance to leaf-roll. S. acaule has some resistance as well (Ross, 1958.).

Only S. chacoense and S. catarthrum and S. andigena give hope of PLRV immune plants (Ross and Baerecke, 1950). All hybrids with S. tuberosum except one with S. chacoense showed leaf-roll. S.

polyadenium and S. tuberosum hybrids appeared remarkable for their high tolerance seldom showing symptoms in the year of infection and possessing only very faint phloem necrosis. Varieties Paraguay and Bukasov (S. chacoense) carried PLRV symptomlessly.

S. demissum hybrids have high resistance, polygenic in nature to PLRV. Aphid populations influence the degree of infection in resistant types (Ross, 1961). Varieties originating from hybrids between S. tuberosum and S. demissum were exposed to heavy and to light populations of aphids carrying leaf-roll. Reinhort was 50 and 20% infected, Apta, 70 and 25%, and Aquila 80 and 30% respectively. In contrast, a susceptible control, Ackersagen, lacking S. demissum was 100 and 90% infected. Of commercial varieties in Germany 70% (Ross, 1966) carry S. demissum genes because of the favorable S. demissum influence in transmitting desirable characteristics.

S. acaule back crossed to S. tuberosum (Ross, 1966) were remarkably resistant to leaf-roll infection.

In greenhouse inoculation trials (Clark, 1963) 6 to 42 S. phureja tested failed to evidence leaf-roll infection. S. raphanifolium contained 3 such accessions.

Resistance to Viruses Y, A and X

Resistance in potato to these 3 viruses follows somewhat similar patterns with: 1) resistance to infection due to unknown factors; 2) hypersensitivity of field immunity and 3) comprehensive resistance (immunity).

The former type is of relatively little use in a breeding programs, but where varieties have this resistance escapes are of relatively common occurrence and tubers may not be infected even though grown on plants carrying the virus. In this respect, mature plant resistance may be somewhat related.

Hypersensitivity is extreme susceptibility evident as a necrotic reaction which may either kill a plant part and thereby exclude the virus, or the entire plant may be killed and the inoculum source thus destroyed. Resistance of this type is often specific to certain strains of the virus (for example, strains B and X of PVX, strain C of PVY). Strain differences have not yet been identified in PVA.

Comprehensive resistance (immunity) is characterized as inability of the virus to establish a relationship with the host except possibly extremely small necrotic spots have been observed. The virus passed through intermediate highly resistant stocks but has not yet been shown to multiply nor survive for any length of time in such tissue.

Resistance to PVY and PVA is frequently combined within the same genotype. R_y is a comprehensive type of resistance in which infection by all known strains of PVY and PVA is prevented. In this, virus is not accepted by the plant and is unaccompanied by any evidence of hypersensitive response. Genes within several species of Solanum may not be identical, but their effect is similar.

The following listing of sources of resistance is taken from Ross (1958) and from Cockerham (1970).

SOURCES OF PVX RESISTANCE

Comprehensive resistance (resistance to all known strains of the virus):

Rx (immunity) inherited as amonomer dominant determines complete resistance to all strains of PVX.

Rx (acl) in S. acaule.

Rx (adg) in S. andigena controls lethal necrosis to all strains of virus X and B.

Rx (adg) in S. andigena and USDA seedling 41956 controls extreme resistance.

Noncomprehensive resistance with resistance to selected strains of the virus:

Nx (tbr) in S. tuberosum and probably also in S. andigena types controls lethal necrosis (hypersensitivity to all strains of X, and not to virus B, is identical to N^x).

Nx (spl) in S. sparsipilum is similar to above.

Nb (tbr) in S. tuberosum and probably also in S. andigena is identical to N^b controls lethal necrosis to strains of virus B.

Nx (chc) in S. chacoense and probably as also in S. microdontum.

Polygenic inheritance with relatively low resistance.

PVA AND PVY RESISTANT TYPES

Comprehensive resistance to all known strains of viruses A and Y.

Ry (immunity) (syn. Ry (sto)) inherited as a monomer dominant in S. stoloniferum controls extreme resistance.

R (chc) in S. chacoense (a monomer dominant) (immunity).

Ryn (extreme resistance to viruses Y and A), in S. stoloniferum

Extreme resistance to virus Y and lethal necrosis, (hypersensitivity) to virus S.

Ry $(sto)^{na}$ in S. stoloniferum.

Extreme resistance to virus Y and non-lethal rusty necrosis to virus A.

Ry $(sto)^{rna}$ in S. stoloniferum.

Extreme resistance to all strains of virus Y.

Ry (hou) in S. hougasii.

Lethal necrosis to virus Y coupled with susceptibility to virus A.

Ry $(sto)^{n1}$ in S. stoloniferum.

Ry $(sto)^{n2}$ in S. stoloniferum.

Necrotic to all strains of virus Y.

Ny (chc) in S. chacoense and S. microdontum.

Ny (dms) in S. demissum.

Necrotic reaction to strain C of virus Y.

Nc (tbr) in S. tuberosum.

Lethal necrosis to virus A.

Na (tbr) (sun. Na) in S. tuberosum.

Na (dms) in S. demissum.

Rym

Non-lethal rusty necrosis to virus A.

Na (sto) in S. stoloniferum (syn. Rym).

Low resistance to virus Y (polygenic inheritance)

in S. tuberosum varieties Cayuga, Franx franziska, and

in S. demissum varieties Apta, Lori.

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BREEDING FOR RESISTANCE TO POTATO LEAF-ROLL AND Y VIRUSES

C. R. Brown

Diseases produced by viruses in potatoes are an important cause of yield and quality losses in developing countries. In countries of the developed world the importance of these diseases has been reduced due to sophisticated seed production and seed certification programs (which reduce the presence of viruses to economically acceptable levels). In developing countries one is faced with essentially two choices in the fight against viruses: 1) the implementation of schemes of seed production or 2) the provision of materials with improved resistance achieved through breeding. Although seed production technology exists to a certain degree in some developing countries (i.e. ten percent of the hectarage is planted in commercial seed in Peru) and may be expected to increase in distribution, it will probably never be available to a majority of potato growing farmers in most developing countries. It is within this context that a breeding effort is thought justified at CIP.

The CIP population approach

CIP's basic goal is not to release new varieties. Rather the basic philosophy is directed at supplying improved materials to national programs. This appears to be a rational approach as quite often a variety that will do well in a particular environment necessarily will need a complex of disease and pest resistance as well as adaptation and yielding ability specific to a soil-temperature-daylength environment. Population methods enable the breeder to increase the gene

frequencies for traits in a series of sampled populations drawn from a base population with a wide genetic composition. Selection for tuber quality and yield is applied during this process of population development. With resistance to viruses we hope to be able to supply raw materials from our population process, i.e. segregating families, to national programs and also be able to funnel materials into our own work on developing materials adapted to the lowland tropics. Resistance to virus diseases will be essential to the success of this program.

Virus Resistance : Background

Attention is concentrated on potato virus Y (PVY) and leaf-roll (PLRV) because they are the most important.

PVY is a non-persistent virus which is very effectively transmitted by the aphid, *Myzus persicae*. In general, chemical means of preventing infection are not possible due to the short acquisition and infecting feeding period of aphid transmitters. Some cultivars are resistant to PVY based on hypersensitivity of the lethal necrosis type. Immunity is also found in *Solanum chacoense*, *S. stoloniferum*, *S. demissum*, *S. microdontum*, and *S. tuberosum* spp. *andigena*. In all cases immunity has been found to be inherited in a monogenic dominant fashion. Cockerham (1970) reported that a total of six alleles distributed over three loci have been identified. This type of resistance has been incorporated into a number of clones that are available from Germany, Scotland, and the United States. Certain alleles control immunity to specific strains while others are comprehensive.

Leaf-roll presents a contrast. The virus is transmitted in a persistent manner. Preliminary studies at CIP indicate immunity in *Solanum brevidens*, *S. Fernandizanum* and *S. etuberosum*. Resistance to infection occurs and has been extensively incorporated into tuberosum cultivars. For example, many leading German cultivars show this type of resistance to leaf-roll. The resistance is polygenically controlled with all varieties becoming infected when heav-

ily infested with leaf-roll bearing aphids, the resistant ones to a lesser degree than others. A hypersensitive reaction has been found in three German varieties which results in plant death with heavy viruliferous aphid infestation. S. berthaultii has been recorded as hypersensitive, also.

Breeding work with leaf-roll is complicated by the fact that field infection may not be accompanied by clear primary symptoms. It is usually necessary to observe clonally propagated plants for secondary symptoms. Traditionally, infection has been carried out in the field and the variability in aphid populations from year to year has necessitated the exposure of breeding materials over several seasons in order to assure that putative resistant genotypes are not escapes. Although some workers have described the genetic variation of infection resistance to be additive in nature, there appears to be no experimental evidence for this, and no estimates of heritability have been reported.

History of Populations at CIP

Work at CIP on populations to improve resistance to viruses began with Dr. Jerome Franckowiak in 1975. Three populations were created utilizing selected materials: 1) one incorporating tuberosum germ plasm; 2) one with a predominance of andigena germ plasm; and 3) a diploid population utilizing S. phureja and dihaploid lines from other breeding programs. Following CIP's philosophy on population breeding, a phenotypic recurrent selection scheme with the following objectives was initiated: 1) utilization of heterosis; 2) improvement of adaptation and virus resistance; 3) increases of frequency of desirable genes in a "panmictic" gene pool. In short, symptomless plants were pollinated with a pollen composite collected from symptomless plants.

Cooperation with CIP virologists resulted in a effective screening technique for resistance to PVY, including successful use of mechanical inoculation. Investigations into the validity of using greenhouse aphid populations to inoculate seedlings and cuttings with leaf-

roll in screening for resistance were initiated in 1975.

At present, it appears that accumulation of immunity to potato virus Y will proceed in a straightforward manner. Inheritance is simple, inoculation effective, and symptoms are unambigous. The situation for leaf-roll is quite the contrary. Several research areas may be explored in this regard. First, a reliable greenhouse screening method capable of being used for evaluation of large amounts of material is being developed. Since we usually are dealing only with infection resistance, graft transmission is not a test for resistance. The present method of checking presence of the virus in individual plants, other than observing symptoms, is aphid transmission to Physalis floridana, an extremely time-consuming operation which occupies much greenhouse space. Our criteria for an adequate greenhouse screening must include a good correlation with field performance. Ideally the study of this correlation should be done by comparing individuals of identical genotype under both environments. Also, the method must work with cuttings and seedlings of diverse genotypes permitting unambiguous scoring of symptoms without the need for transplantation to the field. This will not obviate the need for a field environment that presents a severe leaf-roll bearing aphid challenge to materials identified as resistant in greenhouse screening. At the present time in Peru, a field environment providing sufficient aphid population stability for uniform screening is not available, although several sites are being investigated.

A second major point is that a phenotypic recurrent selection procedure will produce genetic gain only if there are large additive genetics effects and there is, consequently, a high heritability. If non-additive genetic effects are predominant, then one can imagine selecting resistant genotypes each cycle which is disrupted by the reassortment of genes that occurs in the subsequent enforced panmixis. We cannot expect to make progress with a quantitative trait under these circumstances.

It may be pointed out that, besides immunity and infection resistance, two other schemes have been suggested to minimize leaf-roll damage. Muller (1939) proposed breeding for tolerance to the virus

so as to reduce the economic loss with infection. Bald and Hutton (1950) characterized the growth characteristics of a tolerant and so-called intolerant variety, but dismissed Muller's suggestion as a poor compromise. The hypersensitivity of several German varieties led Baerecke (1958) to propose the use of a combination of maximum achievable infection resistance in a genetic background conferring hypersensitivity. The rationale for this proposal lay in the hypothesis that infection resistance would keep inoculation at a low level every year, however, successfully inoculated plants would die or at the most not contaminate the next clonal generation with infected tubers. The weakness in this idea, besides the problems of breeding for the combination of traits, is that late infected plants might substantially contaminate the next generation through infected tubers.

Proposed Future Work

At present, we have 238 clones that have not shown symptoms after one infection attempt in the greenhouse by leaf-roll bearing aphids, and a field planting of a clonal generation. This includes 188 diploids and 50 tetraploid clones. We have 396 clones that have undergone a similar process with PVY. These are all tetraploids.

Most of the tetraploid clones are the results of screening of botanical seed progenies received from North American and European cooperators and progenies of neo-tuberosum x andigena crosses produced by Dr. Franckowiak. A few clones are actual varieties which show substantial resistance to leaf-roll. The diploids are a composite of phureja and dihaploids including some crosses from Germany with leaf-roll resistance.

Crosses will be made to produce families for further evaluation. We are interested, in particular, in evaluating our San Ramón station as a future test site for simultaneous evaluation of leaf-roll resistance and tropical adaptation.

Testing of Greenhouse Screening Method

Greenhouse inoculation and screening for leaf-roll is under investigation by R. A. C. Jones, using seedlings and cuttings, as part of the CIP leaf-roll resistance project. In order to test if our method correlates with field performance, we will be comparing susceptibilities of cuttings of seedlings of ten families in the greenhouse and at San Ramón. The second generation of infected material of each genotype will be scored. In addition, progenies that have been sent to Chile and Argentina in 1977 for leaf-roll evaluation will be tested in the greenhouse and at San Ramón.

Leaf-roll resistance - Genetic studies

A diallel will be produced using eight parents from andigena bred and native cultivars. The botanical seed will be available for planting in March of 1978. These progenies will be exposed to infection in the greenhouse in April. Tubers will be collected from individuals and a second planting will be made in San Ramón in November for scoring of secondary symptoms. The results will be analyzed to obtain estimates of general and specific combining ability and heritability. These results will be used to re-evaluate our present population methods and to initiate changes, if thought necessary.

Hypersensitivity to Leaf-roll Virus

Three German varieties, Apta, Carla and Bismark, will be crossed extensively with other materials to examine the nature of inheritance and effects of different genetic backgrounds on expression of hypersensitivity to leaf-roll virus. Progeny of crosses will be inoculated in the greenhouse.

International Trials and Distribution of Improved Materials

A start was made in 1975 in the distribution of materials for evaluation in various countries. The interest in these trials is testimony to the importance of leaf-roll in many potato growing areas. Problems in the past have centered mainly on the large losses that have occurred with transplantation of seedlings to the field. In the future these trials may also include use of tuber families. Although initial results indicate a need for improvement in practical procedures for carrying out the trials, they indicate that Penobscot, a North American tuberosum variety, is a donor of leaf-roll resistance to its progeny.

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FIELD TRIALS FOR VIRUS RESISTANCE

T. M. W. Davidson

The title of this talk is field trials for virus resistance with reference to leaf-roll and virus Y. With leaf-roll one knows that with few exceptions all genotypes are susceptible and some are more susceptible than others. It is because of genotypes having different degrees of susceptibility that pedigree breeding for leaf-roll resistance has been attempted. What is required is some test or trial to give an accurate assessment of this character not only to enable a degree of choice to be made between potential parents, but also to assess seedlings and parental contribution.

With regard to virus Y monogenic resistance of the comprehensive type[1] derived from S. demissum, S. chacoense, S. microdontum and S. stoloniferum a trial is required only as a safeguard. Adequate testing can be accomplished in the greenhouse either by inoculation of seedling progenies in seed pans at the cotyleden stage or clone testing by hand, graft or aphid inoculation with virus Y. However, material derived from S. phureja with field-resistance to virus Y as well as Y resistance of the Pentland Crown type requires an assessment trial.

Breeding for resistance to leaf-roll is based on breeding from clones with reputed resistance to the disease. As yet there is little knowledge of the combining ability of the leaf-roll resistance character[2]. But breeding from resistant parents is more likely to give resistant offspring than otherwise. With regard to the nature of resistance, there is the possibility that resistance, such as it is, is associated with the deposition in the phloem of callose plugs which in itself is a sensitive reaction and responsible finally for the debilitating effect of the disease.

TABLE 1

This shows the regression of progeny on parents on an 1-12 scale (resistant - susceptible) of mean parental and offspring resistance. As many parents had been selected for commercial attributes, only the number of seedling for which data for both parents was available was limited. You will note that the numbers within progenies are low. Due to selection, it will be seen that the regression of progenies on parents is significant. The evidence of parental-offspring regression is indicative that the character of leaf-roll resistance is quantitative.

In the process of selection of seedlings for commercial attributes, in parallel with leaf-roll resistance it was found that leaf-roll resistance was negatively correlated with desirable tuber characters, as it was also in some cases for flowering and fertility. Whatever breeding and selection policy is adopted, a trial component has to be included. Original breeding at the Scottish Plant Breeding Station was done from reputedly leaf-roll resistant native varieties and German varieties and S. acaule derived resistant clones acquired from Dr. Ross[3]. Also, in using S. demissum and S. chacoense in Y resistance breeding leaf-roll resistance was noted, particularly in S. demissum lines. At this time, many methods of selection for leaf-roll resistance were tried, particularly in the greenhouse. Straightforward inoculation with aphids is laborious and the intense labour requirement limits its scale. We found that results for effort were poor, although there is no reason to suppose that such a method could not be made to work efficiently with sufficient resources. One method that did work well from the point of view of infection rate, was to inoculate young plants at the four leaf stage with one viruliferous M. persicae each. The young plants were later transplanted to the field where infected plants showed up well later in the season. An objection to this as a selection method is that infection is taking place when the plant is very young and is a true seedling as opposed to a mature plant and the infection appeared to be random. From the work of others, particularly the late Dr. Baerecke[4], field testing was started firstly in Scotland then because of lack of infection the trial was switched to Cambridge where annual populations of virus

Table 1. Regression of offspring on mean parental resistance.

Mean parental reaction	Number of progenies	Number of seedlings	Mean progeny score
1	6	9	3.55
2	11	27	2.70
3	17	42	4.57
4	10	50	4.88
5	9	26	6.42
6	3	9	5.44
7	11	47	7.64
8	11	41	7.66
9	6	40	8.43
10	6	36	8.92
11	2	11	8.18
12	2	14	10.29

Equation of best fitting line : $y = 2.55 \quad 0.617x$

Analysis of variation of regression :

Source	df	M.S.	V.R.
Regression	1	54.43	110.9 very significant
Residual	10	0.49	

vectors looked more promising. The trial design was aimed at obtaining a meaningful score for leaf-roll and virus Y infection from a relatively small tuber outlay in order to use the score as an aid to selection early in the career of a seedling and to reduce seedling numbers to manageable proportions. Although the initial screening with small samples may be of dubious value in years other than those of near optimal spread of virus. In practice it provides a workable method of selection for leaf-roll resistance early in the career of a seedling. A further trial at a later date with a larger sample has in many cases confirmed the presence of resistance. A further point the field trials are incorporated in a continuing selection for other characters than virus resistance alone. In breeding new varieties a seedling with leaf-roll resistance but without commercial attributes at least as good as existing varieties is only of value as a potential parent.

The trial also monitors annual infection levels using control varieties in order to adjust annual scores to a suitable datum-line. This is achieved by applying a correction factor to bring the standard control variety Pentland Crown to a 50% infection level.

The trial with which I am acquainted is a combined one for leaf-roll and virus Y. The basic unit is a row of three plants along the ridge bounded at one end by a leaf-roll infector and at the other by a virus Y infector. The scale of the trial annually is around 1,600 replicates of 3 tubers and occupies about a 1/2 acre. The 3 tubers of a replicate are designated A, B or C according to their position relative to the infectors, i.e. A is always adjacent to the LR infector and C farthest from it next to the Y infector. At harvest 3 tubers are kept from plants A, B and C and these are planted and scored in the following year. Three tubers are required to ensure within reasonable limits that partial infection of the mother plant is recorded positively. One, two or all three of these plants infected indicate that the mother tuber whether it is A, B or C had become infected in the previous year.

With all trials in which the forces of Nature hold the whip hand, success depends a great deal on there being little variation from normal

conditions from year to year. Peak aphid populations are greatly influenced by climatic conditions in winter. In this respect weather in February is known to have a direct influence on the size of the summer population of Myzus persicae (Sulz). A mild February leads to an early migration of M. persicae onto potato crops and a cold February to a late migration. The time of the migration then determines the earlyness of lateness of the population peak and the type of summer the size of that peak. This is with reference to counts within crops[5].

There are factors over which one has control, these being cultural and spatial. The trial must be well cultivated and well grown. The leaf-roll and virus Y infectors used should be varieties which are tolerant of the disease and give good plants, although systematically infected. In tropical areas the use of aphicide and/or wider spacing between plants or interspersed blank ridges might be required to reduce the full effect of very high vector populations. The strain of the virus Y infector is a matter of choice but is seems advisable to use the current dominant strain or strains. Ideally, leaf-roll infectors from different sources could be used for each block of the trial. Where infected material has been inadvertently incorporated in a trial it should be rogued out as early as possible as it upsets the balance of the infectors.

The scoring of the infection achieved has to be accurate. In temperate climates this is readily accomplished by visual inspection of the regrown material in the following year and testing for virus Y where there is doubt. In warm climates growing in the winter or in the highlands might reduce the difficulty of distinguishing infected plants. Staining sections of tubers with an ammoniacal-resorcinol stain of Tunmann-Rosenthaler (as described by the late Dr. Baerecke[6] has been found to distinguish leaf-roll infected from healthy tubers, particularly where the infected tubers are from plants with primary leaf-roll infection. In stain testing material from the trial in early-winter for some years the accuracy of the test was ever 90%. Growing eye-pieces in pots is a good method but scoring for leaf-roll is difficult in the greenhouse. There is a report by Sarkar on the use of the electron microscope for the routine diagnosis of leaf-roll and Y infection of sprouted tubers[7].

Figure 1 shows the annual variation in the percentage of leafroll infection of the control varieties Majestic and Pentland Crown from a series of annual trials in the south of England. It shows a pattern of continuous variation in respect of numbers infected and uninfected within each variety. A measure of confidence can be placed in a trial where the correlation between control varieties Majestic and Pentland Crown for a six-year period is very high (0.8982 ± 0.2197) in spite of differing levels of infection over the years. To achieve this result with the control varieties 20 replicates (60 tubers) were required. One can realize that the minimum of 4 replicates (12 tubers) used for clones under test can only give an indication of resistance and a bigger sample is required to confirm the resistance of apparent resisters. It is a reliable method of test if the sample is large enough.

To apply simple statistical analysis to the trial requires a clone sample of about 40 tubers conditions not being extreme. In selection terms this is possible with advanced clones only. The Scientific Services of the Department of Agriculture in Edinburgh use such a method for varieties entered for the National List Trials. They use 40 plants in a randomised block layout with replicates of 2 tubers between the leafroll infector and the Y infector.

Mention should be made of Y infection in the trials at Cambridge. As was mentioned earlier, Y infection is used to identify susceptibles in monogenic Y resistant lines and to this end it serves a useful purpose. Clones with resistance derived from S. phureja or with Pentland Crown type resistance can be scored in the same manner as for leafroll. The control Pentland Crown becomes infected in normal years to about 2-3 per cent and Majestic to 80 per cent with virus Y. The strain of virus Y used for infection is of the Y^o type but Y^n infectors could be used for half of the trial with advantage.

In Table 2 data from the Rothamsted Insect Survey is presented for M. persicae caught at Broom's Barn in a suction trap about 30 miles from the trial site. It gives one a fair idea of previous activity in surrounding crops. Leafroll infection of Pentland Crown is only fairly consistent with the count, and not so in instance. The percentage readings of Pentland Crown infection from 1965 are 30, 40, 42, 52,

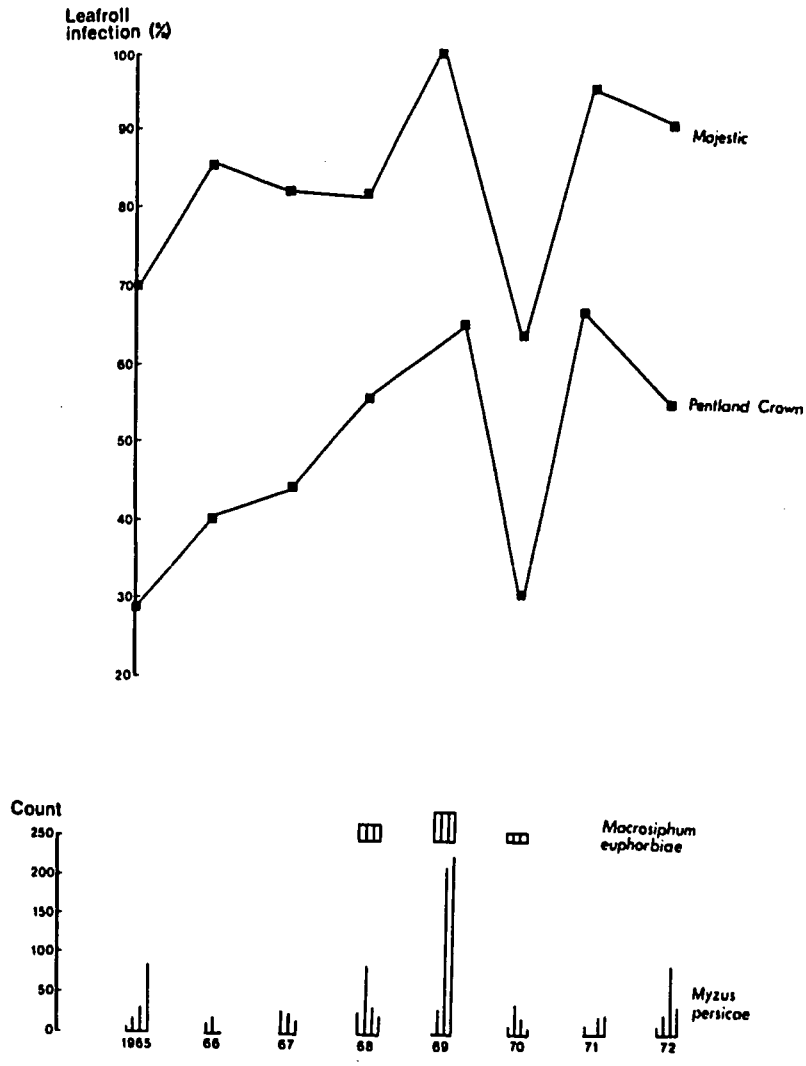

Fig. 1. Annual variation in the percentage of leafroll infection of the control varieties Majestic and Pentland Crown. The field counts for Macrosiphum euphorbiae and Myzus persicae for the corresponding periods are presented.

Table 2

Rothamsted Insect Survey (extracted)

(Myzus persicae (Broom's Barn)

Year		June (weeks) 2	3	4	July (weeks) 1	2	3	4	Aug 1
1965					5	14	27	29	
1966					16	16	0	1	
1967					31	28	18	1	
1968					23	77	38	26	
1969					1	27	212	226	
1970					8	32	18	8	
1971				12	0	18	20	2	
1972				1	13	22	70	30	8
1973	No trial			14	62	42	6	2	
1974	No trial	14	37	34	26	15	5		
1975	No trial			3	3	48	463	52	2
1976		66	434	178	18	10	1		
1977					2	0	13	10	52

64, 30 then in 1971 68% when one would expect 40%. There was no trial from 1973-1975. In 1976 Pentland Crown became 100 per cent infected as was most of the trial after a year with an extreme and prolonged early peak population of M. persicae. From the Table one can see that after 10 years of quiesence with one large count of M. persicae in 1969 the mild winters and hot dry summers of 1975 and 1976 gave unprecedented population explosions. These peaks were traced from the south to the north of the country and left an epidemic of disease in stocks in areas usually relatively unaffected. Fortunately after a cool winter and a very cold spring the picture has reverted from one extreme to the other. A good comparison between 1976 and 1977 is in field counts of M. persicae and Macrosiphum euphorbiae (Thomas) taken in the trial on 100 leaves. In the middle of June 1976, 6,100 M. persicae and 800 M. euphorbiae were found. This is a rate of 69 aphid vectors per leaf. In 1977 only 1.6 leaf were counted on the 6th of July and most of those were M. Euphorbiae . Regular samplings, identifications and counts within the trial are a much surer way of monitoring aphid activity and population flux than suction traps.

Table 3. These are data of leafroll infected plants within the replicates over a 6 year period. Eighty-three per cent of the plants show sequential infection from the infector while 17 per cent show random infection. The plant next to the leafroll infector was 1 1/2 more likely to become infected than the one twice removed. Leafroll spread could be either by apterous aphids or by alates but it is reasonable to assume that it is mainly by the apterous aphids on the evidence of sequential spread of leafroll. The movement of the disease along the ridge is usual with leafroll in trials of this kind.

There are other methods of field trial. Folsom [8] exposed potato seedling between rows of heavily infected Green Mountain and Chippewa potatoes. After many years of exposure his best clone had contracted an average only 7 per cent an 8 year period. He found that leafroll resistance was increased by the use of resistant parents.

Exposure to natural infection alone in the course of propagating varieties and clones should, in areas of relatively high natural spread,

Table 3. Spread of leafroll from the infector as indicated by the relative position and number of plants infected. Plant A being adjacent to the infector and C farthest from it.

Year [1]	Replicates infected [2] single plants and plant combinations [3]						
	A	AB	ABC	B	C	AC	BC
1965	160	142	204	22	18	46	11
1966	131	142	340	33	29	63	29
1967	129	122	134	21	14	33	8
1968	50	88	312	10	11	36	5
1969	84	92	364	9	7	28	7
1970	190	134	227	28	29	82	18
Total	644	720	1581	123	108	288	78
%	18.3	20.3	44.6	3.5	3	8.1	2.2
		83.2				16.8	

	Plants infected [4]		
	A	B	C
1965	552	379	279
1966	676	544	461
1967	418	285	189
1968	486	415	364
1969	568	472	406
1970	552	407	356
	3252	2502	2055
Proportion	1.58	1.22	1

give a rough selection for leafroll and virus Y which might be enhansed. To sum up one may say that field trials for resistance to leafroll and virus Y will function satisfactorily:

1. In years and places where the intensity of infection is such that material in trial can be scored and ranked in order of merit.

2. Where there is adequate replication of material under test.

3. Where the cultural procedures have been reasonably good, and

4. Where there is a reliable method of scoring.

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TURL., L. Verbal

METHODS FOR BREEDING VIRUS RESISTANT POTATOES

H. Ross

In this presentation, I wish to summarize and comment on the various methods that are used for breeding virus resistant potatoes. I will first p· ·sent the methods with respect of each of the three important virus· ·, PLRV, PVY and PVX and later give a general account of classical and population methods of breeding.

1. Breeding for Resistance

1.1 Resistance to potato leaf roll virus (PLRV, Table 1).

For this there exists only one useful type of resistance, i.e. resistance to infection. According to Bawden, this type is based on the fact that a minimal dose of virus is necessary for an infection. This dose differs with the potato genotype. Resistant varieties need more virus i.e. a higher aphid infestation than the susceptible ones.

Many varieties with this type of resistance were bred in several countries. It is highly recommended that CIP be provided with the best of these varieties. Data about the degree (10 notes) of resistance of European varieties were published recently by H. Stegemann and V. Loeschcke: Index Europäischer Kartoffelsorten. Mitteilungen der Biologischen Bundesanstalt, Heft 168. 1976. Moreover, the Netherlands and West-Germany have enlisted the leaf roll resistance of their varieties in variety lists. The following best Dutch resistant varieties are in declining order: Parel, Pimpernel, Ajax, Mara, Climax and Doré. In Germany,

TABLE 1

BREEDING FOR RESISTANCE TO PLRV

Source	Methods	Reaction	Back test
Varieties or breeding lines, resistant to infection; polygenes	a) pre-screening: viruliferous aphids placed on seedling families in greenhouse	a) seedlings with symptoms to be discarded	ELISA-serology or Igel-Lange test in the tuber (resorcin-blue) or in single cases graft onto PLRV, sensitive varieties
	b) viruliferous aphids placed on 3 c[illegible]rge tuber sprouts [illegible]n cuttings in greenhouse	b + c) degree of resistance is estimated by the percentage of infected plants in the tuber progeny	
	c) field exposure of at least 2 x 10 hills with PLRV-infected potato plants each third row; or field plots in a potato growing region with an aphid population peak between 20 and 100 aphids per 100 leaves		

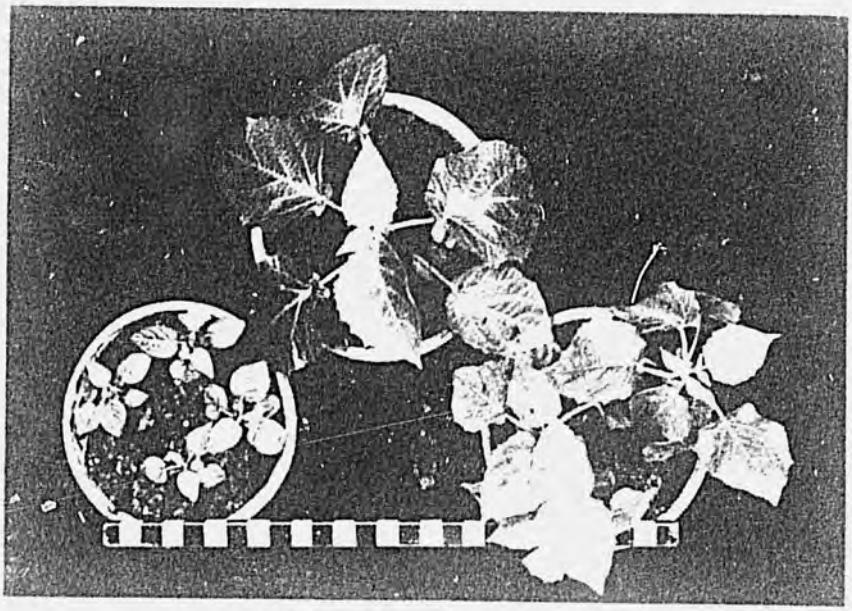
twelve varieties fall in the best category, though not all of them can be termed as very resistant. To get more details and a better knowledge, those who are interested can get in contact with the scientists at the Institut für Virusforschung in Braunschweig. Work in the comparision of best resistant varieties of different countries are missing and it would be very useful for CIP to do this for its own benefit and for that of other countries.

Infection resistance is inherited by polygenes. That means that only a very small percentage of seedlings show the same resistance as the resistant parent, when crossed with a susceptible parent. Both parents influence this percentage. Better results, i.e. a higher percentage is achieved when both parents are resistant. The resistance is greater when the four grand parents were selected for resistance.

This genetic situation is especially important when breeding is done by the population method, i.e. when pollinations are performed by bulked mixed pollen in the field. Every pollen grain from a susceptible parent will reduce the number of resistant seedlings in the progeny. Therefore, the degree of leaf roll resistance should be known for every parental line and should not be too low.

Methods to assess resistance among seedlings can be performed by placing them in a chamber together with virus-bearing aphids, as was done by Hamann and co-workers in East-Germany. Dr. Jones already has used this method here. I too have tried it and found it to be useful for prescreening. However, a standardized method must be elaborated with fixed age and growing conditions of seedlings, strain of PLRV, number of aphids, temperature and humidity.

Tuber lines obtained can be tested in the following years with the same standardized method to get more reliable results because then more than one tuber/plant per genotype will be



[illegible]

[illegible] the stage. The [illegible] at the [illegible] with [illegible] there [illegible]

[illegible]

[illegible] the [illegible] A [illegible] entered [illegible] [illegible] PLPV. [illegible] PLPV spreads [illegible] the eyes finally. [illegible] a very high percentage of the [illegible] dead eyes and will not emerge. A less infected [illegible] will not effect the yield because the [illegible] will emerge.

Fig. 5. Left and right, graft of a PLRV-infected scion on a healthy stock of the susceptible variety Sieglinde, center, healthy control.

1.5 Results and Discussion

Two varieties and breeding strains are mentioned first in the table because of the very favorable inheritance. Immunity is governed by one major gene and this gene prevents the multiplication of the viruses PVA and PVY, so that no plant in the field becomes infected. The gene R_y acts against every strain of both of the viruses including: both the groups Y^C and Y^N, as well as the group C, the strain N 204 (which Dr. de Bokx kindly put at my disposal) and the group of the PVY strains which do not produce local lesions on Ac.

TABLE 2

BREEDING FOR RESISTANCE TO PVY

Source	Methods	1)	Reaction	Back test
Immune varieties and breeding lines based onto *S. stoloniferum* (German cv. Bison, Cordia, Fanal, Franzi, Pirola) 2) gene Ry	a) sap inoculation of seedlings	group Y^N	none	ELISA-serology or
Immune Cornell hybrids based on *S. andigena*; unnamed major gene	b) graft to PVY-infected (and TMV-immune) tomatoes	group Y^N	none or necrotic needle point spots or rusty and necrotic leaves, new growth symptomless	detached leaf-method (A6) or
Scottish breeding lines with comprehensive resistance	grafting and sap inoculation	group Y^N	none or necroses	inoculation of indicator species tobacco cv. Xanthi NN
Varieties resistant to infection; polygenes	field exposure of at least 2 x 10 hills with PVY infected tobacco each third row	indefinite	degree of resistance is estimated by the percentage of infected plants in the tuber progeny	ELISA-or latex-serology or detached leaf-method or sap-inoculation with leaves or tubers to Xanthi-NN-tobacco

1) PVY-strain to be used.
2) Most varieties and breeding lines inherit plasmatically pollen sterility.

Favorable inheritance means that 50% of the progeny is immune. The other parent, of course, can show every degree of resistance or susceptibility without influencing the segregation figures. The 50 percent figure is only an average. The percentage can change by double reduction, by a duplex condition of the gene Ry, by a suppressor gene in the susceptible parent and for other reasons.

The gene Ry was found in some acquisitions of S. stoloniferum, a Mexican wild species. The first crossings were made in MPI in 1944. Beginning with the third back-cross to S. tuberosum the progeny received fairly good combinations of many important properties, so that they could be delivered to private breeders as breeding parents. Nearly every other year new lines with additional properties followed. We have now reached the 7th back-cross. Because of the selection among the different back-cross generations, each comprising up to 50,000 seedlings, unfavorable properties from S. stoloniferum have nearly vanished. In Table 3 the four immune varieties Bison, Fanal, Franzi and Pirola are listed as well as the non-immune ones derived from S. stoloniferum. It illustrates that there is no reason to believe that unfavorable genes from S. stoloniferum would prevent the breeding of varieties. These varieties represent different levels of maturity and some are of high quality. In addition, I could incorporate the gene Fb for resistance to all pathotypes of Globodera rostochiensis in two of these varieties. The MPI-material is also used in East-Germany, Argentina, Czechoslovakia, the Netherlands, Norway, Poland and Scotland. It is also present in the Ir-1 potato collection in Sturgeon Bay.

Another source for immunity exists in the Cornell hybrids of Dr. Plaisted and co-workers, based on a Solanum andigena line and conditioned also by one major gene, hitherto unnamed. While the German lines are often male sterile by plasmatic inheritance, Plaisted's lines are male fertile.

TABLE 3

VARIETIES CARRYING THE GENE R_y

FROM *S. STOLONIFERUM*

Variety	Maturity	Reaction to PVY	PVY-Immune progenitor
Bison	late, high in starch	immune	49.767/9
Ceres	med. late	not immune	49.767/9
Cordia	late	?	61.303/34
Dorado	early	not immune	49.767/9
Fanal	late, high in starch	immune	54.4128/208
Franzi	maincrop	immune	61.303/34
Leander	late	not immune	54.4128/208
Pirola	late	immune	49.767/9
Rosita (Holland)		immune (very prob.)	57.1775/110
Viki	maincrop	not immune	54.4129/288

The best screening method that can be recommended is the sap inoculation of the seedlings from immune parents at a very early stage. (Fig. 3). If the seedlings are in a state where the leaves are laying nearly flat on the soil, the spray gun can be used. If the leaves are higher up on larger seedlings, the air-and sapstream does not press the leaves to the soil but whirls them around so that the inoculation may fail. Therefore, in case the seedlings have grown older or they have become etiolated, it is advisable to infect them with a rubber sponge. As inoculum, a strain of group Y^N should be used because then the appearance of necrosis is largely prevented. After 10 days or later symptoms appear in the susceptible seedlings showing mosaic and leafdrop, and often the growth is stopped. Necrotic seedlings, or those with larger necrotic

Fig. 3. Inoculation of potato seedlings with a spray gun

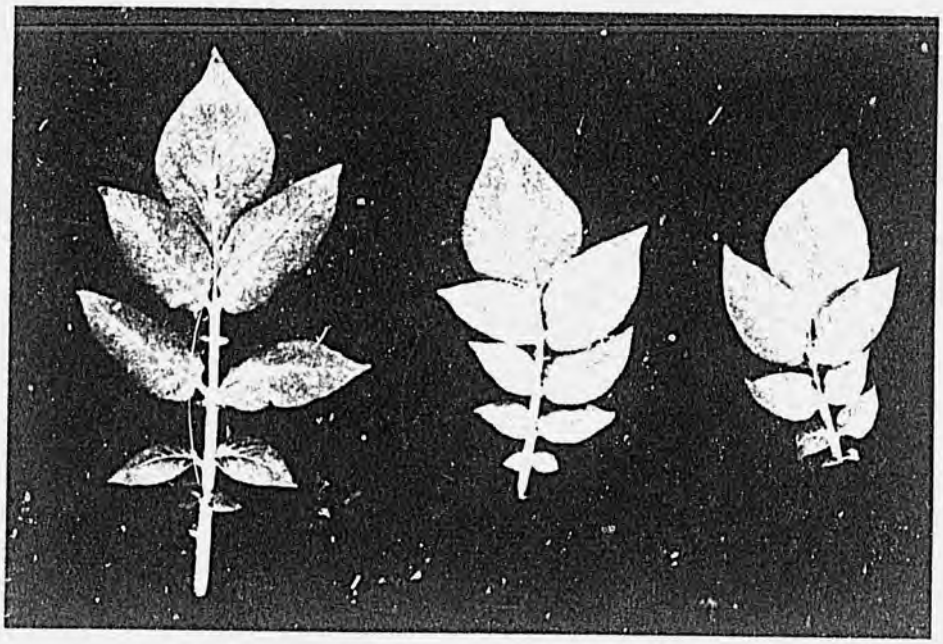


Fig. 4. Upper leaves of a scion with the gene Ry grafted to a PVY - infected stock. Note the length green flecks surrounding a necrotic pin point.

Of the basic test methods the best is the serotest. Although the ELISA-test is the most sensitive one, it is rather complicated and for routine work the latex method may be sufficient. The detached leaf method can be applied if one has already made sure that it works with the applied PVY strain. Tests with indicator plants, of course, are also useful, especially for the detection of aberrant strains not reacting with the genes for immunity or hypersensitivity.

Finally, I will mention breeding with infection resistant varieties. Numerous varieties with a strong resistance exist in several countries. Their relative level of resistance has been reported in occasional variety lists for European varieties in

Fig. 5. Lower leaves of a scion with the gene Ry grafted to a PVY infected stock. Note the necroses spreading from the top of leaves.

the work of Stegemann and Loeschcke. This type of resistance is inherited by polygenes. It means that only a low percentage of resistant seedlings appear inthe progeny and the percentage will be still lower when the non-resistant parent is highly susceptible. I would not recommend to use this type of resistance at the moment extensively as long as the major gene inherited immunity is not broken down by aberrant strains of PVY. Especially when the population method of general breeding is applied, care must be taken to put as much major gene inherited characters as possible into the field of breeding parents, otherwise, the property can easily get lost. A last argument in favor of immunity is that the gene Ry carries immunity to PVY and PVA together.

Nevertheless, it often will be necessary to assess the exact degree of infection resistance to PVY. One should make use of the PLRV exposure trials for this purpose. Not only the percentage of leaf roll infections should be tested but also the degree of PVY-infections. Visual observation leads to errors because PVY-symptoms are often similar to PVX-symptoms. The best test is the serotest. Indicator plants and detached leaves too can be used with sap from leaves. Tubers react best when their inner core is rubbed onto indicator leaves. The outer parts contain a virus inhibitor according to Nienhaus.

1.3 Breeding Methods

About methods to breed potatoes resistant to PVX, Table 4 shows what one should consider. I think it is not necessary to go into the details again. Let me only point out that the field selection with the spray gun works well. Only two plants per genotype are necessary for this screening method. A PVX strain of the mottle group is recommended. The field plants should not exceed 10 cm in height.

As was found by Jones, Salazar and coworkers that PVX-strains exist in Peru able to break the immunity conditioned by the gene Rx from S. andigena and S. acaule. It certainly is necessary to elucidate the occurrence and the behaviour of these strains. However, because of the relatively little importance of PVX, it would in my opinion, not be justified to replace the immunity as a breeding aim by the infection resistance being spread among varieties and breeding parents. The inheritance of PVX-infection-resistance seems to be polygenic. Genetic conditions of this kind are complicated to handle in breeding and should not be initiated unless there is an urgent need as it is with PLRV and - if the immunity type of resistance turns out to be useless - PVY.

TABLE 4

BREEDING FOR RESISTANCE TO PVX

Source	Methods	Reaction	Back-test
Immune varieties based on *S. andigena* cv. Villareola (USA 41956, Tawa, Shoshoni a.o.); cv. CPC 1673 (Dutch varieties) gene Rx_{adg}	sap inoculation of seedlings or spray inoculation of field plants	none or occasional small necrotic lesions on the leaves and aerial tubers	Serology or indicator species *Gomphrena globosa*
Immune varieties based onto *S. acaule* (German varieties Saphir, Moni a.o.) gene Rx_{acl}	graft onto X-infected (and TMV-immune tomato plants		

2. General methods in breeding

Let me add some remarks about the aim to supply developing countries with improved varieties. I understood that true seed will be produced under CIP's supervision in Peru from gene-pools as broad as possible and be sent abroad to Stations and places in many areas for selection by national program investigators.

I would like to restrict myself to the first part of the aim, i.e. a method to produce seed in Peru with as many genes for resistance to viruses incorporated as possible. The success, no doubt, depends on the selection of the best parents and the pairings. I suppose that there are at least two breeding gardens available, one for highland conditions in Huancayo and one for lowland tropic conditions in Yurimaguas.

There are two different methods for breeding:

2.1 First the classical method. In this controlled fertilizations are performed between parents which are proved to be adapted to their respective regions in their yield, maturity, quality traits and resistance properties. Because of the controlled fertilization it can be planned and forseen how many seeds for different required combinations can be obtained. By observing the berry-set, corrections regarding the number of special pollinations to be made are possible at any time. The pollinations usually are made with flowering stalks in bottles in greenhouses. Most important is that the different progenies can be analyzed giving information about the combining ability of the parents.

2.2 The population method. This is the modern method of breeding with bulk pollination and mass selection. I do not deny the importance of this method for producing lines best adapted to a special new region. I think that both these methods are useful, because by the first one the undeniable necessary knowledge about the genotype of a parent can be obtained.

With the population method three variants are possible:

2.2.1 Seed arising from 2x . 2x - crosses. At first, let me say that I do not doubt that varieties can be bred at the 2x-level. Inter-crossings with dihaploids in my breeding garden showed a rising yield from generation to generation. Now in H^3 and H^4, the average yield still is below that of 4x . 4x - crosses, but a considerable percentage of the seedlings yield very close to the 4x - yield. I have no reports about Dr. Haynes' results with S. phureja - and S. stenotomum cultivars. Maybe he has good results with his non-inbred material. The breeding garden is planted with dihaploids and interdihaploids. These are mostly self-incompatible; that means that nearly all berries are produced from cross fertilization. Therefore, artificial fertilization is not necessary. The useful properties of the parents must be known. At any rate, lines which should supply properties inherited by polygenes must be present in far higher proportions than those contributing major gene inherited characters. That means that especially PLRV - resistant lines must be present in a higher proportion than those with PVY - and PVX-immunity. Especially valuable are those parental lines with multiple virus resistance (Table 5). This variant can be applied not only in Peru but in developing countries where people are available with limited training.

2.2.2 The breeding garden comprises 4x - breeding parents and varieties. These are self-compatible. Therefore, berries formed naturally in the field, are generally useless. It is necessary to do handfertilization, using a mixed pollen collection from all flowering plants. It is advisable then to bring the pollen only to the polygenic inherited PLRV-resistant parents. Otherwise this property may be easily lost.

TABLE 5 Breeding lines of the Max-Planck-Institut fur Züchtungsforschung, Köln-Vegelsang, with immunity to PVY.

Clone Nr.	Reaction to PVY	PVX	Resistant to PLRV	Corostochiensis
57.1729/62	immune	-	very res.	-
57.1775/110	immune	immune	res.	-
58.10/119	immune	immune	‡ res.	-
58.229/360	immune	-	res.	-
59.697/20	immune	immune	res.	-
59.704/168	immune	immune	res.	-
60.1237/324	immune	immune	very res.	-
61.511/143	immune	immune	res.	-
61.515/64	immune	immune	res.	-
61.519/53	immune	immune	res.	-
62.47/20	very res.	-	very res.	-
63.634/32	immune	-	res.	-
64.953/74	immune	immune	very res.	yes
64.956/68	immune	immune	res.	yes
66.1043/50	immune	immune	very res.	yes
65.378/23	immune	immune	res.	-
68.711/1	immune	?	res.	yes
69.1409/44	immune	immune	± res.	yes
70.86/39	immune	immune	res.	yes
70.94/124	immune	-	res.	yes
71.234/14	very res.	-	res.	?
72.545/19	immune	immune	res.	yes

1) flowers only when grafted to tomato

e = early, emc = early maincrop, mc = maincrop, l = late. All clones except 62.47/20 are pollen sterile

TABLE 5 (continued)

Maturity	Other properties	yield
emc	res. to P. i. and Synchytrium races	Ø
l	res. to P. i.	> Ø
mc	very res. to P. i., white flesh, high in starch	Ø
emc/ml	res. to P. i., white flesh	Ø
emc	res. to Streptomyces sc.	Ø
mc/l	res. to Streptomyces sc., high in starch	Ø
emc	very res. to P. i., high in starch	Ø
e/mc	res. to P. i., high consume quality	Ø
emc/mc	very res. to P. i. and Streptomyces sc.	> Ø
mc	very res. to P. i.	Ø
e	inherits many good properties	Ø
emc	res. to P. i. including tuber, high consume quality	Ø
l	high consume quality	Ø
e/emc	high consume quality	Ø
emc	res. to Streptomyces	< Ø[1)]
e/emc	res. to P. i.	Ø
emc/mc	white flesh	Ø
e/emc	high protein content	Ø
e/emc	high protein content	> Ø
e/emc	high protein content, very res. to Streptomyces	Ø
e	high consume quality	Ø
emc	very res. to Streptomyces and Fusarium	< Ø

2.2.3 In this variant the breeding garden comprises 2x- and 4x- breeding parents and varieties. Seed should be taken only from the 2x- plants. It will be partly or perhaps mostly 2x from 2x . 2x- fertilization (see 2.2.1). Besides, 3x- seedlings will arise, which are often heterotic but sterile. The diplogynoid tetraploids will also be formed. A high percentage of these show a considerable heterosis in yield but than in itself does not make them of outstanding value. If the diplogametes are formed by the mechanism of parallel spindles, they contain the whole undisrupted genetic constitution and combination of the 2x- parent that transfers them undisturbed, varied only by crossing over, to the zygote. The geneticist will know what it means when nearly all seedlings from such 2x . 4x cross have received all the properties of the selected diploid parent, so far as they are inherited in a dominant manner, which is at least true for immunity to PVY, PVA and PVX. Besides, a few quadragametic tetraploids will be formed from crosses 2x . 2x. These are also often heterotic.

I do not hesitate to propose such a composition out of 2x- and 4x- parents as an excellent solution for a breeding garden aimed at producing seed for a developing country or for a new region. Even for Stations and Institutes engaged for a long time in potato breeding such breeding performance would certainly be progressive.

There is another advantage. Because no artificial fertilization is necessary, there is no need to do the crossing with skillfull people in Peru while the selection of the seedlings for adaptation is going on in the respective countries. With the 2x- 4x- method both, i.e. the parents as well as the seedlings, can be planted in the very region for which it is bred. So they both undergo adaptation selection.

Up to now it would not have been possible to perform this efficient method because the production of unreduced gametes and, therefore, diplogametic tetraploids is low. Three years ago we found only very few interdihaploids, produced in crosses with 4x-parents between 30 and 100 seeds per berry contrary to the majority of 2x- lines which formed only one or less seed per berry. Ir. Jacobsen, at MPI, has worked efficiently in producing 2x- breeding lines with a high production of diplogametes. This year he had 40,000 seeds from 4x.2x- crosses. They came from 35 dihaploids out of 9 families which produced higher percentages of diplogametes. However, all families were of the direction 4x . 2x. But, we know that the tendency to form diplogametes is similar on both sides although generally more diplandroids are formed than diplogynoids. There is no doubt that more investigation will open the way to improve 2x . 4x breeding as a new and practical tool in population breeding. A participation of the International Potato Center in this research would certainly be a benefit for all interested in potato breeding.

DETACHED LEAF METHODS AND NEW INDICATOR SPECIES

J. A. de Bokx

Introduction

The presence of viruses can be detected by the symptoms they cause in potatoes or in special indicator plants, by their reactions with specific antisera or by electron microscopy. In seed-potato growing, symptomatology is still a very important element in certification schemes, since field inspection and roguing are corner stones of any seed program. However, symptoms induced by viruses are not always very clear or may be even absent. Therefore, a number of "laboratory" methods have been developed for the detection of plant viruses, in support of the field inspection.

Particularly for diagnosis of sap-transmissible viruses, lesions on specially selected hosts (indicator plants) are often used. Two types of indicator (= test) plants are common: those that develop systemic symptoms and those that develop local lesions upon inoculation with a virus. In the latter case often necrotic or chlorotic spots or rings are formed. Systemic symptoms appear after the virus has spread to the un-inoculated parts of the plants.

If an inoculated indicator plant reacts with local lesions, there are often no systemic symptoms, viz. Gomphrena globosa after inoculation with PVX. Alternatively, both local and systemic symptoms may appear after inoculation, as in A6 after inoculation with PVY, and in Physalis floridana after inoculation with PVY°. In these instances, detached leaves of the indicator plants may be used.

Detached leaves have the advantage that many samples of infected potato can be tested routinely (Quantz, 1957; de Bokx, 1964; Kratchanova, 1976).

Detached leaf methods

To be applicable in practice indicator plants should be easy to raise in all seasons, they must remain susceptible to virus over a long period of their growth, be convenient to inoculate and give an uniform response.

In principle, detached leaves of any indicator plant can be used for detection of viruses if the indicator reacts upon inoculation with local lesions and if the detached inoculated leaves show lesions before they deteriorate (depends on habit of the foliage and lengh of incubation time of the virus). Generally detached leaves can survive a period of about 10 days if kept at high humidity.

A number of indicator plants, suitable for detached leaf methods are used for the detection of potato viruses, viz. A6, Solanum demissum Y, Solanum demissum A, Solanum chacoense (TE), and Capsicum annuum.

Since the A6-detached leaf methods is generally applied for the detection of PVY in several different seed-potato producing countries, this test will be discussed in detail.

Methods of inoculation

Before inoculation with sap, the test-leaves are dusted with an abrasive, Carborundum powder (350-500 mesh) or Celite, which can be applied to the test leaves with an insufflator. The test leaves can be inoculated with virus-containing sap using cheesecloth, a glass spatula, or a plastic sponge. However, in routine procedures the dry inoculation (= quick inoculation) (Yarnwood, 1953) is recommended.

Squashed sprouts from potato tubers used as inoculum yield reliable results. Cut potato tubers do only if they are secondarily infected and stored at high temperatures (de Bokx, 1974; 1976).

Factors in the susceptibility of test leaves

Leaves, half leaf or leaf pieces from midway up the stem produce the highest number of lesions, whereas on leaves from the top and base of the A6-plant a lower number of lesions will be produced. The same holds for the indicator *Solanum demissum* Y (SdY).

When A6-plants are about 4 weeks old, susceptibility to PVY is high but decreases during flowering. Leaves of SdY and SdA are useless during flowering, since then necrotic spots on the foliage of non-viral origin develop which may interfere with local lesions induced by virus.

A6-plants fertilized with high N-rates (e.g. 5 g ammoniumnitrate-limestone/NH_3NO_3) / pot 8 l) are more susceptible to PVY than those with low N-rates or any fertilized (de Bokx, 1964; Bérces and Keller, 1968). Plants with balanced nutrition (NPK 10-12-18) grow best and produce leaves with high susceptibility over the longest period. The largest plants with the greatest leaf yield are produced by application of complete fertilizer. The different fertilizers variously affect time of flowering which is earliest with complete fertilizer.

Light and temperature before inoculation

Leaves of A6 grown under long-day conditions (16 h light a day) produce 4-20 times as many local lesions as leaves grown on plants lit only for 9 h a day if the temperature is 21-22° C. Plants grown under short-day conditions produce yellowed foliage and do not flower. Leaves of SdY submitted to these conditions do not differ in reaction, when inoculated with PVY^N

Shaded 'A6' leaves (light intensity about 40,000 lx) inoculated with PVY^N yield more local lesions than unshaded A6 leaves (light intensity about 80,000 lx). High light intensity, especially combined with high temperature, is unfavourable for the production of indicator plants with soft, succulent foliage. No effect of temperature on the susceptibility of A6 leaves to PVY could be observed when plants were grown at a constant temperature of 20° C or with a temperature fluctuating between 15° and 25° C.

Light and temperature after inoculation

When detached 'A6' leaves inoculated with PVY^N are stored at 20° or 24° C and illuminated for 24 h a day, a high light intensity (2000 lx) induces more local lesions than a low intensity (500 lx). Local lesions then appear 5 days after inoculation. A short period of illumination (e.g. 8-10 h/day) affects the local lesion production on A6 unfavourably. However, decrease of illumination by about 8 hrs a day does not greatly affect the appearance of local lesions (Table 1).

SdY and SdA do produce local lesions even when kept in the dark, whereas inoculated A6-leaves do not.

Table 1. Effects of daily duration of illumination (1875 lx) on the number of lesions on 'A6' after inoculation with PVY^N.

Average of 20 leaflets (percentage of test leaves showing lesions)

Number of days after inoculation	Duration of illumination (H/day) 24	16	8
4	7 (80%)	6 (80%)	0 (0%)
5	18 (100%)	17 (100%)	3 (50%)
6	20 (100%)	23 (100%)	8 (80%)

Temperature affects the development of local lesions on detached inoculated A6 leaves more than light intensity does. The optimum temperature for development of local lesions on A6 after inoculation with PVY^N or PVY^C is 24° C, whereas A6 leaves inoculated with PVA produce most lesions when kept at 18-20° C. PVY^0 also induce most lesions when kept at 18-20° C. PVY^0 also induces lesions in A6 leaves when kept at 14° C. However, the incubation period is then longer than 5 days.

Humidity

To maintain a high relative humidity, inoculated test leaves are placed on wet filter paper which is kept in suitably sized trays of plastic or of zinc. Plastic trays are put in plastic bags and zinc trays are covered with glass plates. On the bottom of the trays is a layer of water to maintain humidity.

Recently another routine method has been developed to store inoculated test leaves (Bakker et al., 1977): the inoculated leaves on wet filter paper are kept between sealed sheets of plastic. The sheets, which are 3-5 cm apart, hang vertically and are illuminated sideways by a vertically placed light source. The reability of the test is equal to that of the previous one.

Detached leaf methods and viruses detected

Capsicum annum is susceptible to several potato viruses, e.g. PVY, but since plants are raised from seed, contamination of detached leaves with unwanted viruse does not often occur. This method has been described for detection of PAMV, but it has not yet become a routine method.

Solanum chacoense (TE_1), is susceptible to TMV, TNV, TRV and AMV, not susceptible to PAMV, PVA, PVM, PVX and PVS (de Bokx, 1974). TE_1 plants are propagated from tubers or cuttings.

They grow well throughout the year without additional illumination but the foliage production is low. Strains of PVY^C, PVY^N and PVY^0 induce pin-point necrotic lesions on detached leaflets of TE_1 3-4 days after inoculation, when kept under conditions similar to those described for detached A6 leaves. Detached TE_1 leaves are used for detection of PVY to some extent in some Eastern European countries.

Solanum demissum A (SdA) and Solanum demissum Y (SdY) (Cockerham, 1958) are propagated by true seed. Not much information is available about susceptibility to common potato viruses. SdA should react with local lesions upon inoculation with strains of PVA only, whereas local lesions are said to appear on SdY after inoculation with strains of PVA and PVY. Detached leaves of those indicators are not used routinely.

The clone 'A6' (Solanum demissum X Solanum tuberosum cv Aquila) is mainly propagated by tubers. It can be systemically infected with many viruses such as PVX, PVY, PVS, PVM and PLRV (de Bokx & Chrzanowska, 1972), so A6 indicator plants reacting locally with necrotic lesions after inoculation with PAMV, PVX or PVY must be grown under aphid-free conditions. Detached A6 leaves are used for detection of PVA and PVY. The use of detached A6 leaves for detection of PAMV and PVX has not been investigated throughly. A6 reacts with local lesions after inoculation with many strains of PVY^C, PVY^N and PVY^O. For unknown reasons PVY^C does not always induce clear necrotic lesions under "standard" conditions. A strain of PVY^O has been described which does not induce local lesions at all in A6 (de Bokx, 1975).

New indicator species

The search for new indicator plants is time-consuming and can be unproductive. Although there is often a chance that plants closely related to a host of the virus under study can be infected, the results are not predictable and the choice of species empirical. Several

new indicator hosts for potato viruses have been described, although not much is known yet about their practical suitability.

- Nicotiana bigelovii reacts with a characteristic systemic vein clearing and necrotic netting of minor veins, followed by mosaic symptoms after inoculation with several strains of APLV (Fribourg et al., 1977).

- N. bigelovii is also a suitable indicator plant for the detection of APMV (Fribourg et al., 1977).

- Physalis peruviana var. macrocarpa and P. pruinosa is locally and systemically susceptible to PAMV and PVX (Horváth, 1974).

- Paulownia fargesii was found to be a suitable local-lesion host for PAMV, PVX, TRV and TRSV, but it is immune to alfalfa mosaic virus, PVM, PVS (Horváth, 1973).

Potato black ringspot virus causes necrotic local lesions and systemic necrosis in Gomphrena globosa, Chenopodium amaranticolor, C. quinoa and Phaseolus vulgaris cv. Pinto or Sanilac (Salazar and Harrison, 1977).

- Chenopodium amaranticolor produces fine concentric necrotic ring lesions after inoculation with potato mop-top virus (Jones, 1975).

- Lycopersicon glandulosum, L. hirsutum, L. humboldtii, L. peruvianum, L. pimpinellifolium, L. pyriforme, L. racemiflorum and L. racemigerum react with epinasty and crinkle after inoculation with PVM (Horváth, 1971).

- Lycopersicon chilense shows clear systemic symptoms with PVM and PVS. Symptoms of PVM are crinkle and epinasty which appear about 10 days after inoculation with PVM. Epinasty and leaf-drop appear up to 10 weeks after inoculation with PVS (Ross, 1968).

Lycopersicon chilense so far is the most reliable test plant for PVM and Chenopodium quinoa for PVS. In both cases 12-24 days are required for the symptoms to appear. The most rapid development of PVM symptoms results from inoculationon Phaseolus vulgaris cv. Red Kidney (4-8 days) and of PVS symptoms on detached leaves of Solanum demissum Y (6-8 days). The detectability of the viruses on these test plants is, however, lower than that on the ones previously mentioned (Kowalska and Wás, 1976). These authors mentioned Cucumis sativus to be a local-lesion host for PVM. Phaseolus vulgaris cv. Red Kidney a local- lesion host for PVM (Hiruki, 1973) showed also local lesions after inoculation with PVS (Kowalska, 1977).

Potato virus T produces a characteristic systemic necrosis in Chenopodium amaranticolor; it can be assayed in Phaseolus vulgaris cv. the Prince or cv. Pinto, in which local lesions are produced when leaves are heavily shaded after inoculation.

- Chenopodium botrys, C. capitatum, C. foetidum, C. giganteum, C. glaucum, C. opulifolium, C. quinoa var. rubescens, C. quinoa var. viridescens show local lesions 4-6 days after inoculation with PVY and PVX (Horváth, 1968).

Pisum sativum cv. Rondo is found to be a local-lesion host for strains of PVX, however, strain differentiation with this plant is not possible (de Bokx & Janse, 1977).

Useful local-lesion hosts for PVX, showing lesions 4-7 days after inoculation, are mentioned by Fribourg and de Zoeten (1975) viz. Canavalia ensiformis, Crotalaria juncea, Dolichos lablab, Phaseolus lathyroides, P. vulgaris cv. Pinto and some subspecies of Vigna unguiculata.

- Tobacco ringspot virus (Andean potato calico strain) produces unusual necrotic local lesions in Cucumis sativus and systemic epical necrosis in Chenopodium quinoa, C. amaranticolor and Petunia violaceae (Fribourg, 1977).

GENERAL REMARKS

The presence of viruses in potato plants can be determined with the use of indicator plants. If e.g. for grading in seed-potato production, large numbers of plants have to be checked a detached leaf method is preferable to the use of intact indicator plants in pots, since the latter need much greenhouse space. However, test plants alone cannot give a reliable identification of a newly isolated virus nor, on present evidence, can they form any basis for virus classification. They do, however, offer a very sensitive and reliable means of detecting virus: over one hundred times more sensitive than the common serological tests or electron microscopy (de Bokx & Piron, 1977; de Bokx, 1969). Indicator plants can differentiate between virus strains that appear identical with other techniques and are the most reliable means of indexing mother plants to provide virus-free foundation stocks.

It is not possible to give a general advice on the application of one specific indicator plant in various parts of the world with different climates. Although growing conditions for indicator plants can be standardized using growth chambers, it would be cheaper in the long run to use a type of indicator plant which can be grown in an aphid-free greenhouse under local conditions. A6, for example, is used for detection of PVY in various countries with a moderate climate. However, it is possible that in other climates SdA and SdY would yield better results than A6 does.

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SUMMARY OF HISTOLOGICAL STAINING METHODS

Dr. D. Peters

The callose test has been used in the diagnosis of PLRV since 1954. In sieve elements of infected tubers and plants an extra deposition of callose often occurs. This can be detected after staining slices from tubers with resorcine blue and viewing at low magnifications with a light microscope.

The heel end of the tubers is preferred above the other parts of the tuber. In a study, it has been shown that 63% of the slices from the heel end were positive, 50% from the centre part of the tuber and 39% from the rose end. In this study, 10 tubers of plants with primary leafroll virus infection were used. The outermost part of the heel end has to be excluded.

The method is not completely reliable. Positive reactions cannot always be found in sections from infected tubers, whereas sections of healthy tubers may also react positively. The deposition of extra callose in healthy tubers can be promoted by several treatments. After treatments such as heat, herbicides, electric current storage in CO_2 rich environments, extra callose depositions has been found in the sieve elements.

The infection can readily be detected when:

a) the plant has been infected for a period of at least 6 weeks

b) the tuber has been stored after harvesting for three or four weeks at temperatures of 15 - 20° C.

The test can be used in a number of varieties e.g. Bintje to estimate the percentage of infection when this percentage is high. Not all tubers with infection will be found, but this will be compensated by those healthy tubers which reacts positively. With other varieties e.g. Sientje, very unreliable results have been obtained as positive reactions can be found in many healthy tubers. In order to learn to what extent the method can be used, experience has first to be acquired with the strain or variety in question.

To detect one infected tuber out of many the test can not be used.

A new staining method has recently been developed by Ulrychova for the detection of callose. Using Poinceau S a red staining of callose is obtained. Unfortunately, no information if available about the reliability to detect PLRV-infected tubers. I expect that this staining procedure will have the same (un)reliability as the resorcine test.

Many plant viruses induce distinctive intracellular inclusions. They may contain virus particles and/or other products of the viral genome and, in some cases, modified cell constituents. Most inclusions are induced with great constancy over a range of hosts. Their detection can provide a rapid and relatively inexpensive method for determining viral infection.

A technique in which epidermal strips has been stained with acridine orange has been developed by Hooker (Exp. Cell Res. 33, 609-612, 1964). Another method in which excellent staining of all inclusions containing protein, or/and RNA, can be obtained has been developed by Christrie and Edwardson (Light and electron microscopy of plant virus inclusions, Florida Agric. Exp. Sta. Monograph Series Nog, 1977).

Virus specific inclusion bodies are always observed in infections with PVY, PVX, PVM and PVS. Although the occurrence of these inclusion bodies is highly recommended for use in diagnostic work and screening activities, staining methods are seldom used, and it seems that hardly anybody has experience in evaluating staining methods in virus diagnosis and screening. The species S. tuberosum is seldom used in studies of inclusion bodies.

DETECTION OF POTATO VIRUSES BY ELECTROPHORESIS ON POLYACRYLAMIDE GELS

R. P. Singh

When a susceptible plant is infected by a virus, symptoms develop which are characteristic for the given virus-host combination. These symptoms may be either macroscopic or so subtle that of ten it becomes difficult to distinguish infected plants from the healthy ones. But whether or not symptoms are obvious to the naked eye, their expression is often primarily the result of changes in the physiology of the host plant brought on by a specific interaction of the virus and the host genome - directly or indirectly. A virus-infected plant thus may contain proteins specifically coded for by either viral or by host nucleic acids as part of the response to infection. Often these components in infected host do not lend themselves to ready detection by conventional procedures, so the need to develop new and more sensitive detection procedures has become necessary. Witness the advances made in the development of techniques of serology, immunoelectrophoresis, florescent antibody, electron microscopy, and electrophoresis on agrose or polyacrylamide gels in the very recent past.

On this basis, van Loon and van Kammen (1970) tried to apply disc electrophoresis procedure to detect virus-induced proteins from infected plant tissues. However, the resulting protein bands in the gels had little specific diagnostic value because similar qualitative changes in certain proteins were observed after infection with other different viruses (van Loon, 1975). Also, the presence and intensity of protein bands seemed to depend on whether or not tissues were showing necrotic symptoms.

Efforts to detect viral components themselves for diagnostic purposes have been successful, even though only a limited number of studies are available on this aspect. Several of the major ones are briefly described here.

A. Viral coat proteins

Paul (1974) experimented with the detection of coat proteins of plant viruses using electrophoresis on polyacrylamide gels. In his earlier work he observed that proteins from highly purified plant virus particles had produced distinct bands with sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Moreover, the band positions were characteristic for a given virus. Thus, it seemed plausible that SDS-PAGE could be helpful in determining the presence and perhaps the identity of a virus in a plant, provided complete purification of the virions could be conducted using relatively simple procedures, that did not entail substantial losses of the intact virion. Based on the analysis of 9 strains of potato virus X, Paul (1974) made the following observations:

1. Protein bands produced at 50×10^3 MW were host specific, while those at 20 to 30×10^3 MW were PVX specific.

2. A given strain grown in different hosts revealed very similar patterns.

3. The band patterns made by 9 strains seemed less uniform when compared to each other.

4. A strong band at 28×10^3 MW was always present, but it showed anomalous migration in different concentrations of gel (3.5% and 1.5%).

5. A band near 24×10^3 MW was very variable in intensity and sometimes absent, although it behaved normally at various gel concentrations.

6. When different purification procedures were used with one strain, total number of bands were reduced but favored a band at 26 x 10^3 MW.

7. Healthy Solanum demissum cv. A 6, produced bands which can be interfered with PVX - specific band. Questionable bands at 23 and 25 x 10^3 MW range appeared with healthy S. demissum.

From these observations it is clear that virus specific protein bands can be demonstrated by using electrophoresis on polyacrylamide gels, but the band position and intensity varies depending on purification procedure, concentration of polyacrylamide gels, the virus strains and the host plant. Moreover, when other plant viruses were examined, they also produced specific bands in the size range of 20-30 x 10^3 MW, thus, identity of individual viruses based on SDS-PAGE appears of less diagnostic value than other methods. In 1975, Paul analyzed the SDS-PAGE patterns of several other plant viruses, including some potato viruses and arrived at the following conclusions:

1. Detection by SDS-PAGE of potato virus A and potato virus Y necessitated concentrated virus solutions.

2. Potato virus Y always produced more than one specific protein band.

3. Extracts containing potato virus M and tobacco rattle virus failed to produce discernible virus specific bands.

Although only these studies have been reported so far, it appears that even to demonstrate virus specific protein bands two fundamental criteria must be met. First, the virus concentration in the infected tissues must be relatively high; and secondly, the host should be relatively free of any protein band of similar mobility as virus specific proteins. With reference to the potato viruses, it remains to be established whether potato plants

contain proteins which would interfere with virus specific proteins. The nearest relative Solanum demissum used with potato viruses X and M has thus far given poor results.

B. Nucleic acid

To date relatively facile isolation of virus nucleic acid is possible only with those viruses which occur in the form of free ribonucleic acid in the plant tissue and whose nucleic acid size is sufficiently different from those of cellular host RNAs. Among the potato viruses, spindle tuber agent occurs as free ribonucleic acid (Diener and Raymer 1967, Singh and Bagnall, 1968). Also, spindle tuber ribonucleic acid is of low-molecular weight (Diener 1971, Singh and Clark 1971) that migrates discretely in the gel electrophoresis compared to other cellular RNAs (Morris and Wright 1975).

Morris and Wright (1975) developed a procedure which involved extraction of cellular nucleic acids from infected tissue and their separation by polyacrylamide gel. After staining with toludine Blue 0 a PSTV band was detected which was of diagnostic nature because no comparable bands were present in the healthy tissue. They made the following observations:

1. The mild strain and severe strains showing respectively mild and severe symptoms in tomato, had a specific band - "PSTV band" after gel electrophoresis.

2. Dormant potato tubers from plants grown from infected tubers showed a PSTV band.

3. Presence of other potato viruses did not affect position or appearance of PSTV band.

Subsequently, Morris and Smith (1977) reported a modified procedure, permitting rapid extraction of nucleic acid. The modified procedure took only 2 days instead of 3 in the original procedure. The improved procedure detected PSTV band as efficiently as the old procedure. The procedure detected PSTV band from as little as 0.2 gm of infected tissues.

In order to monitor PSTV in large plantings of elite or foundation seed stock, they proposed an intermediate bioassay on tomato seedlings; where the extracted nucleic acid was inoculated to tomato seedlings and incubated for 3-4 weeks to allow the PSTV concentration to build up to detectable levels. Then the nucleic acid was extracted and analyzed by electrophoresis on gels. Using this bioassay procedure, they reported that they were able to detect 1 infected disc which was mixed with 250 healthy discs, thus demonstrating the possibility of combining samples for large scale indexing of PSTV.

Discussion of PSTV indexing procedure

Besides the gel electrophoresis method of PSTV indexing, tomato (Raymer and O'Brien, 1962) and *Scopolia sinensis* (Singh, 1971) indicator plants are also used. In bioassays the inoculation procedure is rapid but 2 to 3 weeks of incubation period is needed before definite results could be known, while in the gel electrophoresis test extraction of nucleic acid is time-consuming but indexing results are known within 3 days. The use of gel electrophoresis for detection of PSTV has been successful so far where plants have been infected and showing symptoms. No information is available in cases where PSTV contamination occurred during the growing season. Whether these "new infections" could be detected by gel electrophoresis is not known.

Secondly, the proposed intermediate step of tomato-inoculation followed by gel electrophoresis for monitoring large number of potato plants is contingent on the assumption that infection from single infected plants in a mixture of 250 or so healthy plants would take place. And even if infection were to take place in these circumstances, an incubation period of 3-4 weeks is still needed for nucleic acid concentration to build up to detectable levels. Moreover, if infection takes place and plants are maintained as described by Yang and Hooker (1977) then even mild strains should develop symptoms, thus eliminating entirely the use of cumbersome nucleic acid extraction and gel electrophoresis.

The intermediate bioassay - gel electrophoresis procedure is suitable only for seed stocks where finding of any infection means discarding of the whole "lot". It should not be applied to various parental or breeding stocks as those maintained in commonwealth potato collections, CIP, and various potato breeding projects which require information on individual clones or lines.

Thirdly, in our own studies, we found that gel electrophoretic patterns are seldom uniform and often difficult to reproduce. For example, when field-grown Bintje plants were used, nucleic acid extracted from tissues failed to yield a definitive PSTV band because of overall variability in nucleic acid mobility. When grown under different environmental conditions, i.e., greenhouse vs. field, bands varied considerably in both number and appearance.

Actually, to date no throughly reliable comparison has been made in which the results of both indicator plants and gel electrophoresis have been compared. Unquestionably much more work is needed to establish a definitive comparison.

Recommendation for PLSTV indexing

Biological tests for infectivity are the most sensitive and specific and can detect fewer than 10^6 virus particles per ml. Electron microscopy can only detect about 10^8 particles per ml, and chemical and serological tests are even less sensitive and specific; they can detect 10^9 particles per ml, or perhaps fewer when radioactive labelling is used, whereas physical tests such as optical assay methods are usually the least sensitive and will only detect 10^{10} or more particles per ml (Gibbs and Harrison, 1976).

Considering the above statement and applying it to the spindle tuber agent (which occurs in extremely low amounts in the infected tissue when compared with the amount of other cellular RNAs), which has extremely high specific infectivity (Sanger *et al.*, 1976), it stands to reason that indicator plants should be used for indexing. For example, with PSTV, tomato and *Scopolia sinensis* are both equally sensi-

tive (Singh, 1971) and both could be used to detect infection within 2-3 weeks after inoculation of the potato plants themselves (Singh, 1977). However, both indicator plants require somewhat special environmental conditions and these are described briefly below.

Diagnosis by Scopolia sinensis test. - The seedlings should be grown in relatively low light intensity (300-400 ft-candles) at a temperature of 18-23° C with a relative humidity of over 60%. It is imperative that the plants be regularly fertilized to ensure vigorous growth, and only fully-developed succulent leaves should be used for the inoculation. Usually inoculum in the form of phenol extract (Singh and O'Brien, 1970) is preferred to crude sap. Inoculated plants should be maintained under the same environmental conditions as described above. With severe strain of PSTV local lesions usually develop in 8-10 days whereas with mild strain these take 14-18 days. Local lesions are invariably followed by systemic symptoms of extensive veinal necrosis and finally by leaf drop.

Diagnosis by tomato test. - No special care should be given to growing tomato seedlings, but it is important that they be inoculated in the cotyledonary leaf stage - preferably using phenol extract. However, after inoculation, the plants should be maintained under continuous light regime (Yang and Hooker, 1977) of 1000-2000 ft-c, at a temperature of 27-31° C with a relative humidity of over 60%. The presence of manganese (Lee and Singh, 1972) in the growth medium enhances the symptom expression and should be included, if possible. The PSTV symptoms appear in 2-3 weeks as a characteristic bunching of the tops and extensive veinal necrosis in the developed leaves.

Conclusion. - It hardly bears mentioning that for most potato viruses, indicator plants have been and continue to be more sensitive and easier to use than gel electrophoresis in detection and diagnosis.

For the potato spindle tuber agent, on the one hand if sample number is low (for example, nucleus stock, etc.), they should be repeatedly indexed using both indicator plants and gel electrophoresis, since none of these tests are by themselves one hundred per cent reliable. On the other hand, if the sample size is relatively large and the

potato stocks represent different Solanums, for example, in breeding stocks, etc., each plant should be tested on the indicator plant.

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ELECTRON MICROSCOPY AND PLANT VIROLOGY

Dr. G. De Zoeten

The traditional approach to answering questions of virus identity has been an attempt to transmit the unknown causal agent to healthy plants of the same genus or to other herbaceous hosts. This, however, constitutes a laborious, imprecise method of identification, that suffers additionally from lack of scientific method in determining what indicator host to use. Results of identification attempts based on host range studies alone are tentative at best.

Serology alone as a method of virus identification constitutes a shotgun approach. This can be alleviated only minimally when the two techniques are combined. However, in many laboratories these two methods are the tools used mainly in virus identification.

Electron microscopy is the most important initial step in plant virus identification that should preceed both host range studies and serology, since it provides information on virus morphology that can be used to guide both the choice of indicator hosts, and antisera to be used subsequently.

Manifestations of virus infection in plant cells are of three kinds:

a) Host generated membranes. These are usually associated with the viral polymerase and the replicative forms and replicative intermediates

b) Virus coat protein and anomalous proteins, and

c) Virus particles.

These virus-related manifestations of infection are virus or virus-group specific and can be used, therefore, as identifying characteristics.

Sectioning techniques for electron microscopy can be used to reveal the presence of all three or any one of these virus infection associated structures. These techniques, however, cannot be classified as "quick", however, much they seem to yield in information about a particular virus.

Host generated membranes

Membrane proliferations constitute most probably the host-DNA-dependent early step in plant virus (SS-RNA viruses) replication. Although membrane proliferations are associated with many virus infections, they have been associated unequivocally with RF, RI and viral replicate for only four plant viruses.

Viral coat protein and anomalous proteins

Viral coat protein in its unassembled form seldom forms identifiable inclusions. Inclusions of empty capsids or crystalline virus coat protein inclusions have been reported for some viruses and can be used as characteristics for identification purposes.

Although there are methods available to detect the location of coat protein in infected cells, they involve sophisticated labeled antibody techniques not applicable to routine quick diagnosis.

The anomalous proteinaceous inclusions used for virus identification are usually assumed to be of host origin unless, as is the case with the inclusions of the potato virus Y group (pinwheels, scrolls and, laminary inclusions), there is evidence to the contrary. Inclusions

associated with potato virus X (PVX) are characteristic and a sensitive aid in PVX identification. We have to remind ourselves that the specificity of inclusions indicative of virus infection is not always a foregone conclusion as was shown clearly for an inclusion associated with pea enation mosaic virus (PEMV) infection which is also associated with the infection of red clover yellow vein mosaic virus (RCYMV) in the same host (Pisum sativum L.)

Virus particles

Virus particles can be observed in sectioned material and much can be learned about their morphology this way, aiding identifications.

Although light microscopy may be able to reveal some of these inclusions, electron microscopy enables one to extract additional information at relative little extra effort, so that it should be prefered over light microscopy when questions of identification are at stake.

Electron microscopy in general does not lend itself to routine application in indexing programs, maybe with the exception of those techniques that extract particles from infected material in direct ways using these for identification.

There are two basic variations in these identification techniques, one utilizing antisera and morphology and one based on morphology only.

The latter has two variations again and is generally referred to as Brandes' quick dip method.

a) A droplet of distilled water is placed on a microscope grid. This droplet is touched with the cut end of a leaf piece showing severe systemic symptoms. The surface tension draws the cell contents onto the grid. Excess fluid is drawn off with filter paper and the grid is further processed for EM (negatively stained, metal evaporation).

b) Tissue showing systemic symptoms is triturated either in buffer, distilled water or negative stain. A droplet is placed on a microscope grid, excess fluid is removed and the grid is viewed in the EM or further processed to be viewed, if for example distilled water was used initially.

Only positive results are useful and negative tests should be retested by means of other testing methods.

The methods used by Drs. Lovisolo and Milne combined serology with the two Brandes dip techniques. Homologous particle-serum combinations show up as clumped icosahedral particles or, in the case of rod-shaped particles, as particles with diffuse outlines resulting from antibody-antigen attachment.

Serological specific grids as used by Dr. Derrick are prepared by floating membrane covered grids on delute antisera. Grids made serologically specific in this manner can subsequently be floated on plant sap to react specifically with homologous antigens in this sap, if present.

The last methods seem to hold some promise for use in seed certification programs, although not quick enough to be applicable in large scale testing.

In conclusion we can say that electron microscopy is the essential first step in identification of unknown viruses but does not lend itself to large scale testing and is too tentative a method to be used as sole testing method in any seed program.

IMPROVED TECHNIQUES FOR THE DETECTION OF POTATO VIRUSES: NEW HIGHLY SENSITIVE SEROLOGICAL TECHNIQUES

R. Koenig

For the highly sensitive serological detection of plant viruses two tests have proved to be especially useful in large scale routine tests: the latex-test (Bercks, 1967; Bercks and Querfurth, 1969) and the ELISA-test (Voller et al., 1976; Clark and Adams, 1977). Both tests have been used successfully, though to a limited extent for the detection of potato viruses X, S, M, Y and A, Andean potato latent (APLV), Andean potato mottle (APMV) and other viruses in potatoes. With the ELISA-test even potato leaf roll virus has recently been detected (Casper, 1977).

The maximum titer increases - as compared with the usual precipitin tests - which we so far have reached with the latex-text were x 5000 with some antisera to APLV and APMV and x 200 with antisera to elongated potato viruses. To obtain optimal results with the latex-test it is essential to be aware of the following points: individual antisera may differ in their suitability for this test; purification of antibodies in addition to ammonium sulfate precipitation may improve the results; the optimum concentration of antibodies for the coating procedure should be determined in pilot experiments; the resuspension of the latex-particles in the centrifugation procedure should be done carefully and, according to A. Murant (pers. communication) excessive centrifugation speeds should be avoided. The assay procedure in the latex-test requires shaking. Poor results were obtained when shaking was done by hand or with vibrating or slanting types of shakers. Optimal results were obtained with rota-

ting shakers at 140-145 revolutions per minute. Higher and lower shaking speeds gave much poorer results. The sensitivity of the latex-test increases with increasing shaking time up to 1 hour. Mumford (1977) found that a temperature of 30-32° C was more favorable for doing the test than was room temperature - with the viruses we used we could not confirm this in a limited number of experiments.

The maximal sensitivity increases which we obtained in the ELISA-test were even higher than those obtained with the latex-test: about x 10,000 in visual assessment. A further advantage of the ELISA-test is that it is not inhibited by antigen excess. Thus, the test can be done with a single dilution of plant sap - whereas in the latex-test the use of two or three different dilutions is necessary. For sap from potato tubers dilutions of 1:4 and 1:40 are recommended.

The number of working steps, on the other hand, is much lower in the latex-test than in the ELISA-test. In the latex-test, the latex-serum which is kept ready for use in the refrigerator and the plant sap are brought together, the mixture is shaken and the results can be read after one hour. This is an advantage especially if testing procedures have to be done by persons with a low training level. The ELISA-test requires a much greater number of working steps: coating of plates, incubation for 2-4 hours, 3 x washing of plates, addition of plant sap, incubation over night, 3 x washing of plates, addition of enzyme-conjugate, incubation of plates for 4 hours, 3 x washing of plates, addition of freshly prepared substrate solution, incubation up to one hour and reading of the results. The test is thus more laborious and more susceptible to mistakes made by persons with a low training level. On the other hand, there are possibilities to shorten the ELISA-procedure, e.g. by the simultaneous addition of plant sap and conjugated antibodies. This procedure may, however, increase the likelihood of nonspecific reactions (M. Clark, pers. communication). In a limited number of experiments with Chrysanthemum viruses we found no adverse effects.

In the latex-test the amount of work increases in about direct proportion with the number of samples to be tested. The ELISA-test, on the other hand, is very uneconomic if only a few samples have to be

tested. However, the development of 4 ELISA-plates (240 samples) means only little more work than the development of 1 plate (60 samples).

Both, the latex- and the ELISA-tests can be automated by the use of appropriate equipment and in both tests the procedure can be simplified by the use of mixed antisera if no specific virus diagnosis is required. In the latex-test the mixing of antisera has to be done before the adsorption to the latex- particles.

In studies with APLV we found that the ELISA-test has a much higher degree of strain selectivity than the latex-test. Thus, in the latex-test with an antiserum to the original isolate of APLV from Gibbs we were able to detect all isolates of APLV which were made at CIP and even the type and Abelia latent strains of eggplant mosaic virus (EMV) which are related to APLV. With the ELISA-test (Fig. 1) this was not the case. The antiserum to Gibbs' isolate of APLV gave strong reactions with its homologous antigen and the isolate Caj, weak reactions with isolates 300 and 376 and no reactions with isolates Hu and the strains of EMV. Similar results were obtained with an antiserum to isolate Caj. An antiserum to isolate Hu gave a strong homologous reaction. The reactions with the other isolates of APLV and EMV were either very weak or completely absent. An antiserum to the type strain of EMV detected the isolate Hu and with a weaker reaction also the Abelia latent strain.

The high degree of strain selectivity of the ELISA-test can be a very useful feature in epidemiological, genetic or chemical studies where a rapid typing is necessary. On the other hand, it may be a problem in quarantine-testing because we do not know whether all strain groups occuring in the vast Andean region are already known.

The high strain selectivity of the ELISA-test apparently does not apply equally to all systems. Thus, we could detect the veinal necrosis strains of potato virus Y without difficulties with antisera to the normal strains and vice versa. With other viruses in potatoes where different isolates show few or no serological differences, such as potato viruses X, S and M, strain selectivity would not be a problem.

In order to make the optimal use of any testing procedure it should be kept in mind that the concentration of viruses may be very different in different parts of the plants (e. g. foliage and tubers) and in different tissues, that it may depend on the physiological stage (e. g. dormancy or rapid growth) and the climatic condition. These factors should be carefully studied before any method is adopted for routine-testing.

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NEW SEROLOGICAL METHOD FOR DETECTION OF POTATO VIRUS DISEASES

Dr. A.M.H. de Lekeu

The important role of serodiagnosis in maintaining potato stocks free of viruses is very well known. We have developed a method which is simple and more convenient than conventional tests.

Serological indexing provides a means of eliminating virus-infected plants from seed stocks on a large scale. However, the rod-shaped viruses which do not readily diffuse through agar present a special problem. Problems in the detection of rod-shaped viruses were investigated by Purcifull and Shephard in 1964, who degraded certain elongate plant viruses into fragments which diffused rapidly through agar and reacted with antiserum against the homologous virus. Hamilton in 1964, reported that barley stripe mosaic virus was detected in sap from infected barley plants in a gel double diffusion system which contained sodium di-butyl naphtalene sulfonate as detergent (Leonil SA).

Shephard (1970) and Shephard and Secar (1969) developed a radial diffusion technique which is more sensitive than commonly used double diffusion test. The method we developed is a modification of a simplified radial diffusion technique reported by Slack and Shephard (1975) for barley stripe mosaic virus.

Agar plates were prepared containing a mixture of the following solutions:

Solution I		Solution II	
* PVY Antiserum	: 1 ml	Ion-agar N° 2	: 0.0217 gr
Distilled water	: 0.7 ml	0.1 M Tris-HCl ph 7.4	: 1.7 ml
Leonil SA	: 0.0217 gr	Na Cl	: 0.028 gr
Sodium ozide	: 0.007 gr		
Acetic Acid	: 0.007 ml		

* Titer of PVY antiserum 1/5 12 diluted ten times.

Also we used antiserum to detect Potato Virus S (PVS), Potato Virus X (PVX) and Andean Potato Latent Virus (APLV); the Solution I was prepared without Leonil SA when I used antisera to detect APLV.

Before mixing Solution II was heated to dissolve the agar and Solution I was heated for three minutes at 50° C; then aliquots of 1-6 ml were poured into 5 cm diameter plastic petri-dishes.

After the agar was solidified small 2 mm^2 pieces of potato leaf tissue were embedded in the agar, after having been cut and pressed out with adapted tweezers. The agar plates were kept in a humid chamber at room temperature and the readings taken after 30 hours. The reactions were observed with a binocular microscope at 10-20 x magnification, using dark field illumination. Figure 1 shows the reaction produced when PVY is present.

Table 1 shows the results obtained after using the new serological test as routine to detect Potato Virus Y (PVY) in comparison with microprecipitation test and tobacco indicator hosts.

TABLE 1

Experiments	N° of samples tested	Number of positive reaction		
		New Test	Mic. oprecipitation	Host Plant
1	40	28	32	33
2	35	22	17	20
3	38	29	25	27
Total Samples	113			

There is a high positive correlation between the results of the three tests as shown in Table 1. In the microprecipitation test 5.3% escape PVY detection; there is a 3.1% spontaneous reaction in the new serological test.

In a plastic petri-dish of 5 cm diameter 60 samples can be tested simultaneously. Inmunospecific reactions were obtained for PVY, PVX, PVS and APLV using this method.

The antisera we used for our tests were prepared against whole viruses; we did not use D-protein antiserum.

Serological tests were shown to be practical for the detection of potato virus diseases.

In seed potato production programs, the tests of microprecipitation and agglutination are being used to detect PVX, PVY, PVS and PVM. We have developed a method which has the advantage of eliminating tissue grinding and the chemical treatment of the antigen. Moreover, the required size of the sample is much reduced and a larger number of samples can be tested simultaneously in a single petri-dish.

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POTATO VIRUS X

C. E. Fribourg

Potato virus X has been found commonly in Andean potatoes showing a diversity of symptoms including mild interveinal mosaic, leaf deformation with necrosis and yellow areas that resemble calico disease. In 1973 four isolates named X_A, X_C, X_D and X_E were characterized (3). More recently a new strain, X_{HB} found in Bolivian potatoes is being studied as part of an M. Sc. thesis by A. Moreira in cooperation with R. A. C. Jones and myself (4). Here I will refer only to strains X_C, X_D and X_{HB}.

Symptomatology in indicator hosts

X_C induced systemic symptoms of strong mosaic and veinbanding in most of the Solanaceous indicator hosts tested, being specially strong in *Datura stramonium*, *Nicotiana megalosiphon* and tobacco. Symptoms induced by X_{HB} were in general milder. However, in some hosts like *Nicotiana debneyi* and *N. glutinosa* they were difficult to differentiate from those of X_C. X_D induced mild mosaics in all instances in Solanaceae. All three isolates induced local lesions in *Chenopodium quinoa* and *C. amaranticolor*. X_C and X_{HB} but not X_D induced local lesions in *Claytonia perfoliata* and X_C and X_D but not X_{HB} induced necrotic local lesions in *Gomphrena globosa* typical of PVX. X_{HB} infected *G. globosa* symptomlessly in inoculated leaves. This unusual behaviour in the normal diagnostic host for PVX was constant at 10 C, 14 C, 18 C, 22 C and 24 C, temperatures at which X_C always produced lesions.

Confirmation that X_{HB} is an isolate of PVX despite its unusual behaviour in *G. globosa* was obtained because:

(1) it produced partial cross protection against X_C in G. globosa inoculated leaves; (2) it reacted strongly with antiserum to X_C; (3) it had particles identical to those of PVX in size and shape when sap was examined in the electron microscope. Also, like typical PVX it was readily transmitted by simple contact but was not transmitted by aphids (Myzus persicae).

Physical properties

X_C and X_{HB} had similar physical properties. Both were still infective on dilution of infective sap to 10^{-6} and after heating sap for 10 minutes at 70 C but not at 75 C. Also, both survived storage for at least 2 months. X_D differed from the other two in having a dilution end point of 10^{-5} - 10^{-6} and a thermal inactivation point of 65-70 C.

Symptoms in Peruvian potato cultivars

Inoculations were made to 8 potato cultivars that are either andigena types or andigena x tuberosum crosses. All were susceptible to PVX showing symptoms of mild mosaic, strong mosaic, top necrosis or mild calico (Table 1). X_C produced strong mosaic in all cultivars with the exception of Huacapa Huahuan that showed top necrosis. X_D induced either infection without symptoms, mild mosaic (in some cultivars) or top necrosis in Chata blanca, Renacimiento and Sipeña. The top necrosis symptoms observed were similar to the hypersensitive response that some European cultivars show when they are infected by some strains of PVX. In European cultivars this reaction is due to two dominant genes Nx and Nb (1). Cockerham (1) has classified PVX strains into groups 1, 2, 3 and 4 using cultivars that had the following genotypes nxnb, nxNb, Nxnb and NxNb, the genes Nx and Nb being responsible for the hypersensitive reaction. This author has also suggested that the genes for hypersensitivity in the andigena cultivars might be the same as those from tuberosum (2). On the basis of this assumption it was deduced that X_C would belong to group 3 and X_D to group 2 (3).

Strain X_{HB} normally induced mild mosaic or no symptoms, but produced calico like symptoms in the cultivar Renacimiento and vein

necrosis followed by mosaic in the cultivar Mi Peru.

Table 1. Reactions of some Peruvian cultivars to inoculation with three Andean strains of PVX.

Cultivar	Isolate		
	X_C	X_D	X_{HB}
Ccompis	SM	C	SS
Chata Blanca	SM, LD	LNS, TN	MM
Huacapa Huahuan	TN	SS	-
Mariva	SM	MM	SS
Merpata	SM	SS	SS
Renacimiento	SM	LNS, TN	C
Revolución	SM	SS	SS
Sipeña	SM	LNS, TN	MM

Symptoms in cultivars with genes for hypersensitivity or immunity to PVX

Strains X_C and X_{HB} were inoculated to some European and North American cultivars or clones with genes for hypersensitivity and immunity to PVX (Table 2). X_C induced a hypersensitive response in cvs Pentland dell and Arran Pilot and mosaic in South Esk.

From this reaction we deduce that this strain belongs to group 2 of Cockerham's classification and not to group 3 as was deduced on the basis of the reactions in Peruvian cultivars.

Table 2. Reactions of some clones and cultivars with genes for hypersensitivity or immunity to two Andean strains of PVX.

Cultivar	Strains	
	X_C	X_{HB}
Pentland dell (Nx:Nb)	LNS	LNS, PTN, SS
Arran Pilot (nx: Nb)	LNS	LNS, SS
South Esk (Nx : nb)	M	M
Saphir	NI	SS
44/1016/10	NI	SS
USDA 41956	NI	-

The reactions of X_{HB} in these cultivars were similar to those of X_C with respect to the presence of mosaic in cv South Esk and of local necrotic spots in cvs Pentland dell and Arran Pilot but differed in that the virus also became systemic in Pentland dell and Arran Pilot as was demonstrated by backtesting to N. glutinosa or N. debneyi. X_C did not infect clones which carry two different genes for immunity to PVX, USDA 41956, cv Saphir and 44/1016/10 behaving in this respect like typical PVX. In contrast, X_{HB} infected

cv. Saphir and clone 44/1016/10 both of which carry the gene for immunity to PVX originally from Solanum acaule.

This is the first time a strain has been found which breaks the S. acaule immunity gene and this finding has special significance for the breeding of PVX immune cultivars locally should X_{HB} prove to be common in the Andean region. X_{HB} is also likely to be a problem for seed programs which rely only on G. globosa for indexing for PVX.

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TOBACCO RINGSPOT

Calico - like symptoms are common in potato fields of several cultivars in the Andean highlands of Peru. On the coast they are common only in the cultivar Ticahuasi planted with seed from the highlands. Symptoms are yellow areas starting at the leaf margins. The yellowing then tends to progress inwards until most of the leaf is affected. No stunting or leaf deformation is observed. Preliminary inoculation to indicator plants done in 1970 indicated that the virus found was different from the viruses that cause calico symptoms in other parts of the world: potato mop top, tomato black ring, alfalfa mosaic, potato aucuba mosaic and tobacco rattle. A study was therefore started in 1971 to characterize the virus (2).

Host range and symptomatology

The virus has a wide host range infecting 24 species belonging to the families Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Leguminosae and Solanaceae but does not infect *Capsicum pendulum* and tomato. It induced symptoms typical of those reported for tobacco ringspot (TRSV) in *Nicotiana tabacum*, *Phaseolus vulgaris*, *Vigna sinensis* and *Cucumis sativus*. However, some reactions were not typical e. g. the necrotic spots produced in the inoculated cotyledones of *C. sativus* and the systemic apical necrosis produced in *Chenopodium quinoa*. It also induced rings in detached leaves of the potato clon A6, an indicator commonly used to detect PVY and PVA. *Solanum chancayense* was also infected producing local necrotic spots followed by extensive necrosis. Seven other tuber-bearing *Solanum* species and the *Andigena* cultivars Ccompis, Huagalina, Imilia negra and Sipeña were not infected. When the virus was mechanically inoculated to healthy Ticahuasi plants calico symptoms were not reproduced but the virus became systemic and could be recovered by back inoculations to *Chenopodium amaranticolor*. However, calico might be a secondary symptom and infected tubers were not planted

to test this possibility. It also infected the Andigena cultivar Renacimiento inducing systemic necrotic spots in young plants.

To determine if the virus could be isolated only from plants showing symptoms, 17 samples of the cultivar Ticahuasi with calico were inoculated to V. cylindrica plants. Sixteen of the 17 induced local lesions followed by a sistemic necrosis typical of TRSV, in contrast only one of 10 samples taken from normal looking plants produced these symptoms.

Physical properties

The dilution end point was 10^{-4} - 10^{-5}, thermal inactivation point 55-60 C and longevity in vitro 9-11 days.

Purification and serology

The virus was purified and an antiserum prepared to it with a titer in microprecipitin tests of 1/128. It was compared in agar double diffusion tests with 6 other serological strains of TRSV at present recognized: NC-38, NC-39, NC-72, NC-87, Eucharis mottle and Texas (3). The antiserum produced clear precipitation lines and spurs with all the other antigens when tested side by side with the calico isolate and titer varied between 1/32 to 1/128 indicating a close relationship of calico to all TRSV strains. In the reciprocal tests, precipitation lines and spurs were also obtained in all cases (1).

Distribution and importance

The virus has been isolated from plants with calico symptoms in the seed producing areas of Huánuco, Huasa-Huasi and Huancayo in the highlands. In these regions wide daily fluctuations in temperature occur and nights are cold. Under these conditions several cultivars develop calico when infected with TRSV. In contrast only the cultivar Ticahuasi shows symptoms in the potato growing areas on the coast probably because there the daily fluctuations in temperature are not so pronounced and the nights are warmer than in the highlands.

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ANDEAN POTATO MOTTLE

In a study of four viral isolates from Peruvian potatoes all thought to be Andean potato latent virus (APLV) (1), it was found that one of them (Lm) differend from the other three in its host range, symptomatology in indicator hosts and in potato plants, lack of transmission by Epitrix sp. (a flea beetle) and absence of definite reactions when tested serologically against APLV and other tymovirus antisera. Physical properties were similar, however, and particles viewed under the electron microscope could not be distinguished from those of APLV. A detailed investigation was started to determine the true indentity of the virus in cooperation with Dr. R.A. C. Jones at CIP and Dr. R. Koenig at Braunschweig, Germany.

Distribution and incidence

The virus has been detected in plants of the cultivars Renacimiento, Chunqui and Revolución growing in the localities of La Molina (coast), Apurimac and Huancayo (Andean highlands) and is also present in Bolivia. It was common in the International Potato Centre germplasm collection which includes potatoes from all parts of the Andean region occuring in about 12% of the clones.

Host range and symptoms in indicator hosts

Andean potato mottle infected 23 species all in the Solanaceae but 12 other species in the families Amaranthaceae, Chenopodiaceae, Cucurbitaceae and Leguminosease were not infected. The best host for detecting the virus was N. bigelovii which produced a mosaic characterized by large green blotches; tip leaves sometimes developed necrotic areas and holes. N. clevelandii reacted with a similar mosaic but no necrosis. Lycopersicon chilense developed mosaic, vein clearing and chlorotic spotting. No host was found that developed local lesions suitable for infectivity assays.

Symptoms in wild and cultivated potatoes

The most typical symptoms produced in plants of six wild potato species and eight Peruvian potato cultivars inoculated with APMV was a mild mottle. The strongest symptoms developed in the cultivar Revolución. These were initial systemic necrosis of shoots resulting in stunting. Later axillary shoots showing strong leaf mottling grew out from near the base of the stem. Wild species and cultivars observed for secondary symptoms showed severe mottle (S. brachycarpum, S. chacoense, S. demissum, S. mochicense, S. tuberosum subsp. andigena cvs. Chata Blanca, Renacimiento, Sipeña, and S. tuberosum subsp. tuberosum x andigena cvs. Mariva, Ranrahirca and Revolución). Other secondary symptoms were leaf deformation which was accompanied by sistemic necrosis in S. mochicense and delayed emergence plus initial severe stunting in Revolucion Plants of cvs. Chata blanca, Participación, Revolución, and Renacimiento grown in the field from infected tubers showed symptoms that resembled those observed in the greenhouse except that in Chata blanca and Participación they were more severe.

Physical properties

APMV was stable and reached high concentration in infected indicator hosts. Sap remained infective when diluted to 10^{-6} in distilled water, stored for 4 weeks at room temperature or heated for 10 min at 65 but not at 70 C.

Transmission

Attempts to transmit APMV by the flea beetle Epitrix sp. were unsuccessful. By contrast, the virus was transmitted by contact between plants when systemically infected D. stramonium or N. bigelovii were brushed against healthy N. bigelovii. It was also transmitted by brushing infected plants of cv. Participación against healthy plants of the cvs Participación and Mariva. The virus was not transmitted through true seed of N. bigelovii or potato cv. Renacimiento.

Serology

The virus was purified and antisera prepared to it for comparison with several different comoviruses. The titer when tested in gel diffusion tests with the yellow and Arkansas strains of cowpea mosaic virus and with bean pod mottle was in the range of 1/2 to 1/8 while the homologous titer was 1/4,096. No reactions were observed with squash and radish mosaic viruses. Antisera to the Arkansas and El Salvador strains of cowpea mosaic virus, to bean pod mottle and to quail pea mosaic reacted with APMV. No heterologous reactions were found with antisera to squash mosaic, radish mosaic, Echtes Ackerböhnenmosaic, red clover mottle and bean rugose mosaic (2).

Electron microscopy and biophysical properties

Purified preparations gave an absorption spectrum typical of a nucleoprotein with the peak at 254 nm and a ratio A 260/280 of 1.5. When viewed under the electron microscope the particles were isometric about 28 nm in diameter. SDS polycrylamide electrophoresis of the protein yielded two bands whose molecular weights were 20,800 and 40,100 daltons respectively, values very similar to those of two other comoviruses (squash mosaic and radish mosaic) that were checked at the same time.

Importance

Andean potato mottle virus is a new member of the comovirus group. Like other comoviruses it causes mosaic and mottle symptoms, has a narrow host range, reaches high concentration in sap and has isometric particles about 28 nm in diameter. Besides, it shows definite serological relationships with some members of this group and has two proteins which are similar in size to the proteins of other comoviruses.

Andean potato mottle resembles other potato viruses in being readily transmitted by plant to plant contact. This is probably the most important means by which the virus spreads in potato crops. However, because the comovirus group is characterized by having beetle vectors,

it also may spread by this means, although it is not transmitted by Epitrix sp. Andean potato mottle seems to be widely distributed in potato-growing areas of the Andes. Like APLV, potato mop - top and tobacco ringspot it shows evidence of adaptation to the climatic conditions of this region multiplying well only at cool temperatures such as those encountered in fields in the highlands.

APMV produces stronger symptoms than APLV and therefore can be readily eliminated by roguing during seed potato propagation. However, it probably has greater economic importance because of the severe disease it causes in most cultivars and because most farmers in the Andean region do not use healthy seed potatoes.

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POPULATION BREEDING FOR VIRUS RESISTANCE

H. A. Mendoza

Virus infection are to the potato crop one of the mos important causes of losses in production and one of the main reasons to justify seed production schemes to obtain non infected seed or at least low virus content planting materials. In developing countries, seed production is a costly process and in many instances the amount of seed produced and/or the quality of it may not be enough to have an impact on raising the productivity of the crop. An alternative way to alleviate the problem of scarcity of good quality seed would be by selecting varieties with high levels of resistance or immunity to two or three of the most important virus diseases which attack the potato crop.

Within the International Potato Center's breeding philosophy a concentrated effort is underway to maximize the utilization of the genetic resources contained in the genus Solanum. The proper utilization of this genetic variability permits the selection not only of a high yielding and well adapted germplasm but also the combination of resistances to the main pests and diseases to which the potato crop is so vulnerable.

To produce an improved germ plasm a population approach has been chosen with the purposes of:

a) Increasing gene frequencies of desirable alleles.
b) Producing the recombination of valuable traits present in the various taxonomic groups.
c) Conserving a germ plasm with a wide genetic variability on which high yielding materials can be selected.

To illustrate the progress achieved in the population breeding to increase gene frequencies for potato virus Y, we shall present some results obtained with a diploid phureja-stenotomum population.

To test for resistance to PVY a sample of 35 families from a random mating population was taken. Twenty four seedlings per family were mechanically inoculated with the common strain of

PVY. The inoculated seedlings were maintained in a "walk-in" growth chamber at 20°C for 30 days. Readings were made taking counts of plants showing systemic symptoms, necrotic symptoms or no symptoms at all, which were classified as susceptible, hypersensitive or immune respectively. The results obtained are shown in Table 1.

Table 1. Population phenotypic distribution for reaction to PVY

Phenotypic Reaction	N° of Individuals	Phenotypic Frequency
Immune	60	.0714
Hypersensitive	87	.1035
Susceptible	693	.8250
Total	840	.9999

From this table, one can observe that there were immunity genes in this population but in a rather low frequency. Only 7.14% of the individuals exhibited an immune reaction. However, this phenotype is represented not only by one genotype but by three. It has been established that the reactions to PVY is under the control of an allelic system where:

$$Y^I > Y^H > Y^S$$

The allele for immunity Y^I is dominant over Y^H, the allele for hypersensitivity, and this is dominant over the allele for susceptibility, Y^S.

Since the sample analyzed came from a random mating population, it is assumed by theory that this was a population where the gene frequencies were at equilibrium. So if we call p, q, and r the frequencies of the alleles Y^I, Y^H and Y^S, respectively, we could find the gene frequencies by the following expression:

$$(p-q-r)^2 = p^2 + 2pq + q^2 + r^2 + 2pr + 2qr$$

Table 2. Expected genotypic frequency corresponding to each phenotypic reaction

Genotypes	Genotypic Frequency	Phenotypic Reaction	Phenotypic Frequencies
$Y^I\ Y^I$ $Y^I\ Y^H$ $Y^I\ Y^S$	p^2 $2pq$ $2pr$	Immune	.0714
$Y^H\ Y^H$ $Y^H\ Y^S$	q^2 $2qr$	Hypersensitive	.1035
$Y^S\ Y^S$	r^2	Susceptible	.8250

From this table, it is possible to establish the gene frequencies p, q, and r, i.e.

$$r = (.8250)^{1/2} = .908$$

Therefore, the gene frequencies obtained for each of the alleles were estimated as:

$$p = .036$$
$$q = .055$$
$$r = .908$$

These gene frequencies show that in this population the frequency p for the allele for immunity Y^I was low. However, since the genetic control of the resistance to PVY is simple, it is possible to rapidly upgrade the level of resistance in this population.

If an assortative mating scheme using only immune genotypes is carried out for various generations, the expected changes in the frequency of the various phenotypes as well as the changes in gene frequencies are illustrated in the following table:

Table 3. Changes in phenotypic and gene frequencies in various breeding generations

Phenotypes	Breeding Generations				
	0	1	2	3	4
Immune	7.14	75.61	89.00	93.71	95.98
Hypersensitive	10.35	2.61	1.15	.63	.41
Susceptible	82.50	21.50	9.69	5.84	3.52
Gene Frequencies					
p (Y^I)	.036	.507	.669	.749	.801
q (Y^H)	.055	.026	.017	.010	.008
r (Y^S)	.908	.464	.311	.241	.187

Table 3 illustrates the rapid progress which can be reached in

PVY resistance in a few generations of selection. After 4 breeding cycles one individual taken at random from this population would have 96% probabilities of bein immune. This approach not only provides the opportunity to identify immunity to PVY, but on the same time, to select for other attributes of economical importance to potatoes such as yield as well as resistance to other pests and diseases.

In tetraploid populations of Andigena (both native Andigena and neo-tuberosum) the frequency of the allele for immunity to PVY appears to be slightly higher than in the diploid Phureja-stenotomum diploid populations. Table 4 shows results of inoculations carried out in three different sets of materials composed by varying numbers of families and individuals. In set I, a random set of Andigena clones used as females was crossed with bulk pollen from high yielding selected clones of neo-tuberosum. No selection for PVY immunity was carried out in any of these materials. Set II was formed by a set of neo tuberosum clones selected for yield, crossed with bulk pollen from clones which were inoculated with PVY and those showing symptoms were eliminated. However, no further confirmation of immunity was performed. The third set was formed by crosses of selected females with bulk pollen of clones in which their immunity was confirmed.

Table 4. Reaction to PVY inoculation of materials of various origins

Set	N° of Families	N° of Seedlings		Frequency of Immunity
		Immune	Susceptible	
I	12	42	294	.12
II	7	49	107	.31
III	5	43	32	.57

Set I shows the natural occurrence of the PVY immunity allele in this population which is slightly higher than the 7% detected in the diploid population. Set II, shows that with a simple inoculation of clones with elimination of those which show symptoms but without a further confirmation of the resistance of those which did not show symptoms was enough to raise to .31 the frequency of immune seedlings. Finally, Set III shows that when the resistant parents were of a proven immunity the frequency of immune offsprings was .57 which would be the expected when one crosses susceptible x immune genotypes which carry the immunity allele in a simplex condition. This frequency can be readily upgraded if the selection for immunity is carried out in both males and females. If one selects only simplex genotypes, the outcome of these matings would provide a frequency of .75 immune progenies. These frequencies could be significantly increased to .97 by selecting genotypes which carry the immunity alleles at a duplex condition. This work is presently underway at CIP and increases the likelihood of obtaining by combination with other similar traits the multiple resistant clones.

A similar study was carried out utilizing potato virus X. In this case, only two sets of families were used. Set I was formed by families obtained by crossing high yielding clones taken at random. The second set was formed by PVX immune females crossed to a random sample of high yielding clones unselected for PVX resistance. Results are presented in the following table:

Table 5. Reactions to PVX inoculation of materials from various origins

Set	N° of Families	N° of Seedlings		Frequency of Immunity
		Immune	Susceptible	
I	7	23	134	.17
II	3	68	29	.70

Results of Set I demonstrate that the natural occurrence of the allele for PVX immunity in these populations is even higher that for PVY which makes the selection for immunity to this trait not very complicated. This is also supported by results for Set II in which one could have expected only a frequency of .5 of immune offsprings. However, it was .7 and this is explained by the contribution of the male parents which in spite of not have been selected for resistance still were contributing some alleles for immunity which increased the expected ratios.

The population breeding provides a unique opportunity when the screening is applied to seedlings at an early age to combine resistances and later field select for adaptation and other agronomical characters. Also, further evaluations for pest and disease resistance are confirmed under natural infection conditions.

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