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Technology Evaluation Series No. 1982–6

Field-Screening CIP Clones Developed for Resistance to Bacterial Wilt

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INTERNATIONAL POTATO CENTER (CIP)

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TECHNOLOGY EVALUATION SERIES

The purpose of the Technology Evaluation Series is to acquaint scientists in national potato programs with technologies that have been generated at the International Potato Center (CIP) and that are ready for evaluation under local conditions.

Each technology is presented with relevant research results and with sufficient information on materials and procedures that can be used for evaluation. Several experiments including field layouts are suggested. Guidelines and data recording sheets are included.

Through this series CIP is attempting to improve the process of technology transfer to scientists of national potato programs, and to facilitate the adaptation of the technologies to local conditions. It is important to CIP's efforts for improving potato productivity that national scientists feed back the results of their technology evaluations. This information will help to orientate CIP's research and will result in technologies more appropriate to farmers' conditions and needs.

Primo Accatino
Coordinator, Research Transfer

FIELD-SCREENING CIP CLONES DEVELOPED FOR RESISTANCE TO BACTERIAL WILT

E. R. French

INTRODUCTION

Bacterial wilt of the potato is, after late blight, the most damaging potato disease in the tropical belt of the world. The development of resistant clones is a priority objective of the International Potato Center (CIP). Because resistance is specific to the strain or strains of the bacterium in a given geographical region, an essential step for national programs to develop resistant varieties is to screen and select the most suitable among tuber families and clones that CIP can supply. The following description of field screening methods is provided for the recipients of such materials.

SPECIFICITY OF RESISTANCE

The resistance breeding program has developed clones with resistance to wilt derived from Solanum phureja. This resistance is controlled by a few genes and is specific for one or more strains of the bacterium. These strains may be in either race 1 or race 3 of Pseudomonas solanacearum. Race 1 is characterized by having many hosts in several plant families, including crops and weeds; many solanaceous hosts are often susceptible to race 1. This race occurs more frequently in hot climates (lowland tropics and subtropics). Race 3 is generally specific to potato, but sometimes it attacks another crop or weed in a given location. It occurs principally in cool climates such as the highland tropics and temperate zones, or in potato plants grown in warmer locations from seed tubers coming from cool climates.

Race 1 usually persists in the soil for many years because of its numerous hosts, whereas race 3 tends to drop off in a few years (it may even do so very rapidly and make screening difficult). You may be able to judge which

race you have by observation and/or prior knowledge, but if in doubt request instructions from the author on how to collect and send specimens to CIP for identification.

Both races may occur in a given field in the mid- to low-elevation tropics (0 to 1500 m). Locating a cultivar with resistance to strains in each of the two races is possible but less probable than to any one strain in either race. It is more likely that the clones developed so far will provide resistance to race 3 strains than to those of race 1. Also, resistance to race 1 strains, because they most often occur in hot climates, will most likely not be a complete resistance but a degree of resistance only--that is, fewer plant will become diseased. Bear in mind also, that the incidence of root-knot nematode is greater in hotter climates, and the resulting damage to the root system increases the chances of bacterial wilt incidence.

ESTABLISHING A FIELD SCREENING SITE

Screening for resistance requires a field with reasonably uniform infestation. Choose a field in which bacterial wilt can be seen and its distribution recorded or with a known history of wilt incidence. If you have located a field of low inoculum potential, you can plant a susceptible crop and observe the natural incidence of wilt. If less than 30% or poorly distributed, compensate by inoculating.

If bacterial wilt is not available conveniently close by, then a soil may have to be infested, but this may give unpredictable results: the inoculum potential could either be insufficient or so great that everything you plant dies quickly.

To infest a field, plant potato tubers harvested from a wilt-diseased crop and let nature take its course, aided by watering if necessary to maintain high soil moisture. An alternative is to plant a susceptible crop and then inoculate with *P. solanacearum*.

Inoculation may be difficult if the facilities or expertise to isolate and grow the bacterium are not available. A good source of inoculum is tubers from a diseased crop. Cut healthy looking tubers from a wilting plant and

choose those that ooze at the vascular ring. This ooze can be used to prepare a milky suspension for inoculations. Inoculation can be done by either cutting 2 to 3 leaf tips per plant with a scissors dipped in the inoculum, or by using a syringe and thick needle to inject into 2 to 3 leaf axils. The disease should develop rapidly if temperatures average 20°C or more.

Once plants begin to wilt you can plant a screening test a month later. Uproot every other plant and replace it with test plants. Bacteria in soil and in the remaining roots will serve as inoculum, and bacteria will exude from the roots of the remaining diseased plants.

PLANTING A SCREENING EXPERIMENT

The plot design will depend first on the amount of tubers available per clone. We will consider three cases: 1) tuber families, where each tuber is a different clone; 2) selected clones, previously screened and multiplied, 5 tubers each; and 3) advanced clones, for which 20 or more tubers of each are available (see Appendix A for definitions).

Tuber Families

Interplant with a susceptible crop or potato cultivar, planning for a final within-row distance between test plants of 80 cm, and 90 to 100 cm between rows. The susceptible may be planted 80 cm apart a few weeks ahead of the test plants. Plant members of a family in sequence along a row, continuing if necessary into the next row. Carefully record this sequence and stake each plant or at intervals of no more than five plants (Fig. 1).

Selected Clones

With five tubers per clone there are two options: 1) plant the five in one plot, which may lead to unreliable results when inoculum potential varies from plot to plot; or 2) use one-plant plots and thus establish five replications in a completely randomized, block design (Fig. 2). The second option is more difficult to set up and manage, but it gives reliable data.

BORDER	S	S	S	S	S	S	S	S	S	S	S	S
Family A	S	T A1	S	T A2	S	T A3	S	T A4	S	T A5	S	S
Families A & B	S	S	T A6	S	T B1	S	T B2	S	T B3	S	T B4	S
Family C	S	T C1	S	T C2	S	T C3	S	T C4	S	T C5	S	S
Family D	S	S	T D1	S	T D2	S	T D3	S	T D4	S	T D5	S
BORDER	S	S	S	S	S	S	S	S	S	S	S	S

Figure 1. Field design for planting tuber families. A susceptible (S) alternates with test plants (T) both along and between rows, except at the perimeter where all are S. Distance between plants is 40 cm, and between rows 100 cm.

BORDER	S	S	S	S	S	S	S	S	S	S	S	S
Row 1 Plot No.	S	A3 1	S	A1 2	S	A4 3	S	A1 4	S	A2 5	S	S
Row 2 Plot No.	S	S	A4 6	S	A2 7	S	A3 8	S	A2 9	S	A4 10	S
Row 3 Plot No.	S	A3 11	S	A1 12	S	A2 13	S	A4 14	S	A3 15	S	S
Row 4 Plot No.	S	S	A2 16	S	A1 17	S	A3 18	S	A1 19	S	A4 20	S
BORDER	S	S	S	S	S	S	S	S	S	S	S	S

Figure 2. Completely randomized planting of five tubers per clone, interplanted with a susceptible (S). Distance between plants is 60 cm, and between rows 100 cm.

Advanced Clones

Clones that have been through tuber family and clone tests and have proven useful not only for their resistance, but also agronomically, are multiplied further and distributed for tests where good testing sites have been established. These are limited in number and thus can enter randomized block plantings in comparison with previous selections or local varieties. These experiments should employ general agronomic practices, possibly with variation only in a greater plant-to-plant and row-to-row distance, to more readily count the number of diseased plants per plot. The 20 tubers can be utilized, five per replication in four blocks. Interplanting a susceptible may not be necessary. Major differences in yield potential can be determined and agronomic characters recorded (type of foliage and plant shape, tuber shape, skin and flesh color, utilizing the code numbers shown in Bryan, J. E. 1981. Pathogen-tested potato cultivars for distribution. CIP, Lima, Peru).

RECORDING DATA

Three factors are of interest: 1) wilting of plants, 2) infection of tubers (visible symptoms or latent vascular infection), and 3) yield of marketable tubers.

Plant-by-plant weekly records must be kept to determine when each plant wilts and dies as a result of P. solanacearum, or if death is due to other causes. Other causes such as insects and fungi should be controlled. Since late blight resistance will differ in the clones and cultivars being tested, it may be possible to record differences in spite of control measures, or to increase the time between fungicide applications as of two weeks after flowering to observe the differences in the new, unprotected foliage. However, if late blight is not controlled, wilt resistance may not be recordable.

A data form has been prepared (Appendix C) with data shown for tuber families, selected clones, and advanced clones. An additional blank form is inserted (see Appendix B) that can be photocopied for use; more are available from CIP. A second set of forms may be modified for recording agronomic characters.

If uncertain about disease symptomatology, consult the Compendium of Potato Diseases (Hooker, W. J., ed. 1980. American Phytopathological Society, St. Paul, MN, 125 p).

DATA INTERPRETATION

Results will differ with different climatic factors (temperature, rainfall); therefore, choosing the correct season and planting date is important. Under cooler climatic conditions, it may be feasible to provide the most favorable conditions for other factors that enhance the capacity of the bacterium to cause disease and to still select fully resistant clones. In warmer climates it may only be possible to select clones that suffer less than others (lower infection rate, slower wilting).

Examples of results are given in Appendix C. Family member A1 is apparently resistant and a reasonably good yielder. A2 is susceptible and is discarded. A3 is apparently resistant and yields well, thus it is also selected.

Selected clone A1 when tested in five one-plant replications was diseased in four of the five replications and was discarded.

Selected clone A3 performed well in the five replications, except for some latent tuber infection that appeared after storing the tubers. It was further tested with other selections as advanced clone A3.

Advanced clone A3 poses a dilemma--hardly any wilt, good yield, small amount of latent infection (all replications were similar to the one shown). It would be a risk if used without an adequate seed program.

Advanced clone A7 (the one replication shown is representative of all five) is easy to interpret. No plants wilted nor did any tubers develop symptoms. It can be tentatively considered highly resistant under the particular testing conditions employed (climate, bacterial strain).

APPENDIX A

DEFINITIONS

Tuber Family

A set of individual tubers from a single progeny identified with a six-digit number (the first digit indicates the CIP breeding program, the second and third the year of the cross, and the last three its family number). Selections from a family become clones with the same number plus a decimal and its own number, which may have one to three digits. For example, tuber family 382508 is composed of 100 individual tubers (each a different genotype), and four of these are selected as clones numbered 382508.7, 382508.31, 382508.54, 382508.86.

Selected Clones

Individuals selected from tuber families that have undergone additional testing. They keep the same nomenclature as in the tuber family but are tested in sets of five or more tubers.

Advanced Clones

Selected clones that have undergone field tests and have been increased as potential varieties. These may be identified by the clone number as shown above under Tuber Family, or by a six-digit number when not from CIP's breeding program.

APPENDIX B

BACTERIAL WILT RECORDING CHART

Plot No./Code	Weeks after planting[a]								Harvest[b]			Storage[c]			
									D	H	Wt. H	D (3 w) H		D (6 w) H	

[a] Rate as follows:

0 = no emergence yet
1 = emerged
W = wilting
X = wilted beyond recovery
Z = dead, other cause

[b] D – H: number of diseased tubers (D) or healthy (H) according to external symptoms.

Wt. H: weight of healthy tubers, in grams.

[c] Number of diseased (D) and healthy (H) tubers after storage for 3 and 6 weeks (cutting for final rating).

APPENDIX C

BACTERIAL WILT RECORDING CHART

Plot No./Code	Weeks after planting[a]								Harvest[b]			Storage[c]			
	3	4	5	6	7	8			D	H	Wt. H	D (3 w)	H	D (6 w)	H
Family A1	1	1	1	1	1	1			0	9	990	0	9	0	9
A2	1	1	1	W	W	X			3	4	210	4	0		
A3	1	1	1	1	1	1			0	10	1150	0	10	0	10
etc.															
1. Clone A3	1	1	1	1	1	1			0	11	1220	0	11	1	10
2. A1	0	1	1	1	W	X			3	5	760	2	3	1	2
3. A4	1	1	W	W	X				0	0	0				
etc.															
1. ADV. CL. A7	Med. height fills rows, flowers white														
Plant No 1	1	1	1	1	1	1			0	9	920	(Bulk, 4 tubers			
2	1	1	1	1	1	1			0	7	850	each plant)			
3	0	1	1	1	1	1			0	12	1080	0	20	0	20
4	1	1	1	1	1	1			0	11	1150				
5	1	1	1	1	1	1			0	8	900	Tubers smooth, white,			
TOTAL										47	4900	cream flesh.			
Ave.										9.4	980				
2. ADV. CL. A3															
Plant No 1	1	1	1	1	1	1			0	11	1150				
2	1	1	1	1	W	W			2	10	1100	3	17	2	15
3	1	1	1	1	1	1			0	16	1400				
4	1	1	1	1	1	Z			0	14	1450	Med. deep eyes, dark pink			
5	1	1	1	1	1	1			0	12	1320	Light pink skin			
TOTAL										63	6420	Yellow flesh.			
Ave.										12.6	1284				

[a] Rate as follows:

0 = no emergence yet
1 = emerged
W = wilting
X = wilted beyond recovery
Z = dead, other cause

[b] D – H: number of diseased tubers (D) or healthy (H) according to external symptoms.

Wt. H: weight of healthy tubers, in grams.

[c] Number of diseased (D) and healthy (H) tubers after storage for 3 and 6 weeks (cutting for final rating).