

# FINAL REPORT

(OCTOBER 1985 TO JANUARY 1988)

" PAPITA GUERA " A POTENTIAL NEW CROP OF GREAT IMPORTANCE  
FOR THE ARID WARM REGIONS OF MEXICO AND OTHER COUNTRIES.

AID/ACI PROPOSAL 4.205

BY

JORGE GALINDO A. PH. D.  
PRINCIPAL INVESTIGATOR

AND

AGR. A. CÁRCAMO, BIÓL. E. ESTAÑOL, BIÓL. S. FERNÁNDEZ,  
Q.B.P. G. HUERTA, BIÓL. D. KLEVEZAS, AGR. C. LÓPEZ,  
BIÓL. R. ORTÍZ, BIÓL. G. RODRÍGUEZ, AGR. P. RODRÍGUEZ,  
AGR. C. BUCIO, SRA. TERESA AGUILAR.

PARTICIPANT INVESTIGATORS

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## INTRODUCTION

We may conclude that the main objective of the project was actually accomplished in the three years work. We were able to gather an outstanding germoplasm for resistance to the aggressive pathogens of "papita guera". Thus we are confident that multiclonal varieties (mixture of clones with diverse kinds of resistance) will be able to overstand the severe attacks of Phytophthora infestans under short humid periods; and those of Alternaria tenuis under long dry periods. Unfortunately we were unlucky in the last field experiment in which it was expected to demonstrate that "papita guera" can be cultivated with our resistant materials. The almost total lack of rain, during months after the establishment of the experiment, frustrated our demonstration of 1987.

Perhaps the most significant accomplishment of the whole three years AID # 4.205 project was the discovery of a plant with a tuber yield of 511 grams, about 20 times the common yield. This has shown a tuber production potential far away from the most optimistic expectation. Thus, it strengthens the confidence that "papita guera" will be a new crop to produce food in the arid regions of the world where human populations are suffering undernourishment and hunger.

A detailed report of these aspects and all the other actions of the project are presented as follows:

### 1. GENETICAL RESISTANCE

Since the "papita guera"-crop will be mainly on the hands of poor farmers living in arid regions, the only practical and economically possible control of pathogenic fungi is by means of plant resistance.

In three consecutive years, a field plot was established in Texcoco, Méx., Fig. 1, with tubers representing wild "papita guera" populations growing in Central Mexico. Each plant was individually tested for resistance to Phytophthora infestans and to Alternaria tenuis. In the case of P. infestans the tests were done by inoculations on detached leaflets in the laboratory.



Fig. 1. "Papita guera" selection-plots established annually at Montecillos, Texcoco, Mex. For single plant analysis in regard to resistance against P. infestans (vertical + horizontal); resistance to A. tenuis toxin; and search for high yield plants.

In order to detect vertical resistance, the leaflets were inoculated with race O. Positive infection indicated the presence of vertical resistant genes, and negative infection their absence.

The horizontal resistance was partially detected by evaluating one of its components, the restriction of the amount of sporulation produced in the lesion of infected leaflets. Leaflets infected with race O for vertical resistance determination were also used for horizontal resistant evaluation. For those cases in which no infection took place with race O, a new set of leaflets were inoculated with the wide pathogenic or complex race 1,2,3,4,5,6,7,9,10,11.

In the case of resistance to *A. tenuis*, the toxin of this pathogen was used for a biosay in which the petioles of leaflets were immersed in a toxin extract during 48 hrs. The toxin causes an increase of leaflet transpiration. Thus leaflets with low or without loss of weight indicated high resistance, and the loss of most of its fresh weigh indicated high susceptibility.

The total of plants tested in the three years were 7457, belonging either to *Solanum cardiophyllum* and to *S. ehrenbergii*.

#### 1.1. Results on Vertical Resistance to *F. infestans*.

We found among the 7457 "papita guera" plants tested, a 77% with vertical resistance genes. This high resistant percentage suggests that *F. infestans*, in spite of its occasional and short incidence due to limited humid periods in arid regions on the "papita guera" population in central Mexico appears to be a pathogen which as an important natural selecting pressure.

The *S. cardiophyllum* species resulted to have a 85% of the plants with R genes whereas *S. ehrenbergii* had only 54%, (table 1). An explanation for this large resistant difference between species can not be given at present. But, perhaps it could be related to the possible fact that each species appears to have its own inhabiting niches within their geographical region. Thus *S. cardiophyllum* habitat could be more humid, favoring a higher selecting pressure by *F. infestans* than that of *S. ehrenbergii*.

Table 1. Number of "papita guera" plants found with vertical resistant R. genes.

Botanical special	Plants tested	Plants with R genes	Plants without R genes	Resistant Percentage
<i>S. ehrenbergii</i>	1884	1015	870	54%
<i>S. cardiophyllum</i>	4532	3856	677	85%
Not determined *	1041	896	145	86%
TOTAL	7457	5765	1692	77%

\* Due to technical failure in 1986, a portion of the plants of the field plot were not identified. But most of them appeared to be *S. cardiophyllum*.

Large resistant differences were also found among population of the same species. This may suggest the existances of niches with different humidity within each species habitat. Another possible explanation could be linked to the existence of clones with premature tuber formation which could scape and make less effective the *P. infestans* selecting pressure, because they are exposed to a shorter period to the damage of the pathogen than those clones with late tuber formation.

The massive search for resistance didn't permit the detailed and time consuming genotype determination of selected materials. However, it appears that most of the plants containing "R" genes have a complex genotype for vertical resistance, as suggested by the failure of the complex race 1.2.3.4.5.6.7.8.9.10.11. to infect them, table 2.

Table 2. Number of "papita guera" plants found to have a very complex vertical resistant genotype or particular genes.

Botanical Species	Plants tested with complex races	Plants not infected	Resistant Percentage
<i>S. ehrenbergii</i>	819	184	22%
<i>S. cardiophyllum</i>	2710	36	1%
Total	3529	220	

Dr. John S. Niederhauser made a breakthrough in breeding potatoes for resistance to *P. infestans* when he demonstrated in the 50's the superior value of horizontal resistance to the vertical resistance. Since that time, an effective control of *P. infestans* has been achieved with horizontal resistant varieties, and consequently, the vertical resistance has practically been ignored. However for the "papita" multiclonal varieties, the numerous clones with vertical resistance and their very complex genotypes found, appear to be of great value because all these clones are very similar and their tubers are undistinguishable. Thus the mixture of clones with various and complex vertical resistant genotypes will exclude most of the *P. infestans* races which could arrive to the heterogenous plant populations. If some plants are infected, the horizontal resistant genes also found, will act to slow down the infection progress.

### 1.2 Results on Horizontal Resistance to *Phytophthora infestans*.

As it is known, the horizontal resistance is evaluated when the pathogen is infecting the host plant. Since 5047 plants out of those 7417 inoculated were infected by either race 0 or by the race 1.2.3.4.5.6.7.8.9.10.11. they were only the available plants for searching horizontal resistance. However, due to technical limitations not all of them were evaluated, and only the extent of sporulation was scored as very low, low, abundant and very abundant. Thus, the very low sporulation of the pathogen corresponded to the highest resis-

lence of the plant, and the very abundant sporulation to the very low resistance, or high susceptibility. Very high resistance was found in 151 plants out of the 2231 plants tested, that is a 7%, table 3.

Table 3. Horizontal resistance evaluation of "papita guera" plants, in regards to the exten of *P. infectans* sporulation.

Botanical species	Extent of sporulation			
	very low	low	abundant	very abundant
<i>S. ehrenbergii</i>	39	91	416	424
<i>S. cardiophyllum</i>	25	35	54	55
No. identified	31	35	143	55
Total	95	161	613	534

### 1.3 Results on Resistance to *Alternaria tenuis* toxin

The information obtained in a previous work by Huerta, (1984), related to the conditions found for high toxin production and the development of a biosay to detect the toxin, was the basis for our massive individual plant testing for resistance to *A. tenuis*. After exposing the leaflets to the toxin, the weights retained were recorder. The data was arranged in eleven resistant classes. The class "I" goes from 0 to 9% of retained weight, corresponding to the most susceptible plants and the class "XI" is from 100 to 115% retained weight, corresponding the most resistant plants which din't loss weight, on the contrary, there was a weight-gain during the 2 days test. There were 62 plants in this XI class out of 7109 plants tested in the 3 years work, that is a 0.9%, table 4.

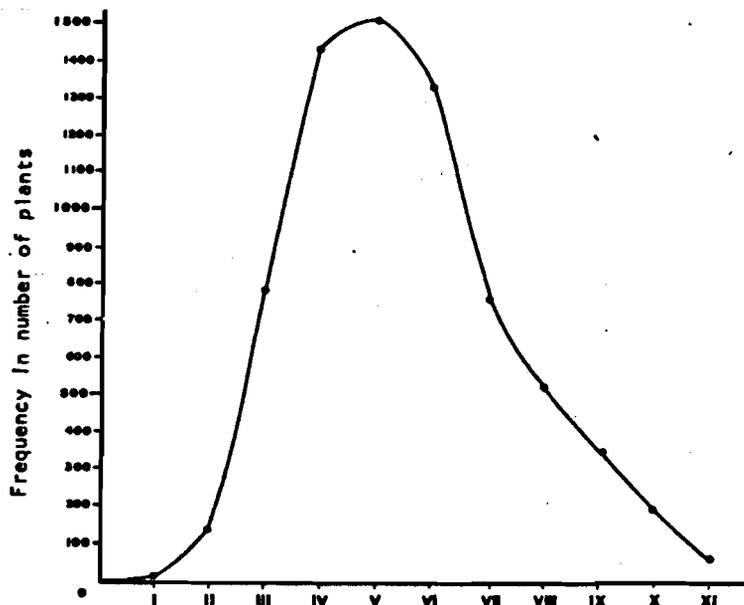


Fig. 2. Papita Guera resistant classes to *A. tenuis* toxin. Class I, highest susceptibility, class "IX" highest resistance.

The frequencies found among the resistant susceptible classes give rise to a bell shape curve, fig. 2. This may suggest a quantitative factor in the genetical nature of toxin resistance. Perhaps the resistance could be cytoplasmic and its gene(s) could be in mitochondrial DNA and not in chromosomal DNA, Table 4.

Table 4. Resistance evaluation of Papita Guera plants to Alternaria tenuis toxin.

Percentage of leaflet weight retained.	Resistant class	Number of plants in each class			
		1985	1986	1987	3 years total
0 - 9	I	5	1	2	8
10 - 19	II	16	34	88	138
20 - 29	III	140	366	282	788
30 - 39	IV	205	781	456	1442
40 - 49	V	167	887	457	1511
50 - 59	VI	231	740	358	1329
60 - 69	VII	89	392	279	760
70 - 79	VIII	54	262	205	521
80 - 89	IX	38	155	163	356
90 - 99	X	26	86	82	194
100 - 115	XI	13	28	21	162
					7109

## 2. RESEARCH ON Alternaria tenuis

On the contrary to Phytophthora infestans, our experience and knowledge on Alternaria tenuis was very limited when we started this project. Thus, it was considered important to do some exploratory research on this very aggressive pathogen in order to have a better understanding on its biology and interactions with papita guera, particularly those aspects which could benefit our "papita" breeding for resistance to its severe attacks. The results obtained in different research actions are presented below.

### 2.1. Disease development.

The first lesions caused by A. tenuis are usually observed at the apical leaflet of the lower leaf in contact with the ground, suggesting that primary inoculum is in the soil. They start as a black necrotic pin point which later turns in a large necrotic area with concentric rings, in most cases have a yellow halo in the margin of the lesion. The necrosis usually covers the whole leaflet and continues to other leaflets. The affected tissues dries completely and becomes brittle. The infection continues to upper leaves, and when the lower three leaves have died the rest of the plant turns yellow. At this stage the buds and flowers drop, and after about eight days the whole plant die.

It was observed in different years, that S. cardiophyllum plants become infected earlier than S. ehrenbergii plants but the latter died earlier (47 days after infection) than the former (33 days a.i). It was also observed that A. tenuis infection occurs mainly just after blossom, 63 days after planting S. cardiophyllum and 71 days for S. ehrenbergii.

## 2.2. Parasitism.

When lower and upper sides of leaflets were inoculated with drops of spore-suspensions a higher amount of infection was obtained on the upper side, table 5. This showed that spores are able to infect either side of "papita" leaflets and suggested that infection takes place by direct epidermis penetration, because the higher number of stomata on the lower side didn't favor a higher infection. Also, by microscopic observations, it has been found germination tubes of spores passing above stomata without entering into the tissue.

Although a single spore was able to infect a leaflet the percentage of success was only 5% as compared with 83% obtained with groups of 15 spores. This phenomenon is not due to a low germination of spores because 19 spores placed individually on water agar had a 90% germination, thus some unknown factors may interfere with the process of infection.

Table 5. Effect of inoculum density of *A. tenuis* spores on leaflets infection of "Papita species".

Botanical species	spores per ml	upper side infection	lower side infection
<i>S. cardiophyllum</i>	2000	5/15=33%	4/15=26%
	6000	6/15=40	6/15=40
	18000	10/15=67	3/15=20
	28000	10/15=67	11/15=73
	42000	11/15=73	9/15=60
<i>S. ehrenbergii</i>	2000	5/15=33%	5/15=33
	6000	11/15=73	10/15=67
	18000	11/15=73	10/15=67
	28000	9/15=60	9/15=60
	42000	12/15=80	10/15=67

## 2.3. Sporulation.

After infection takes place, this fungus parasitizes with its mycelium the living tissue and after some vegetative growth it starts an abundant sporulation which can continue even when the tissue is dead.

The spore production of the saprophytic phase was found to be favored by light, although it was able to produce a good number of spores under continuous dark table 6. A 100% of relative humidity (rh) favored the maximum sporulation, at 76% rh was able to sporulate about half its potential, at 33% rh can do a little sporulation, but it was not able to sporulate at 5% rh.

## 2.4. Pathogenic races of *A. tenuis*, and its correlation with its toxin production.

It was observed the existence of different pathogenic levels among various *A. tenuis* isolates, thus, it was considered important to see if the pathogenicity was correlated with the amount of toxin production.

Table 6. Effect of light on *A. tenuis* sporulation.

<i>A. tenuis</i> isolate	Number of spores in eight agar discs	
	continuous light	continuous darkness
1	50 000	34 000
2	18 000	12 000
3	62 000	58 000
4	40 000	22 000
5	80 000	70 000
6	102 000	78 000
7	78 000	52 000
8	42 000	22 000
9	30 000	18 000
10	34 000	10 000
11	36 000	22 000

The toxin extracts of three different *A. tenuis* isolates were used to evaluate their effect on leaflets of *S. cardiophyllum* and *S. ehrenbergii*. At the same time, spores of the same *A. tenuis* isolates were inoculated on another set of "papita guera" leaflets. The loss of weights shown by the leaflets exposed to the toxin appeared to correlate with the loss of weight lost by the corresponding inoculated leaflets, table 7.

Table 7. Weight losses of leaflets exposed to the *A. tenuis* toxin as compared with the weight losses of leaflets inoculated with *A. tenuis* spores.

<i>A. tenuis</i> isolates	Papita guera species	Percentage of weight lost			
		inoculated with spores (average)		exposed to toxin (average)	
A-1	<i>S. cardiophyllum</i>	14.64		24.84	
A-1	<i>S. ehrenbergii</i>	21.19	17.7	12.71	18.77
A-7	<i>S. cardiophyllum</i>	15.35		10.23	
A-7	<i>S. ehrenbergii</i>	26.77	13.3	5.23	7.7
A-10	<i>S. cardiophyllum</i>	3.11		1.27	
A-10	<i>S. ehrenbergii</i>	6.11	4.7	2.07	1.6

### 2.5 Parasitic races of *A. tenuis*.

*A. tenuis* lesions on juvenile leaves were found in "papita guera" plants growing wild in Central Mexico in 1986. Since this parasitic behavior is deviated from normal infections on old leaves, it was decided to find out if such juvenile infections were the result of a special parasitic race of *A. tenuis*. Two isolates of *A. tenuis* were obtained from lesions of young leaves and inoculated on young and old leaves of "papita guera". Also, six additional *A. tenuis* cultures isolated previously from old leaves were inoculated in the same manner. Each treatment had four replications and the whole experiment was repeated to confirm the results, table 8.

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Table B. Parasitic variation found among *A. tenuis* isolates

Isolate	Infections on:	
	Young leaves	old leaves
A-38 from young leaf	+	-
A-27 "	+	+
A-18 from old leaf	+	+
A-31 " "	+	+
A-33 " "	+	+
A-01 " "	-	+
A-07 " "	-	+
A-10 " "	-	+
A-21 " "	-	+
A-22 " "	-	-

#### 2.6. Spore dissemination and its epidemiological significance.

In the winter season, when is dry and there are very few herbaceous plant species, some *A. tenuis* spores were trapped every day. The origin of such spores, appear to be mainly the sporulation occurred on "papita guera" plants infected in our previous field plots. When the plants dry, the spores fall into the soil, and from there, they are dispersed continuously by winds to other crop lands. The spores laying on the ground appear to be an important primary inoculum source as suggested by the first lesions observed frequently on papita leaflets touching the soil.

The significant increase of spores trapped at the end of April and beginning of May, when no *A. tenuis* infections has yet taken places on the papita plants, may have had its origin on the saprophytic activity of *A. tenuis*. At this time of the year, some rains have already fallen, so plant debries left on the soil get wet and they become a suitable substrate to be colonized by *A. tenuis*.

It was found experimentally, that maize debries were the best substrates for *A. tenuis* sprophytic growth. Since maize crop has a wide geographical distribution, its debries appear to be of high significance for *A. tenuis* spore production and consequently for epidemics development on papita populations.

#### 2.7. Effect of age on leaflet susceptibility to *A. tenuis* toxin.

With the aim to check the fidelity of the biosay for testing resistance of papita clones to *A. tenuis* toxin, it was decide to test the effect of leaflet age on its reaction to *A. tenuis* toxin. For such purpose, seven leaflets from leaves of differente ages were exposed to the toxin. The weight lost by leaflets after 48 h of toxin exposure was recorded as shown in next table 9.

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Table 9. Percentage of weight losses shown by leaflets of different age after their toxin exposure.

Relative leaflet age	leaflet weight *		Percentage of weigh loss
	before toxin	after toxin	
1 (oldest)	1.5509	1.2920	83.3%
2	1.2078	0.7810	64.6
3	0.7659	0.4111	53.6
4	0.6722	0.1810	26.9
5	0.3345	0.0494	14.08
6	0.1760	0.0251	14.3
7 (youngest)	0.5766	0.679	11.8

\*

Weigh average of two leaflets.

It is clear from data on above table, that leaflet age has a large influence on the tissue reaction to the toxin. When the leaf is in a juvenile stage is highly resistant and as it is becoming older its reaction change gradually until turns as a very susceptible. This knowledge was useful to establish the criteria for collecting the leaflets to be tested in the bioassay for resistance to the toxin. In that way the resistant values could be within the limits of variation that may permit to consider them valid for the purpose to detect those clones with high levels of resistance. In a preliminary observation in the field on the resistant behavior of our selected clones appears to support the validity of our selecting method based on the toxin bioassay.

### 3. TUBER YIELD

#### 3.1. Search for high tuber-yielding clones.

Most of the "papita guera" plants have very long stolons and produce yields of less than 50g. Undoubtedly these are features for a better fitness in their wild life in an adverse arid environment. The long stolon was interpreted as a useful structure to disseminate the tuber progeny, avoiding in that way a lethal competence of crowded seedlings, as could happen in short stolon plants. However, for papita guera plants under cultivation, the long stolon won't be essential because man can disperse the plants according to distances determined by agronomical methods. Thus, it was thought that short stolon plants must have higher tuber yields, because those photosynthates which are not used for a long stolon development, can be used for increasing tuber production. On basis of this hypothesis, it was decided to obtain short stolon plants. For such purpose, three approaches were attempted: 1) sexual crosses with *S. infundibuliforme*\* to transfer the "topiary" gene to our papita materials. 2) the somaclonal variation by protoplast regeneration\*\* and 3) the search of short stolon

\*/ We express our gratitude to Potato Introduction Station Sturgeon Bay, Wisconsin, for providing us with seeds of *S. infundibuliforme*.

\*\*/ Based on a rather frequent appearance of short stolon plants of *S. tuberosum* through somaclonal variation. Personal communication J.F. by Shepard, Kansas State University.

plants among papita guera populations in which wide variation was expected to find. In the first approach, clones of the double recessive topiary genotype of *S. infundibuliforme* were obtained, but we had no success in the first attempts to make the cross with papita clones. In the second approach we didn't succeed also in the first attempts to regenerate plants from papita protoplasts. On the other hand, we succeeded to find short stolon plants with high yields by single-plant harvest, in which the tuber yield found around the stem, no farther than 20 cm., was the criterium to select high yielding clones. The most outstanding plant found was the 86-136 with a tuber yield of 511 g. fig. 3.

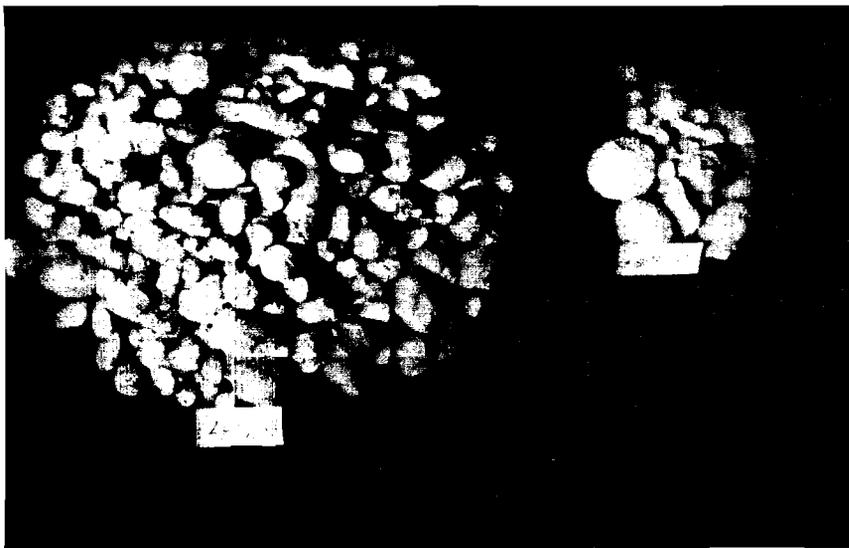


Fig. 3. Left, outstanding single plant yield from clone 86-136; right, common low yield of its neighbor plant, in the selection plot of 1986.

### 3.2. Comparative characterization of the high yielding 86-136 clone with three common clones.

Shortly after the discovery of the outstanding high yielding clone 86-136, it was decided to try to find a morphological or physiological attribute which could be linked to its abundant tuber production. For such purpose, five plants of 86-136 clone were grown under greenhouse conditions, together with same number of plants of three normal yielding common clones.

The results of this comparative study show that there was not a relevant difference in all features observed between 86-136 and the other clones, except the number of stolon branches, table 10 and fig. 4.

Since each stolon branch is a structure with a tuber formation potential, the high stolon branching of clone 86-136 appears to be one of the traits responsible for the very high tuber yield. This conclusion was strengthened after finding that 86-136 clone is not a tetraploid, as was thought due to its exceptional yield, but it is a diploid with 24 chromosomes.

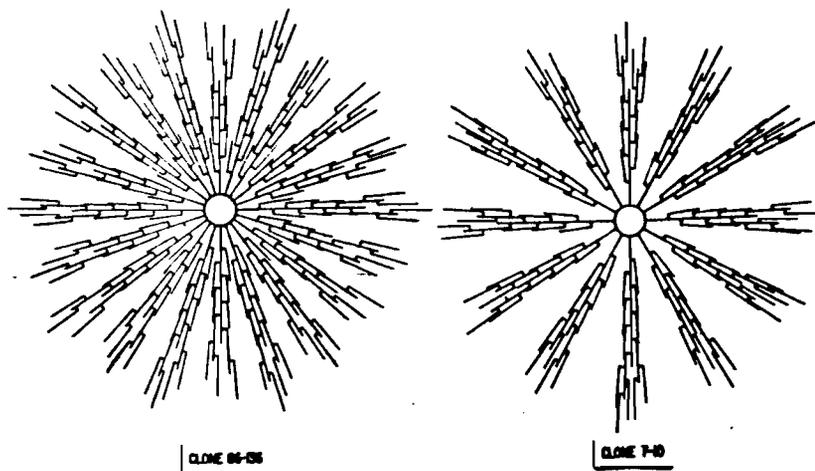


Fig. 4. Diagrammatic representation of stolon branching systems. Left, clone 86-136 (high yield) with 244 stolon branches; right, clone 7.10 (common low yield) with 163 stolon branches.

Table 10. Features shown by clone 86-136 (high yielding) and clones 7-10, 7-4 and 85-055 (low yielding).

Feature	Clone 86-136 <i>S. cardioph.</i>	Clone 7.10 <i>S. cardioph.</i>	Clone 7.4 <i>S. cardioph.</i>	Clone 85-055 <i>S. ehrenbergii</i>
Emergence start	5 days	5 days	5 days	5 days
Emergence period	3 "	9 "	2 "	6 "
Blooming start	25 "	23 "	2 "	2 "
Blooming period	74 "	71 "	51 "	78 "
Senescence start	124 "	124	114	124
Stem (main) length	73 cm	78	79	67
Stem (main) internodes	20 "	22	27	22
Branches*	7-3-4 Tot (14)	5-5-4 (14)	2-5-4 (11)	5-3-3 (11)
Branches internodes	13-3-4	13-3-7	7-2-4	12-3-5
Leaves	82	80	50	79
Stolon** branches	20-150-74 Tot. (244)	12-87-64 Tot. (163)	12-88-55 Tot. (155)	6-67-44 Tot. (117)

\* Lower, middle and upper branches.

\*\* Main, secondary and tertiary stolon branches.

5.1. Verticillium dahliae, causing wilt and dead of plants in the experimental plot of 1925, its incidence was 0.2% in S. cardiophyllum populations and 17.1% in S. ehrenbergii. Unfortunately this pathogen was almost absent in 1926 and 1927 experimental plots established at different places in the same Texcoco zone.

5.2. Sclerotinia sclerotiorum, this was observed as a cause of a wet rot in some tubers. Its incidence was also low and fortuitous.

5.3. Potato virus X. It was detected in plants from central Mexico showing very small leaves and a severe stunt. By electron microscopy two kinds of virus particles were observed, a rod flexuous shape and a polyhedral shape. The long rod flexuous particle was separated by inoculation on Datura stramonium, because it was infections and not the polyhedral virus. By host range and serology it was identified as virus X of potato. It caused very mild mosaic on either S. cardiophyllum or S. ehrenbergii plants. So it was not the cause of the severe disease observed in the field.

5.4. "Papita oreja de raton virus" (mouse ear virus) this polyhedral virus, co-infecting papita plants with potato virus X, as mentioned above, was separated by sap inoculation on Gomphrena globosa which was infected by this virus and not by PVX. When inoculated on papita plants produced the severe symptoms of "ear mouse" leaflets, fig. 5.

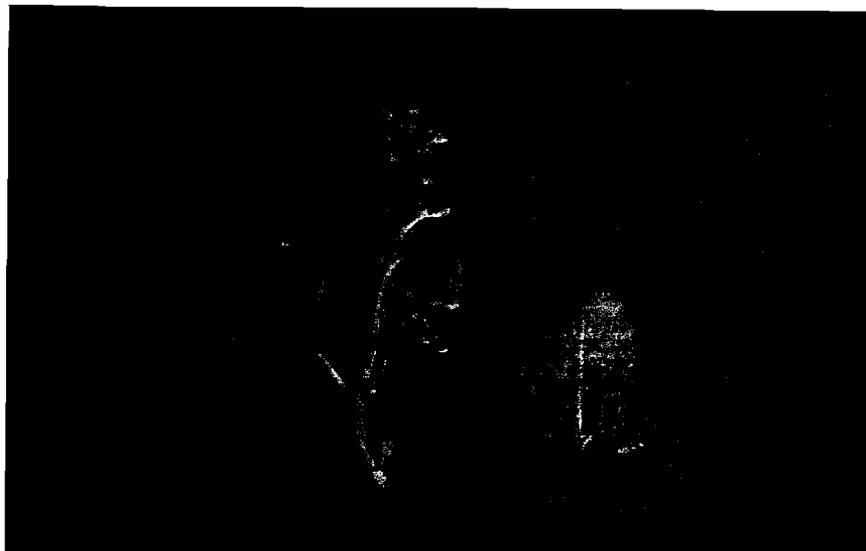


Fig. 5 Left, leaf from virus infected plant showing the "oreja de raton" (ear mouse) symptoms; right, leaflets of healthy plant.

This virus also infected plant species belonging to Amaranthaceae, Chenopodiaceae, Scrophulariaceae and Solanaceae. In the latter family, all 14 species inoculated were infected. The biological characteristics of this virus were compared with those of nine polyhedral viruses pathogenic to common potato, no close relationship was found. So it appears to be a new virus, but further characterization is needed for a final conclusion.

#### 4. TISSUE CULTURE WORK.

The aim to start using tissue culture techniques on "papita guera" project was motivated by the following interests: i) to keep a set of elite clones of the germoplasm bank in vitro, in order to avoid the risk of a total virus or viroid infections of a given clone as could happen with clones multiplied in the greenhouse; ii) to have always available the elite clones for an immediate and rapid multiplication when needed for experimental of research purposes, particularly when tubers are not sprouted; iii) to produce micro-tubers for distribution of germoplasm to other countries and iv) to advance in the basic methods for the use of biotechnological approaches in the breeding for the "papita guera" domestication. The achievements obtained in the three year period are as follows.

The best callus medium found experimentally for papita clones belonging to either *S. cardiophyllum* or *S. ehrenbergii* species was the Murashige-Skoog (1962) with 2.5 mg/l of 2,4-D and 1 mg/l of naphthalen acetic acid.

The best explants found for the above medium were pieces of stems or leaflet.

The best propagative medium found was the Murashige-Skoog with 1 mg/l of benciladenine 1 mg/l of indolacetic acid and 3% sucrose. The best explants for propagation resulted to be the apical meristems and axiliary buds.

The plant regeneration from calli was achieved by transferring callus tissue to the propagative medium.

The rooting of shoots was succeeded with Murashige-Skoog medium containing half the amount of salts, without growth regulators and with 2% of sacharose.

It was obtained a 90% of rooted plants in soil by rooting the shoots outside the glass bottle. The basal part of the shoot stem was dipped in Raizone and kept in agrolite under high humidity. After roots developed, each plantlet was transfer to soil and the very humid environment was gradually changed into a normal environment.

The best medium found to obtain friable callus was the callus medium, described before, with 4.5% additional sacharose.

After some experiments, it was possible to obtain large number of protoplasts from "papita" callus by using the enzymatic solution described by Shepard (1981) for the common potato.

A good result was obtained for mantaining papita shoots in vitro by using the slow growing medium developed for common potato by Espinosa et al (1984).

#### 5. PROSPECTION OF PAPITA GUERA PATHOGENS, OTHER THAN *Alternaria tenuis* AND *Phytophthora infestans*.

By searching papita diseased plants during three years, the following pathogens were found and identified.

It was unfortunate to have a decrease instead of an increase of yield by removal of tuber sprout. But at the same time we were unfortunate to become acquainted with this phenomenon, because the tuber sprout of papita guera fall down easily, so now, special handling of tuber-seeds will be taken to avoid a serious yield loss caused by the of loss sprout lost at planting time.

Table 11. Effect of tuber sprout on crop yield

Treatment	Tubers planted	Emerged plants	Total Yield	Yield per plant
Tubers with sprout	144	130	9,859 g	75 g
Tubers without sprout	144	94	3,967 g	42 g

### 6.2. Effect of the size of tuber-seed on crop yield

In order to find out the less amount of papita tubers that should be allotted as seed for the next year crop, a small trial was done to compare the yield obtained by small tuber-seeds (0.5-10.g) with that of a common size tuber (4-12g). The results showed that by using small tubers there was less plant emergence and also the tuber yield was less than 50% of that obtained with common tubers, table 12.

We can conclude from these results that common size tubers should be used as tuber seed in order to get better yields.

Table 12. Effect of the size of tuber-seed on crop yield.

Size of tuber seed	Tuber planted	Emerged plants	Total yield	Yield per plant
Small tuber (0.5-1.0)	144	109	2134	21 g
Common tuber (4-12 g)	144	130	5220	40 g

## 7. GERMOPLASM BANK.

Most entries to the "papita guera" germoplasm bank are the clones selected in the selection plots established in Montecillos, Tex., from 1985 to 1987. Today there are a total of 539 selected clones. This number of clones will be reduced in the near future because a further characterization and a more strict criteria fixed for their selection. The number of clones left will be those needed for the multiclinal varieties and also for the breeding needed to achieve a high degree of domestication based on monoclonal varieties. With a fewer clones in the bank, a more efficient attention can be given to them for their better maintenance. Also, it will be easier to have a set of most of the selected clones in tissue culture, in order to certify the virus and viroid free condition for their safe distribution.

5.5. Alfalfa Mosaic Virus. This virus was found in experimental plots in Texcoco, causing brilliant yellow spots similar to the so call "calico" symptom of common potato,. The incidence was low, and apparently determined by accidental transmissions caused by migrating aphids from nearby alfalfa crops.

5.6 Papita viroid, with exception of potato spindle tuber viroid (PSTV) all the known viroids infect plant species from warm weather regions. Since potato (*S. tuberosum*) is severely infected by this viroid only in warm summers, it suggested that potato might not be a natural host of PSTV but a circumstantial host resulted by man agricultural activities. These considerations motivated the hypothesis that Mexico could be the geographical origen of PSTV, because "papita guera" which has a rather hot habitat could be its natural host. In order to test this hypothesis a join project was established with MC. Juan Pablo Martinez from " Instituto Nacional de Investigaciones Agricolas" and Dr.T.O. Diener from USDA Beltsville.

We had positive results in leaflet samples taken from "papita" plants without any symptom, which were growing in Central Mexico, and also from sprouts of tubers harvested from the same area. A total of five positive results were obtained from about 1000 samples, that is a 1:200 frequency, these isolates were able to infect Gomphrena globosa and to cause flower variegation in Nicotiana glutinosa, features corresponding to PSTV. However by nucleic hybridization using cDNA probes of PSTV and Tomato Planta Macho viroid (TPMVO) it was found a higher degree of hybridization with the latter probe in spite of having more pathogenic PSTV features. Thus this "papita" viroid appears to be an intermediate form between PSTV and TPMV. Further work is needed for a more precise identification of this viroid. Also with further search of viroids in "papita" populations still the positibility fo find a typical PSTV.

The virus causing "ear mouse leaflet" and the papita parasitic-viroid appear to be the most important pathogens found in this prospection. The virus has a high destructive potential for the future papita crop, because the severe damage it causes in infected plants and its potential acumulation by the papita vegetative propagation. The viroid appears to be, at present, no important for papita crop because no symptoms have been observed on the infected papita plants. But it could be of high importance for other crop plant. This information indicate the need of rigorous handling of papita materials to avoid the discomination of these two parasites within Mexico and to other countries.

## 6. AGRONOMICAL WORKS

### 6.1. Effect of tuber sprout on crop yield.

It was found an increase of yield in common potato when the tuber sprout was removed from the tuber seeds before planting. On this basis it was decided to do an exploratory trail on such agriculture practice with papita tubers. The results shown in table 11 indicate that "papita" plants originated from tuber seeds without sprout din't increase the tuber yield as expected, but on the contrary, the yield was about three times less than that obtained with tuber-seed with sprout. Twenty nine percent of the yield loss was due to a lower plant emergence, and 44% was apparently due to a physiological disturbance caused by the removal of the sprout, as suggested by the lower yield per plant.

F The clones in the bank have the following attributes: 181 belong to *S. ehrenbergii*; 211 to *S. cardiophyllum*; 397 have resistance to *P. infestans*; 315 carry vertical R. genes; 115 show horizontal resistance; 397 have resistance to *A. tenuis* toxin at the 40-100% level of retained weight; and 5 with high tuber yield at the 160 to 511 g range. A few clones with no resistance to pathogens and with very low yield also are kept for research purposes.

#### CONCLUDING REMARKS.

The information obtained in the three years work is in concordance with the hypothesis used as a basis for developing the project. The wide variation expected among papita guera populations according to theoretical considerations, resulted to be a fact. Thus our plant selection based on what nature has done nicely in thousands or millions years, was a simple and rewarding approach for a primogenial and fast breeding to obtain multiclonal varieties.

The discovery of clone 86-136, with a tuber yield more than 20 times of that produced by the common papita plants, is beyond the most optimistic yield expectations.

The Germoplasm Bank conformed in the project is now a rich source of genes for resistance to both agresive papita pathogens and genes for high tuber yield. It offers now the oportunity to start a further breeding towards a higher level of "papita" domestication in a monoclonal form.

The abundant and diverse papita gene sources for resitance to its main pathogens; the genetic high yield potential existing in papita populations, as shown by 86-136 clone; the high food quality of tubers (4% protein) and its excelent drouht resistance, constitute a solid evidence to generate a general conviction that papita guera is a plant which will help to aliviate underfeeding and hunger of people living in arid regions of the world.

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