PN-ABK-952
76603

USAID 4TH REPORT DECEMBER 1990

Utilization of Somaclonal Variants in a Backcrossing Program For Trichome-mediated Insect Resistance in Potato

DPE 5542-6-55-8014-00

INTERNATIONAL POTATO CENTER (CIP)

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L-246-NE-90

November 23,. 1990

Dr. R.L. Plaisted
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U.S.A.

Dear Bob,

As promised here is our progress report for the glandular trichome project supported by USAID funds.

Myself, Maria Palacios and Veronica participated in the screening trials. We have included both a Spanish and an English version of the results. Hope you find them useful.

Please let me know if you need any other additional details. Best regards.

Sincerely,

K.V. Raman
Acting Head,
N & E Dept.

KVR/rg

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The International Potato Center (CIP) is a scientific, autonomous, and non-profit institution dedicated to develop and disseminate knowledge for greater use of the potato and other tuber and root crops as basic foods in the developing world. CIP was established by agreement with the Government of Peru and is supported by the Consultative Group on International Agricultural Research (CGIAR) whose members provide funding for international agricultural development.

PROGRESS REPORT 1990

COLLABORATIVE RESEARCH BETWEEN CORNELL UNIVERSITY AND INTERNATIONAL POTATO CENTER ON UTILISATION OF GLANDULAR TRICHOMES FOR INSECT RESISTANCE IN POTATO

SUMMARY

Five glandular trichome developed potato clones, K 411.2, K 419.8, K 432.5 and K 434.1 were evaluated for resistance to the Broad mite (BM): Polyphagotarsonemus latus, Red spider mite (RSM): Tetranychus urticae, Green peach aphid (GPA): Myzus persicae, Potato tuber moth (PTM): Phthorimaea operculella,and Leaf miner fly(LMF): Liriomyza huidobrensis. Clone K434.1 was less damaged to BM. For RSM clone K432.5 was found resistant. GPA populations were significantly reduced in clone K419.8. In three clones K421.2, K432.5 and K434.1 non-preference (antixenosis) to PTM oviposition was detected. PTM larval antibiosis was high in the foliage of clone K434.1. Tubers from clones K411.2 and K421.2 affected PTM larval development. For LMF four clones, K434.1, K432.5, K419.8 and K 411.2 were selected for early season resistance.

METHODS

Clones K411.2, K419.8, K421.2, K432.5,K434.1, S. berthaultii (PI 265858 with glandular trichomes type A and B) and Desiree (without glandular trichomes) were multiplied using stem cuttings.

Resistance tests for pests were first done for Broad mite, followed by screening for Red spider mite, Green Peach Aphid, Potato Tuber Moth oviposition, and larval damage, and then resistance in tubers. The last test was for Leafminer resistance in field.

The first six tests were conducted using controlled infestations. The test for Leafminer fly was done using natural infestations in field. At the end of each test glandular trichome densities were determined by excising the third terminal leaflet. Trichomes were then examined in an area of 8.8mm^2 at a magnification of 60X under stereomicroscope. Polyphenol oxidase (PPO) activity was evaluated using the catechol test. Cultures of all pests were maintained under greenhouse conditions. From these cultures the various pests were collected and used in resistance tests.

Resistance to Broad Mite (BM):

Field cages were used (see fig 1). 10 plants for each clone were randomised with these cages. One potato leaflet containing a colony of BM was placed on top of each of the plants. 20 days after infestation BM damage in each plant was evaluated .

Resistance to Red Spider Mite (RSM):

Field greenhouses were used for this test. A susceptible bean variety was sown and at 30 days after emergence these bean plants were infested with RSM (see fig 2). The test material was grown in between the rows of beans to allow the RSM to move and damage potato plants (see fig 3). 10 plants/clone were sown. These were distributed at random. Two evaluations, the first at 15 days and the second at 20 days after plant establishment were made for RSM damage.

Resistance to Green Peach Aphid (GPA):

This test was done in field cages. 10 plants/clone were infested with 10 GPA third to fourth stage nymphs on the bottom surface of the third apical leaflet. 10 days later GPA mortality was evaluated by counting visually the total number which were alive vs dead.

Resistance to Potato Tuber Moth (PTM) oviposition:

The third apical leaflet of 10 plants/clone was caged with 2 pairs of adult PTM (2 males + 2 females) using a dialysis tubing (see fig 4). After 3 days egg laying was evaluated in these leaflets.

Resistance to Potato Tuber Moth (PTM) larval damage:
In the greenhouse 10 plants/clone were infested individually with 10 first instar PTM larvae. To prevent escape tangle trap was applied at the base of each plant (fig 5). 7 days after infestation each plant was caged with a mesh bag to prevent pupation outside the caged plant. At approximately three to four weeks after caging larval mortality was evaluated by counting the total number of pupae

Resistance to Potato Tuber Moth (PTM) damage in tubers:
In the laboratory 10 tubers/ clone were individually infested with 6 first instar PTM larvae in plastic cups. These cups were kept at 28 C. 4 days after infestation we evauated larval mortality and tuber damage by counting the holes on surface to tuber.

Resistance to Leafminer fly (LMF) damage in field :

Three plots of 15 plants/clone were grown in field. A randomised block design was used. Five evaluations were made in total. Damage was estimated using a 1-5 visual scale. Class 1 = 0, 2-25% 3=26-50, 4=51-75 and 5=76-100% foliar damage. 5 terminal leaflets collected from each plant were evaluated for feeding punctures created by the ovipositor of insect and larval infestation.

RESULTS:

Table 1 presents data for BM resistance, PPO activity and trichome densities for the clones evaluated. One clone K434.1 has significantly less damage. Glandular trichome density in this clone was low ($5/mm^2$)

Table 2 gives results for RSM resistance. The best clone selected in this trial was K432.5 (Fig 6).

Table 3 gives results for GPA resistance. Mortality of GPA in two clones K419.8 and K411.2 was >80%. In S.berthaultii mortality reached 40.9%.(see fig 7 for trichome exudate on GPA).

Table 4 shows results on PTM oviposition. Clones K421.2, K432.5, K434.1 and S.berthaultii exhibited antixenosis to PTM oviposition. Apparently trichome type B seems to play some role in influencing PTM oviposition.

Table 5 gives mortality data for first instars of PTM. Clone K434.1 and S. berthaultii had the highest mortality to PTM larvae.

Table 6 gives preliminary data on tuber resistance to PTM Clones K411.2 and 421.2 were identified as promising . Table 7 gives data on LMF resistance. Clones K434.1,432.5, 419.8, 411.2 were significantly less damaged than the susceptible variety "Revolucion". (Fig 8 and 9). Damage differences are most clear during the early season. At maturity the differences are not that obvious.

FUTURE WORK:

1. Screen for resistance in field for LMF, PTM and Andean Potato Weevil.

2. Screen for resistance to thrips in Puno.

3. Study in detail the mechanisms of resistance.

INFORME DEL PROYECTO TRICOMAS : CORNELL - CIP

María Palacios
Verónica Cañedo

Resumen:

Se evaluaron 5 clones (K 411.2, K 419.8, K 421.2, K 432.5 y K 434.1) con tricomas, con la finalidad de determinar su resistencia al ácaro blanco Polyphagotarsonemus latus, al ácaro rojo Tetranychus urticae, áfidos Myzus persicae, polilla de la papa Phthorimaea operculella y la mosca minadora Liriomyza huidobrensis. En las pruebas realizadas se encontró que el clone K 434.1 es resistente al ácaro blanco. El clone K 432.5 es resistente al ácaro rojo. El clone K 419.8 mostró resistencia a la multiplicación y sobrevivencia de los áfidos. Los clones K 421.2, K 432.5 y K 434.1 mostraron resistencia del tipo antixenosis a la oviposición de la polilla. El clone K 434.1 mostró igual porcentaje de mortalidad que el testigo S. berthaultii. Los tubérculos de los clones K 411.2 y K 421.2 mostraron resistencia al desarrollo de larvas de polilla. Los clones K 434.1, K 432.5, K 419.8 y K 411.2 mostraron resistencia a la mosca minadora en las primeras etapas del cultivo.

Metodología: El material evaluado incluye 5 clones con tricomas, K 411.2, K 419.8, K 421.2, K 432.5 y K 434.1; la especie S. berthaultii como testigo con tricomas y la variedad Desiree o Revolución como testigo susceptible sin tricomas. Todo este material fué multiplicado por la técnica de esquejes. Las pruebas para detectar resistencia se realizaron secuencialmente de acuerdo al siguiente orden : purebas con acaro blanco, con ácaro rojo, áfidos, adultos de polilla, larvas de polilla en follaje, larvas de polilla en tubérculos, mosca minadora.
Las seis primeras pruebas se desarrollaron en condiciones de infestacion controlada, la última en condiciones de infestacion natural en el campo. Al realizar cada prueba se evaluaba la densidad de tricomas en el foliolo apical de la tercera hoja de la parte superior de la planta, contando el número de tricomas en un área de 8.8 mm con un aumento de 60X . Además se evaluaba la producción de polifenoloxidasa utilizando catecol. Para el desarrollo de las pruebas fué necesario establecer la crianza de los ácaros, a partir de material colectado de campo, los áfidos y las polillas fueron obtenidas de las crianzas que se mantienen en el CIP.

Efecto de tricomas en el desarrollo del ácaro blanco:
Esta prueba se realizó en jaulas en el campo. Fig. 1, se utilizaron 10 plantas por cada clone, las cuales se intercalaron al azar. Se colocó un foliolo de papa infestado con ácaro blanco sobre cada planta, las plantas así infestadas se mantuvieron por 20 días con la finalidad de que el ácaro desarrollara. Se evaluó el porcentaje del follaje dañado en cada planta (hojas secas por efecto del ácaro + hojas verdes pero con síntomas de daño) .

Efecto de los tricomas en el desarrollo del ácaro rojo :
La prueba se realizó en una jaula de campo, .
Previamente se sembró frijol, el cual fué infestado a los 30 días con el ácaro rojo . Cuando el ácaro se estableció en el frijol, Fig. 2, se intercalaron entre los surcos las plantas de los clones a evaluar con la finalidad de que los ácaros pasaran del frijol a la papa Fig. 3. Se utilizaron 10 plantas por cada clone y éstas se distribuyeron al azar. Se realizaron 2 evaluaciones ,la primera a los 15 días y la segunda a los 20 días. Se evaluó el porcentaje del follaje dañado (follaje seco por efecto del daño + follaje con picaduras) y el porcentaje del follaje seco por efecto del daño.

Efecto de los tricomas en la resistencia a áfidos:

La prueba se realizó en jaulas en el campo. Se utilicaron 10 plantas por cada clone las cuales fueron infestadas con 10 ninfas del tercer estadío, las ninfas fueron colocadas en la tercera hoja del tercio superior. Después de 10 días se evaluó el número de áfidos vivos y áfidos muertos en cada planta.

Efecto de los tricomas en la resistencia a adultos de la polilla de la papa:
La prueba se realizó en invernadero, se utilizaron 10 plantas por cada clone, en cada planta se aisló la tercera hoja del tercio superior con tubos de diálisis, en cada tubo se introdujo 2 parejas de polillas (2 machos y 2 hembras) de 48 horas de edad para que ovipositaran, después de 72 horas los adultos fueron retirados y se evaluó el número de huevos sobre las hojas, Fig. 4.

Efecto de los tricomas en el desarrollo de larvas de polilla:
La prueba se realizó en invernadero, se utilizaron 10 plantas por clon. Cada planta fué infestada con 10 larvas recién emergidas, las larvas fueron distribuídas en la planta a razón de una larva por foliolo. Para evitar que las larvas colocadas sobre una planta pasaran a otra, se inpregnó con tangle trap el perímetro que rodeaba cada planta Fig. 5 . Siete días despúes de realizada la infestación cada planta fué cubierta con una bolsa hecha con malla plastificada para evitar que las larvas empuparan fuera de la maceta. Cuando las larvas del testigo susceptible empuparon se procedió a la evaluación contándose el número de larvas que lograron terminar su desarrollo.

Resistencia de los tubérculos a la polilla de la papa:
La prueba se realizó en el laboratorio, se utilizaron 10 tubérculos por clone, los cuales se colocaron individualmente en vasos de plástico. Cada tubérculo se infestó con 6 larvas de pollia recién emergidas, los vasos conteniendo los tubérculos infestados se colocaron en una incubadora a + 28 C. Cuatro días despues se realizó la primera evaluación de mortalidad de larvas y número de huecos por tubérculos.

Efecto de los tricomas en la resistencia a mosca minadora:
Esta prueba se realizó en condiciones de campo. Se sembraron 3 parcelas de 15 plantas cada una por clone. En un diseño de bloque completo ramdomizado. Se realizaron 5 evaluaciones durante el desarrollo de las plantas .Se evaluó el daño causado por las larvas en el follaje utilizando una escala de 5 grados: 1, 2, 3, 4, 5 lo que equivale a 0, 1 - 25, 26 - 50, 51 - 75, 76 - 100 % de follaje dañado respectivamente.
Se colectarón 5 foliolos del tercio superior de la planta por parcela para contar picaduras de alimentación y determinar preferencia de las moscas para alimentarse y 5 foliolos del tercio inferior o medio para contar número de

larvas por foliolo y determinar la intensidad de infestación.

Resultados:

La tabla 1 muestra la densidad de los tricomas, producción de polifenoloxidasa y el porcentaje del follaje dañado por el ácaro blanco Polyphagotarsonemus latus. En esta tabla no se observa diferencias significativas en el porcentaje de follaje dañado entre los testigos con y sin tricomas (S. berthaultii y Desiree), lo que indicaria que no existe relación entre densidad de tricomas y el porcentaje de follaje dañado. El clone K 434.1 presentó el menor porcentaje de follaje dañado y su densidad de tricomas es bastante baja (5 tricomas por mm) diferenciándose significativamente de la densidad de tricomas de S. berthaultii (11.7 tricomas por mm) lo cual apoya lo expresado en los párrafos anteriormente citados.
La tabla 2 muestra los resultados de la prueba con el ácaro rojo Tetranychus urticae. Se puede ver que no existe diferencias en el porcentaje de follaje dañado por el ácaro entre los clones con tricomas (K) y el testigo susceptible (Desiree). En relación a la intensidad de infestación evidenciada por el follaje muerto se observó diferencias, los clones con tricomas muestran el menor porcentaje de follaje seco que el testigo susceptible lo cual indicaría un efecto negativo sobre el ácaro. El mejor clone en esta prueba fué K 432.5 . (Fig. 6)
La tabla 3 muestra los resultados de la prueba con áfidos Myzus persicae. La mortalidad de los áfidos en los clones K fué superior o igual a la del testigo con tricomas (S.berthaultii). El incremento de población en el clone K 419.8 fué inferior en forma significativa al incremento de población en S. berthaultii. La resistencia a áfidos está condicionada a la densidad de tricomas de tipo A, Fig. 7.
La tabla 4 muestra los resultados de la prueba con adultos de Phthorimaea operculella . Los clones K 421.2, K 432.5, K 434.1 y el testigo S. berthaultii tuvieron menor porcentaje de oviposición que el testigo sin tricomas (Desiree). Aparentemente los tricomas tipo B desempeñan un rol importante en la resistencia a la oviposición. Los resultados de esta prueba muestran que el insecto prefiere ovipositar lejos de la planta (en el suelo o en los tubérculos) ya que en el testigo sin tricomas (Desiree) la oviposición en las hojas fué tan solo de 16.3 % , esto implicaría que los tricomas no son determinantes en la resistencia a la oviposición.
La tabla 5 muestra los resultados de la prueba con larvas de polilla. El testigo con tricomas S. berthaultii y el clone K 434.1 tuvieron los porcentajes de mortalidad de larvas más altos. No se observa una relación directa entre la densidad de tricomas y la mortalidad de larvas, aparentemente existe un efecto parcial de los tricomas de tipo A y otros

elementos químicos de la planta (posiblemente glicoalcaloides).
La tabla 6 muestra los resultados preliminares de la resistencia a larvas de polilla en tubérculos. En los clones K 411.2 y K 421.2 el porcentaje de mortalidad fué más alto lo cual indicaría un efecto combinado de antixenosis y antibiosis.
La tabla 7 muestra los reasultados de la cuarta evaluación de campo en la prueba de resistencia a mosca minadora Liriomyza huidobrensis. Se puede observar que en los clones K 434.1, K 432.5, K 419.8 y K 411.2 el daño en el follaje, las picaduras de alimentación y el número de larvas por foliolo son menores que en el testigo susceptible (Revolución) Figs. 8 y 9 . Cuando las plantas con tricomas están madurando el efecto del daño se evidencia en una aceleración brusca de la maduración,lo que se observó en la quinta evaluación. Estas diferencias en las primeras etapas del cultivo son importantes ya que condicionan niveles bajos de población de insectos, lo cual puede ser complementado con un buen manejo de campo.

Proyecciones

1. Se evaluarán los clones K en condiciones de campo y de laboratorio para determinar su resistencia al gorgojo de los andes Premnotrypes sp.

2. Se estudiará en forma detallada los mecanismos de resistencia para cada plaga

RESISTENCIA A INSECTOS EN CLONES K CON TRICOMAS

Clone	Acaro R	Acaro B	Afido	Polilla A	Polilla Lf	Polilla Lt	MM
K 411.2						+	+
k 419.8			+				+
K 421.2				+		+	
K 432.5	+			+			+
K 434.1		+		+	+		+

A = Adultos de polilla
Lf= Larvas de polilla en follaje
Lt= Larvas de polilla en tubérculos
MM= Mosca minadora

Tabla 1 .- DETERMINACION DE RESISTENCIA AL DAÑO DEL "acaro blanco" *Polyphagotarsonemus latus*

	TRICOMAS / mm^2						CATECOL		% Follaje dañado	
	A + B		A		B					
	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E
K 411.2	8.4 bc	3.1	5.5 a	1.9	2.9 d	1.3	3.5a	0.5	63.0 ab	28.3
K 419.8	11.1ab	6.2	4.7 ab	2.5	6.5 b	3.9	2.8 b	0.6	66.0 ab	39.2
K 421.2	9.3abc	5.3	4.6 ab	2.2	4.8 c	3.0	3.2a	0.5	75.0 a	35.9
K 432.5	7.2 cd	3.3	3.6 bc	1.4	2.3 c	0.4	2.3 c	0.4	44.0 bc	37.8
K 434.1	5.0 d	2.3	2.5 cd	1.1	2.5 d	1.3	2.7 b	0.4	37.0 c	35.1
Desiree	1.4 e	0.8	1.4 d	0.8	0. e	0.	1.0 d	0	61.3 abc	35.2
S.berthaultii (Check)	11.7a	3.1	3.4 bc	1.5	8.2a	1.8	1.2 d	0.4	56.6 abc	28.4
DLS 0.05	2.78		1.34		1.58		0.35		25.02	
C.V. (%)	49.38		50.11		53.40		20.06		59.97	

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Tabla 2 .- DETERMINACION DE RESISTENCIA AL DAÑO DEL "acaro rojo" *Tetranychus urticae*

	Porcentaje de follaje dañado				POrcentaje de follaje muerto			
	1°		2°		1°		2°	
	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E
k 419.8	100	0	100	0	60.0abc	0	85.0 ab	0
K 421.2	100	0	100	0	46.5 bc	0	90.0 ab	0
K432.5	98	6.32	100	0	37.5 c	0	79.0 b	0
K 434.1	100	0	100	0	69 ab	0	94.0 ab	0
K 411.2	100	0	100	0	79.5a	0	100.0 a	0
Revolucion	100	0	100	0	79 a	0	100.0 a	0
DLS 0.05	NS		-		23.04**		15.06**	
C.V.	2.59		0		41.51		18.39	

Tabla 3.- DETERMINACION DE RESISTENCIA A *Myzus persicae*

	DENSIDAD de TRICOMAS													
	A + B		A		B		CATECOL		Poblacion total		%Mortalidad afidos	
	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E
K 411.2	6.6 c	3.1	4.7 bc	2.4	2.0 d	0.7	2.2 ab	0.7	52.1 bc	75.6	83.2a	29.2
K 419.8	13.8 a	3.5	6.8 a	2.5	6.9 b	1.7	2.6 a	0.4	33.1 c	24.5	89.9a	20.9
K 421.2	9.9 b	4.2	5.1 b	1.7	5.1 c	2.7	2.1 b	0.9	71.9 b	64.2	57.0	27.7
K 432.5	6.5 abc	2.0	3.5 cd	1.2	2.9 d	1.0	1.7 b	0.8	279.9 a	208.2	56.2 b	25.6
K 434.1	12.5 ab	6.2	5.4 ab	2.8	6.1 bc	3.7	2.0 b	0.8	120.3 b	109.5	50.0 b	21.2
S.berthaultii	12.2 ab	3.7	2.8 d	1.0	9.1 a	2.4	1.0 c	0.2	59.0 bc	40.9	64.3 b	17.6
DLS 0.05	2.72		1.41		1.55		0.49		71.16		16.78	
C.V. (%)	41.77		45.79		46.30		37.33		109.42		32.72	

Tabla 4 .- DETERMINACION DE RESISTENCIA A LA OVIPOSICION DE ADULTOS DE *Phthorimaea operculella*

	TRICOMAS / mm^2						CATECOL		% OVIPOSICION			
	A + B		A		B				Hojas		T.D + E	
	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{X}$	D.E	$\bar{X}$	D.E
K 411.2	8.3 bcd	2.9	5.8 a	1.9	2.4 c	1.1	3.0 ab	0.7	12.12ab	12.1	87.8 bc	12.2
K 419.8	9.8 ab	4.0	4.2 b	1.5	5.6 a	2.7	1.6 de	0.9	8.5 bc	13.4	91.4 ab	13.4
K 421.2	11.6 a	4.5	5.6 a	2.5	5.9 a	2.3	2.7 bc	1.0	4.2 c	7.5	95.7 a	7.5
K 432.5	9.3 bc	2.9	4.2 b	1.3	4.9 a	1.9	2.2 cd	0.9	1.7 c	3.6	97.6 a	4.3
K 434.1	7.1 cd	3.2	3.4 b	1.7	3.5 bc	1.6	3.3 a	0.6	4.7 c	5.4	95.2 a	5.4
Desiree	1.2 e	0.8	1.2 c	0.8	0. d	0.	1.0 f	0.	16.3 a	14.3	83.6 c	14.3
S.berthaultii	6.6 cd	1.9	1.8 c	0.9	4.7 ab	1.1	1.2 ef	0.4	1.7 c	2.4	98.2 a	2.4
DLS ^0.05	2.28		1.18		1.28		0.54		6.93		6.96	
C.V. (%)	40.69		43.25		45.50		34.48		135.25		10.35	

Tabla 5 .- DETERMINACION DE RESISTENCIA A LARVAS DE LA "polilla de la papa" Phthorimaea operculella

	A + B		A		B		CATECOL		% Mortalidad	
	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E
K 411.2	10.1 ab	8.3	7.3 a	6.2	2.7	1.6	3.6a	0.4	48.6 de	18.0
K 419.8	12.4 a	3.6	6.3 ab	1.8	6.0 b	2.0	3.2 ab	0.5	50.6 cd	14.8
K 421.2	10.5 ab	4.2	5.0 bc	2.3	5.4 b	2.1	3.0 bc	0.7	37.3 e	14.3
K 432.5	7.6 bc	3.4	3.8 c	1.9	3.9 c	1.6	2.6 de	0.8	52.0 bcd	24.2
K 434.1	6.4 c	3.1	3.4 cd	1.7	3.0 cd	1.4	2.7 cd	0.5	61.3abc	17.2
S.berthaultii	12.0 a	2.3	3.9 c	1.1	8.0 a	1.4	2.2 e	0.5	70.6a	15.8
DLS 0.05	3.10		2.06		1.17		0.42		12.6	
C.V. (%)	49.35		63.44		38.80		22.29		31.76	

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Tabla 6.- DETERMINACION DE RESISTENCIA EN TUBERCULOS A LARVAS DE *Phthorimaea operculella*

	Mortalidad (%)		Huecos / Tuberculo (N°)	
	$\bar{x}$	D.E	$\bar{x}$	D.E
K 411.2	36.40 a	20.27	3.3 a	1.49
K 419.8	29.90 ab	10.33	3.7 a	0.82
K 421.2	36.40 a	7.17	2.1 b	1.10
K 432.5	20.20 bc	6.75	3.7 a	0.67
K 434.1	21.60 bc	13.53	3.2 a	1.32
Revolución	10.10 c	16.5	4.2 a	0.92
DLS 0.05	11.91		0.97	
C.V. (%)	51.54		32.44	

Tabla 7 .- DETERMINACION DE RESISTENCIA A LA "mosca minadora" *Liriomyza huidobrensis*

	DAÑO EN FOLLAJE									
	Grado		Porcentaje		P.A./area		Población recup./folíolo	
	$\bar{x}$	D.E	$\bar{x}$	D.E	$\bar{x}$	D.E	x	D.E
K 434.1	2.0 d	0	13.8 c	3.8	4.9 d	3.7	5.1 b	1.6
K 432.5	2.0 d	0	9.9 c	2.8	32.9 b	0.9	3.7 b	0.6
K 421.2	4.0a	0	60 a	0	28.0 bc	6.7	2.2 b	0
K 419.8	2.0 d	0	11.6 c	6.0	13.6 bcd	6.4	3.8 b	0.5
K 411.2	2.3 c	0.3	15.7 c	5.8	-	-	3.1 b	1.5
Revolucion	3.1 b	0.17	43.3 b	8.8	81.1á	15.8	17.8	5.8
DLS 0.05	0.25**		9.49**		14.94**		4.58**	
C.V (%)	5.51		20.75		26.12		43.06	

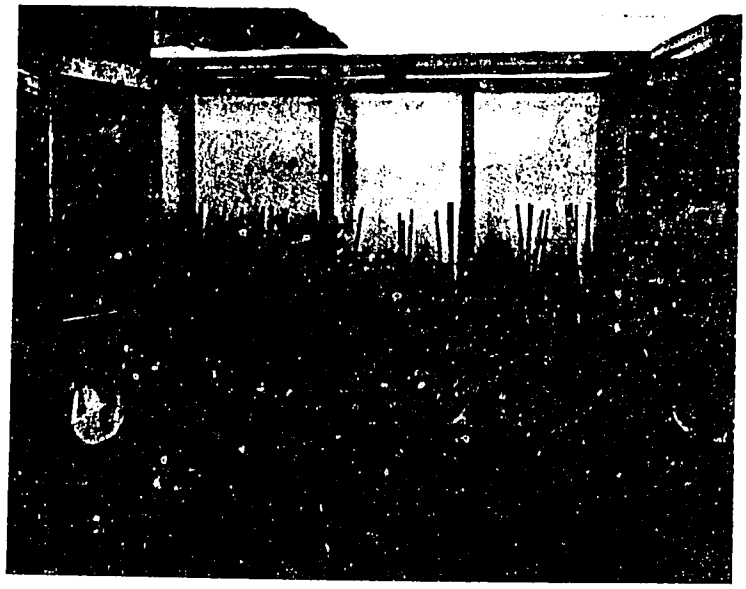


Fig. 1 Prueba con ácaro blanco Polyphagotarsonemus latus

Fig. 2 Metodología de Multiplicación del Acaro rojo.

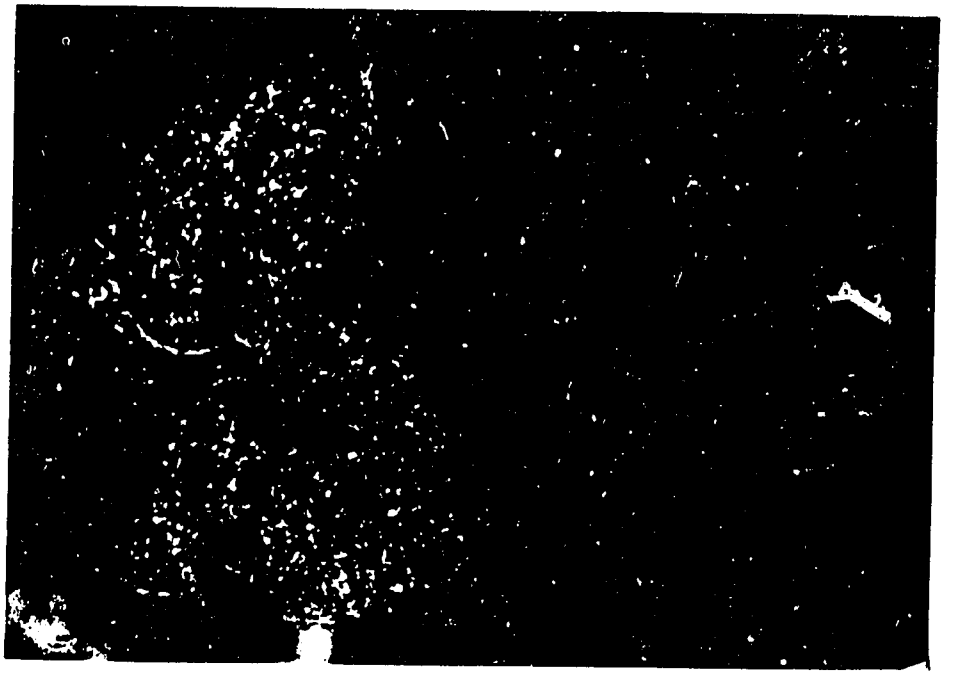


Fig. 3 Prueba con ácaro rojo Tetranychus urticae

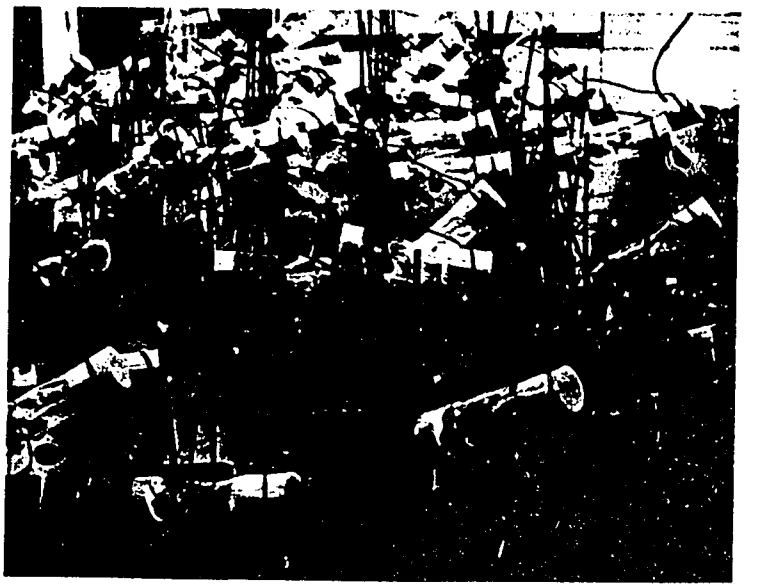


Fig. 4 Prueba con adultos de Pólilla de la papa Phthorimaea operculella

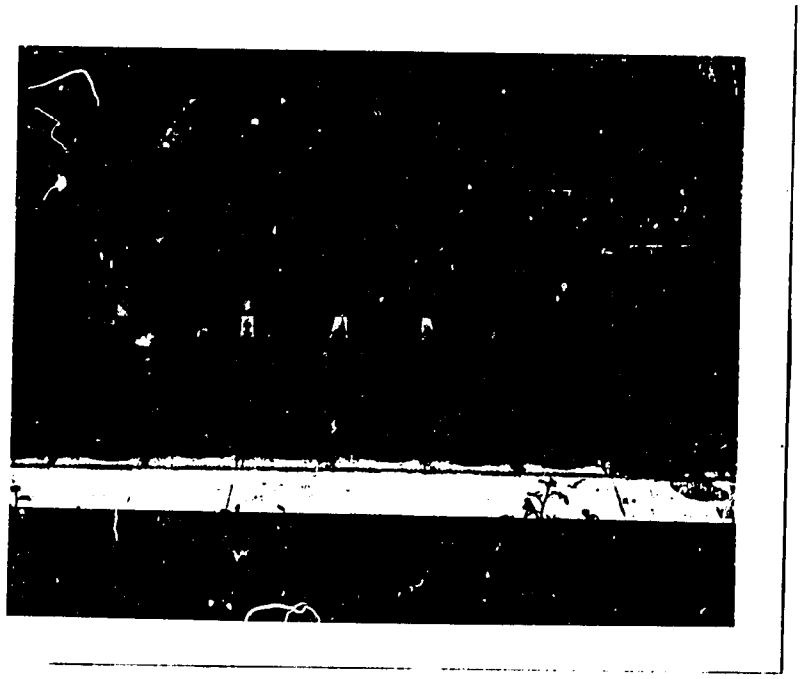


Fig. 5 Prueba con larvas de polilla de la papa Phthorimaea operculella

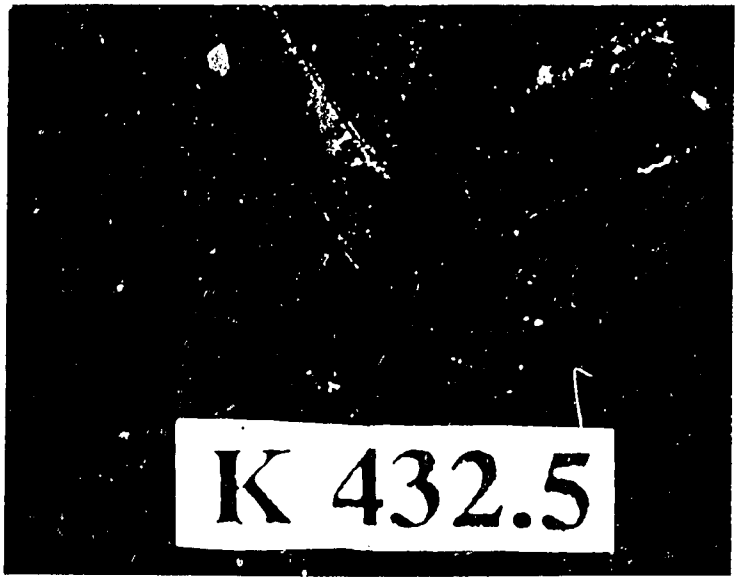


Fig. 6 Resistencia al ácaro rojo Tetranychus urticae

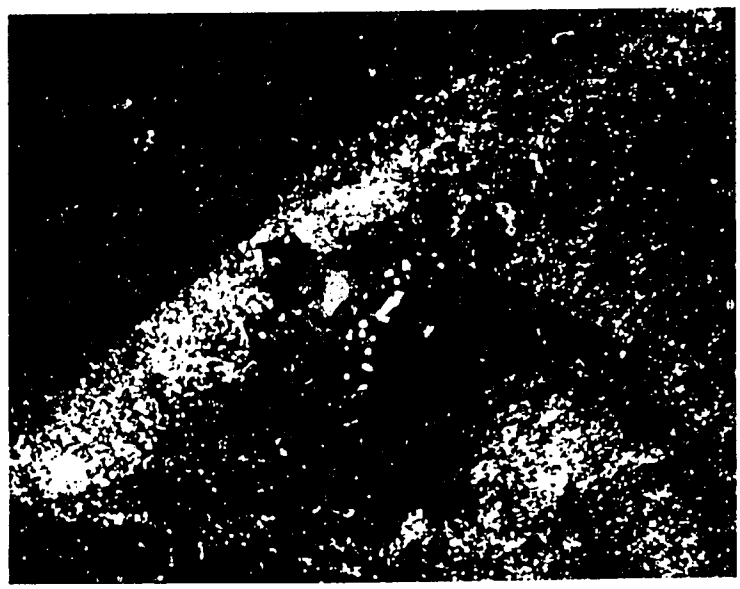
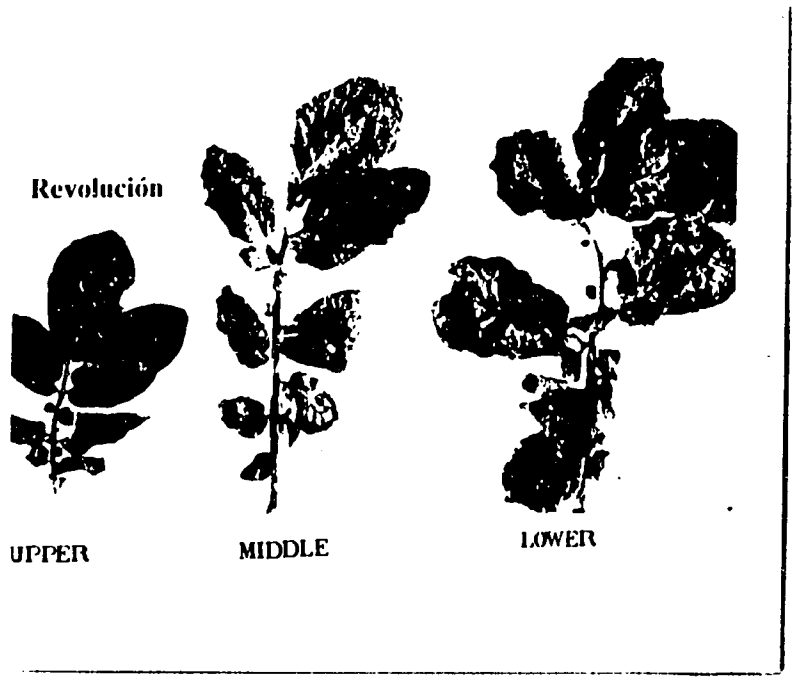


Fig. 7 Efecto de los tricomas en los áfidos.



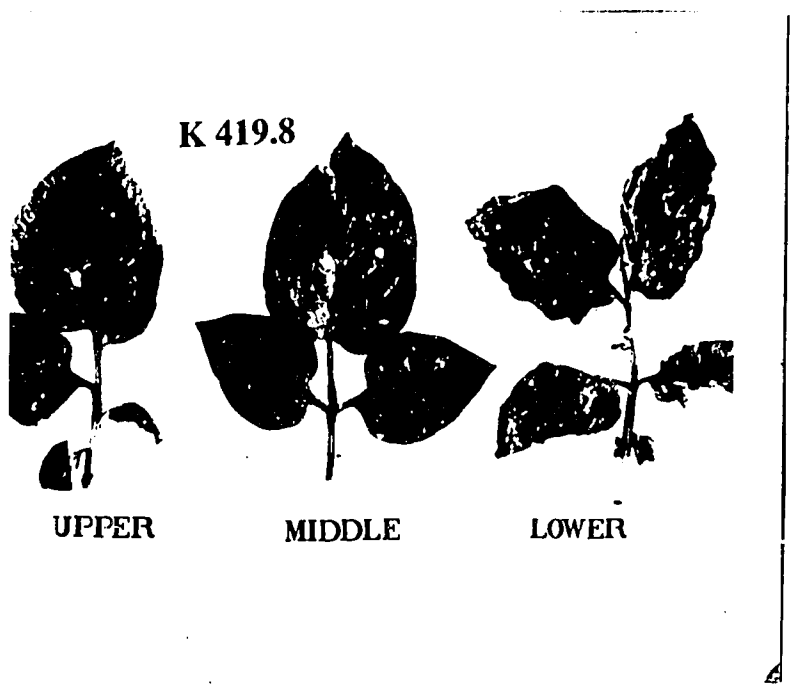


Fig. 8 Clone resistente a mosca minadora.

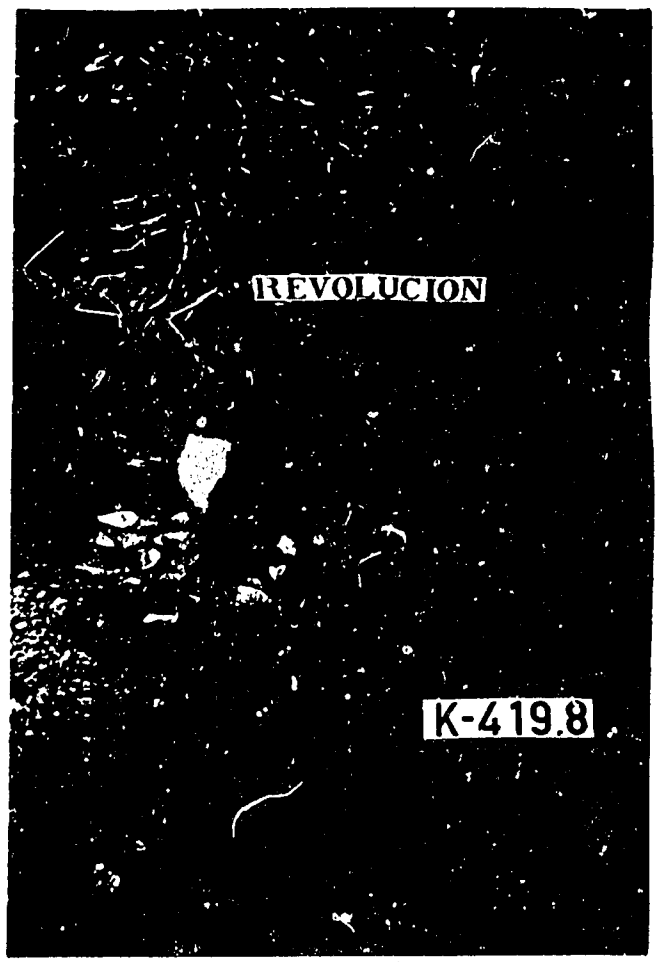


Fig. 9 Clon resistente a mosca minadora.

UNIVERSITY OF THE PHILIPPINES AT LOS BAÑOS
College of Agriculture
College, Laguna, Philippines

INSTITUTE OF PLANT BREEDING

08 March 1990
90053 - ETR

Dr. Robert L. Plaisted
Department of Plant Breeding
and Biometry
Cornell University
Ithaca, NY 14850
U. S. A.

Dear Dr. Plaisted:

Herewith is a narrative report about the five clones that we received from you through Peter van der Zaag. All the clones except K-411-2 responded very well to in vitro multiplication and we now have enough tubers for evaluation during the warm months. We are also planning to use these for crosses with our heat tolerant and bacterial wilt resistant clones.

The regular season evaluation for TPS and clonal materials in our breeding project has just been completed. We may be able to release one clonal variety each for highland and low elevation within the year after assessing the multilocation data. The season has been generally good.

Please give my regards to the plant breeding staff and students.

Sincerely,

EUFEMIO T. RASCO, JR.
Director

cc.: P van der Zaag
Mr. EC Altoveros

SUMMARY REPORT OF CLONES RECEIVED FROM CORNELL UNIVERSITY

One test tube each of *in-vitro* shoot cultures of five promising clones, viz., K-434-1, K-419-8, K-432-5, K-411-2 and K-421-2 with trichome character were received on April 13, 1989 from the Cornell Potato Breeding Program. The *in-vitro* cultures were given to the Tissue Culture Laboratory of Institute of Plant Breeding, UP Los Banos for germplasm maintenance and for initial multiplication *in-vitro*. In September 1989, mother plants obtained from *in-vitro* plantlets were established and multiplied in Benguet State University, La Trinidad, Benguet (1,350 m asl) using the stem cutting technique under screenhouse conditions. Clone K-411-2 did not respond positively to multiplication *in-vitro* and multiplication was significantly delayed for this clone. In November 1989, 40 rooted stem cuttings (RCS's) for each of the four clones were planted in 6" clay pots (2 RCS's to each pot) to produce tuberlets. The RCS's were grown under screenhouse conditions using heat-sterilized soil medium. Harvesting was done on the first week of February and seed tubers are now being treated with calcium carbide to break dormancy. Half of the tubers harvested will be used in the screening for thrips and mites resistance during summer (mid-March) where the insect population is expected to be high. Controlled (screenhouse) and field evaluations will be done in several seasons if conditions allow. The remaining half will be used in further seed tuber multiplication and hybridization.

Insect-resistant plants with improved horticultural traits from interspecific potato hybrids grown *in vitro*.

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Dept. of Plant Breeding and Biometry
Cornell University, Ithaca NY 14853-1902, USA

Introduction

Many attempts to use somaclonal variation as an aid to plant breeding have sought to recover novel traits not present in the original plant material. Our work has a more limited and perhaps more realistic goal: to generate reassortment of genes already present in the original material.

The plant materials used were interspecific hybrids of potato (*Solanum tuberosum* L.) and *S. berthaultii*, an insect resistant wild species from South America. Glandular trichomes on the foliage of *S. berthaultii* confer resistance to insects, including aphids, Colorado potato beetles, leafhopper, mites and thrips. Two types of trichomes are involved in the resistance: type A (short-stalked, with a gland on the tip that can exude a phenolic substance) and type B (longer, with a viscous sticky droplet consisting mainly of sucrose esters). Earlier studies in the Cornell potato breeding program showed that backcrosses of *S. tuberosum* x *S. berthaultii* hybrids to *S. tuberosum* gave predominantly the cultivated phenotype without insect resistance (Kalazich, 1989). There appeared to be restricted recombination between the wild species and the cultivated genomes, which made it difficult to combine the horticultural characteristics of potato with the resistance of the wild species. Because phenotypic and chromosomal alterations have often been seen after tissue culture of potato (reviewed in Karp et al., 1989), we have tested *in vitro* culture of the interspecific hybrids as a strategy for aiding introgression of insect resistance into potato.

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Studies with tetraploid hybrid materials

Plant materials

Tetraploid hybrids of *S. tuberosum* x *S. berthaultii* were used in most of the work. Hybrid F743-4 was 50% *tuberosum* (but not an F_1); hybrid J114-1 and five others tested were 75% *tuberosum*. Callus was initiated from petioles of *in vitro* grown virus-free plantlets of these hybrids using a Murashige-Skoog medium with 3% sucrose and 0.2 mg/L α-naphthaleneacetic acid (NAA), 2.3 mg/L 6-benzyl-aminopurine (BAP) and 10.0 mg/L gibberellic acid (GA_3). Regeneration was evaluated after transfer of callus to medium with different growth regulators (no NAA, 2.3 mg/L BAP, 5.0 mg/L GA_3). Most petioles from *S. tuberosum* controls formed callus, and about 25% of the petioles regenerated one or more plants. *S. berthaultii* petioles produced less callus and almost no plants. The seven hybrid lines tested varied substantially in callus formation and in plant regeneration. Hybrid J114-1 produced 56 plants/10 petioles while F743-4 produced only 5 plants/10 petioles. Plants regenerated from J114-1 and F743-4 after short (2 weeks) or longer (up to 14 weeks) periods of callus growth were used to obtain clonal or sexual progeny for evaluation in the greenhouse and field.

Evaluation of clonal progeny of regenerated plants

Clonal progeny were evaluated in replicated trials conducted in 1987 and 1988, using two populations of plants propagated by nodal cuttings from the original regenerates maintained *in vitro*.. Progeny of two of the 58 R_0 plants recovered from calli of hybrid J114-1 showed stable and heritable differences from the original hybrid over two cycles of evaluations in the field. One regenerate showed significantly higher marketable yield (approx. 2 fold) than the J114-1 hybrid and retained trichome characteristics comparable to the hybrid. The other regenerate had higher levels of phenolic exudate in the A type of trichome. This second regenerate also showed a heritable mutation in flower color, from blue to

white with blue flecks.

Evaluation of sexual progeny of regenerated plants

Sexual progeny of some regenerates showed desirable changes not seen in their clonal progeny. A backcross of hybrid F743-4 to *S. tuberosum* gave very few (0-3%) progeny with droplets on trichome B and only 18% with browning in trichome A. In contrast, seven of 14 regenerates from hybrid F743-4 had more backcross progeny (up to 15 fold) with these desirable trichome traits than the original hybrid. One of the seven had been regenerated from 2-week old callus, suggesting that even a very brief period in culture may give rise to genetic changes. In field studies, these culture-derived plants showed better resistance to leafhoppers than controls, and some had good vine type and yield.

Progeny from a second backcross of at least one regenerated plant again showed a high percentage of plants with droplets on type B trichomes and high browning of type A trichomes. Further backcrosses and evaluation of these culture-derived materials are in progress in the Cornell potato breeding program.

We do not know the genetic basis of the altered phenotypes but they are not associated with changes in ploidy; the regenerates with higher levels of insect resistant progeny are tetraploid like the original hybrid lines.

Studies with diploid F_1 hybrids

The studies described above were done using tetraploid advanced hybrids because diploid F_1 hybrids were not available. We are now working with diploid F_1 hybrids produced by a cross between a diphaploid *S. tuberosum* line and *S. berthaultii*. Petiole explants from 14 different F_1 hybrid plantlets showed great differences in callus growth and regeneration. We have regenerated over 150 vigorous plants from five of the F_1 hybrids, some after more than 7 months of callus culture. Plants are currently being evaluated in the greenhouse for trichome characters. Selected plants will be used for selfs, intercrosses or

backcrosses with *S. tuberosum.*

It will be possible to do the backcrosses either at the diploid or tetraploid level because both diploid and tetraploid R_0 plants were recovered. The ploidy of small regenerated plantlets was determined by a convenient procedure: nuclei were isolated from small amounts (~50 mg) of chopped leaves, stained with a fluorescent DNA-binding stain, and passed through a flow cytometer for analysis of DNA content (Galbraith et al., as modified by Arumuganathan et al., in preparation). Many of the R0 plants (~50%) were tetraploids, rather than diploids. Tetraploid plants were recovered from some cultures maintained as callus for only one month; other calli still produced diploid plants even after 7 months of unorganized growth. Some stunted octoploid plants were also obtained.

Backcross progeny of the R0 plants from the diploid F_1 hybrid explants will be compared to backcross progeny from the non-regenerated F_1 hybrids. The aim is to determine whether changes similar to those observed in the earlier work with tetraploid advanced hybrids occur, whether work at the diploid or tetraploid level and/or with F_1 or more advanced hybrids gives better results, and whether a longer period of callus culture before regeneration is helpful. These studies should help determine which type of explants and culture procedures to use in further work.

Conclusions

The results from this project suggest that callus culture of interspecific hybrids, followed by plant regeneration, may aid in the introgression of desirable traits from wild species into crop plants. The callus phase also facilitates chromosome doubling that may aid in further breeding procedures. Our work has been limited to potato, but the same approach may also be useful for other crops.

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Acknowledgements

This work was supported by U.S.A.I.D., the International Potato Center, Hatch Project 422 (E.D.E.), the NY State College of Agriculture and Life Sciences, and the NY State Integrated Pest Management Program.