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

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Summary. Plants were regenerated from petiole calli of interspecific hybrids of *Solanum tuberosum* × *S. berthaultii*, an insect-resistant wild species. Callus culture was used to generate genetic changes to overcome the restricted recombination between the two genomes. Two plants out of 58 (3.5%) from calli of hybrid J114-1 showed stable and heritable differences from the hybrid over two cycles of evaluations in the field. Replicated trials were conducted in 1987 and 1988, using two populations of plants propagated by nodal cuttings from the original regenerates maintained in vitro. One regenerate showed insect resistance and increased marketable yield (approximately two fold) in the field. The other had higher levels of phenolic exudate in one of the two types of foliar trichomes associated with the insect resistance mechanism. Some desirable changes were discernible only in sexual progeny of regenerates, not in the regenerates themselves. In a backcross to *S. tuberosum*, 7 of 14 (50%) regenerates from hybrid F743-4 showed more progeny (up to 15-fold) with improved trichome traits and horticultural characteristics than the original hybrid. The variations were not associated with changes in ploidy. Fifteen plants obtained from these crosses are currently being incorporated into breeding lines. These results suggest that a period of callus culture followed by plant regeneration may aid in the introgression of desirable traits from wild species into crop plants.

Key words: *Solanum tuberosum* – *Solanum berthaultii* – Insect resistance – Introgression – Potato somaclonal variation

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Introduction

The presence of glandular trichomes on the foliage of *S. berthaultii* confers resistance to aphids, Colorado potato beetles, leafhoppers, mites, and thrips (Gibson 1971; Gibson and Turner 1977; Tingey and Gibson 1978; Tingey and Laubengayer 1981; Wright et al. 1985). The type A trichome is short-stalked with a gland on the tip (Fig. 1). Type A exudate is composed mainly of a polyphenol-oxidase (PPO) and its phenolic substrate and product (Ryan et al. 1982). The type B trichome is longer, with a viscous, sticky droplet exuded from the apex (Gregory et al. 1986) (Fig. 1). The sticky exudate from type B, composed primarily of sucrose esters (King et al. 1986), traps insects and aids the rupture of type A trichomes (Tingey and Laubengayer 1981). Upon rupture, the phenolic substance exuded from type A trichome hardens, immobilizing and preventing feeding of insects. The release of volatile compounds, mostly sesquiterpenes, by type A is associated with insect repellance (Avé et al. 1987).

Earlier studies in our breeding program showed that predominantly the cultivated phenotype without insect resistance was recovered when *S. tuberosum* × *S. berthaultii* hybrids were backcrossed to *S. tuberosum*. There appears to be restricted recombination between the wild species and the cultivated genomes, which greatly constrains the use of backcrossing to incorporate the horticultural characteristics of potato into the resistant clones (Mehlenbacher et al. 1983; Kalazich 1989).

The genetic changes generated during callus culture (i.e., somaclonal variation, Larkin and Scowcroft 1981) may be useful to overcome barriers to hybridization such as those restricting the introgression of the desirable traits into the cultivated crop. The changes generated during callus culture may include altered chromosome

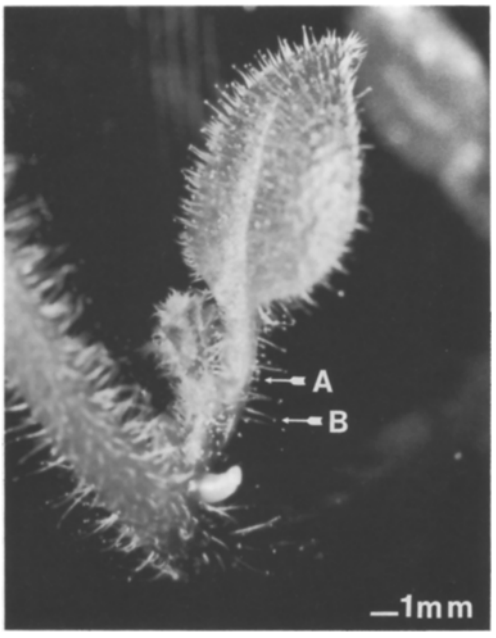

Fig. 1. Glandular trichomes on leaf of a *Solanum tuberosum* × *S. berthaultii* hybrid growing in a culture tube. *A* Type A trichome; *B* type B trichome

number, as well as structural changes (e.g., translocations, etc.) and/or changes in nucleotide sequence (cryptic changes) (Scowcroft et al. 1987). These types of genetic changes have been recovered in potato regenerates (Calberg et al. 1984; Creissen and Karp 1985; Hermsen et al. 1981; Karp et al. 1982; Landsmann and Uhrig 1985; Sree Ramulu et al. 1983, 1984, 1985; Pijnacker and Ferwerda 1987). Thus, callus culture may induce intrachromosomal and/or interchromosomal changes enhancing gene recombination in interspecific hybrids (Orton 1980; Orton and Steidl 1980; Lapitan et al. 1984, 1986, 1988), and may influence the regulation of crossing-over and modify its local control (Sibi et al. 1984). The enhancement of recombination induced by culture seems not to rely on homeology since nonhomeologous exchanges occur frequently (Lapitan et al. 1984; Lee and Phillips 1987).

Many attempts to use somaclonal variation as an aid to plant breeding have sought to identify novel traits not present in the original material. Our work had a different goal: to generate reassortment of genes already present in the original material. Plant regeneration was attempted from petiole calli of seven *Solanum tuberosum* × *S. berthaultii* hybrids. Results indicated that a short cycle of callus culture may be used to accelerate the introgression of insect resistance from *S. berthaultii* into potato. The phenotypic changes noted on regenerates in the field, as well as their stability and heritability through clonal propagation and sexual crosses, are described.

Materials and methods

Plant material

In vitro shoot cultures of tetraploid (2n = 4x = 48) *S. tuberosum* L. (cv Atlantic, NY 75 and NY 76) and interspecific hybrids *S. tuberosum* × *S. berthaultii* [F743-4 (50% *S. tuberosum*); J106-1, J114-1, J115-1, J129-8, J133-1, and J145-2 (75% *S. tuberosum*)], and diploid (2n = 2x = 24) *S. berthaultii* Hawkes (PI 265858 and PI 473331) were used. *S. tuberosum* and hybrid clones were obtained from the in vitro maintenance stock of the Cornell University potato insect resistance breeding program. The clones have been tested for freedom from potato spindle tuber viroid and viruses X, Y, S, A, M, and PLRV. Shoot cultures were maintained in culture tubes on propagation medium (Hussey and Stacey 1981) with 3% (w/v) sucrose, pH 5.9, and solidified with 0.35% (w/v) Gelrite (Scott Laboratories, Inc.) (medium A). Seeds of *S. berthaultii* (from the Potato Introduction Station, Sturgeon Bay, WI) were surface sterilized and germinated (Haberlach et al. 1985). Seedlings (1–2 cm tall) were transferred onto medium A. Shoot cultures were initiated from single stem nodes 4 weeks later (Hussey and Stacey 1981). Plants previously grown in culture tubes were clonally propagated in glass culture dishes sealed with a porous tape (filter tape 19-9708, Carolina Biological Supply Co.) before use as explant donors. In vitro plants were kept in a culture room [25 °C day/night, 16 h/day, 84 μmol m^{-2} s^{-1} provided by Cool White (20 W) and GRO-LUX (40 W) bulbs].

Callus cultures and plant regeneration

Callus cultures were established from leaf petioles from the third to the seventh node of 4- to 6-week old plants grown in vitro. Petioles were placed abaxial side down onto medium containing Murashige and Skoog (1962) (MS) salts, 0.2 mg/l 1-naphthalene acetic acid (NAA), 2.3 mg/l 6-benzyl-aminopurine (BAP), 10.0 mg/l gibberellic acid (GA_3), 3% (w/v) sucrose and 0.22% (w/v) Gelrite (medium B) and cultured in the light in the culture room. After 2 weeks, some calli (short-term calli) were transferred to a medium of the same composition, but without NAA and with 5.0 mg/l GA_3 (medium C) for plant regeneration. Other calli (long-term calli) were transferred to MS medium with 0.1 mg/l NAA and 2.3 mg/l BAP and no GA_3 (medium D). Calli were subcultured onto medium C or D every 3 weeks. After 12 weeks on medium D, long-term calli were transferred to medium C for plant regeneration. Individual adventitious shoots/plantlets (ca. 1.5 cm height) were excised and transferred to propagation medium A for root formation. Regenerates were maintained in vitro as shoot cultures. Plants were potted in Cornell soil-less mix (Boodley and Sheldrake 1973) plus vermiculite (1:1 w/w) in 72-cell Speedling trays watered with 1/4 MS salts weekly and kept in a mist chamber. After 1–2 weeks, plants were transplanted into Cornell soil-less mix in 18-cell Speedling trays and grown under greenhouse conditions for about 3 weeks. Plants were moved from the greenhouse to outdoors for 1 week before transplanting into the field.

Clonal propagation

Increased numbers of each regenerate were used for replicated field trials. In 1987, regenerates were clonally propagated by nodal cuttings in the greenhouse. Cuttings were treated with Rootone F (Amchem Products, Inc.) to enhance rooting, placed in a mist chamber for 1 week, and moved to the greenhouse. In 1988, plants were clonally propagated in vitro by nodal cuttings of the original regenerates, which had been maintained as shoot cultures. Controls consisted of *S. tuberosum*, *S. berthaultii*, and hybrid clones that had been maintained in vitro as shoot cultures

Fig. 2 a and b. Vine type of offspring plants from backcross to *Solanum tuberosum* of regenerates in the field. **a** *Berthaultii* type (score 1); **b** *tuberosum* type (score 5)

and clonally propagated in the greenhouse or in vitro like the regenerated plants.

Sexual crosses and greenhouse evaluations

Plants from in vitro cultures were grown to maturity in a greenhouse (16 h/day, 1,000 W metal halide lamps, 20° ± 2 °C day/night). Hybrids and their corresponding regenerates were backcrossed to *S. tuberosum* cv Atlantic or selfed. Offspring seeds were treated with 2 g/l GA_3 overnight, sown in Quadripaks and then transplanted onto 72-cell Speedling trays in the greenhouse 3 weeks later. Four-week-old seedlings were evaluated for the presence or absence of droplets (i.e., exudate) on type B trichomes in the greenhouse. The abaxial surface of two fully expanded leaves per seedling was examined with a Panasonic hand lens (30 ×). One hundred and eight seedlings were evaluated per cross (total of 55 crosses, 5,940 seedlings). Some regenerate crosses were then selected, either because they had a higher frequency of seedlings with droplets than the progeny of the corresponding hybrid or because they covered the range of variation in droplet frequency observed. The selected crosses as well as the progeny of each hybrid were further evaluated in the field.

Terminology

Regenerates (R_0 plants) are designated by a number identifying the hybrid source (4000 corresponds to F743-4, 5000 corresponds to J114-1, etc.), and a plant number (4001, 5052, etc.). Sexual progeny of R_0 plants are termed R_1. Offspring from selfing of R_0 plants are referred to as S-R_1 family and those from backcross as BC-R_1 family.

Field trials

Plants were evaluated for trichome density and head size (Lentini 1989), concentration of PPO in trichome A [using an ELISA test (Kowalski 1989)], presence or absence of phenolic exudate in trichome A, and leafhopper resistance in field plots subject to natural infestations without chemical control. Phenolic exudate was scored with a modified enzymic browning assay (MEBA), which measures the absorbance (OD at 470 nm) per approximately 28 mm^2 leaf surface (Avé et al. 1986); plants with MEBA ≥ 0.21 OD are referred to as with browning (Kalazich 1989). Leafhopper resistance was rated by the presence of reddish coloration on leaflet tips and margins (hopperburn) and/or the rolling of leaves, and scored from 0 (immune) to 5 (highly susceptible or dead). Scores ≥ 3 (> 25% of leaf tissue with visible damage) were referred to as susceptible. Plants were also evaluated for leaf area, flower color, tuber skin color, marketable yield per plant (tubers ≥ 5.0 cm in diameter), and vine type, which was rated from 1 to 5. Score 1 refers to the *S. berthaultii* phenotype characterized by acute leaves and narrow leaflets (Fig. 2a) and very late maturity indicated by large number of stems, green vines, and active flowering. Score 5 refers to the *S. tuberosum* phenotype represented by a prostrate growth habit and broad leaflets (Fig. 2b) and earlier maturity based on the presence of fewer stems, senescing vines, and post-flowering stage. More emphasis was placed on maturity than on phenotype.

Four clones in 1987 and eight clones in 1988 were evaluated per each R_0 regenerate or control. Thus, 132 plants were scored from 33 R_0 plants randomly selected from the total regenerated in 1987, and 504 plants from 63 R_0s in 1988. In 1987, these plants were spaced 1 × 1 m in two completely randomized plots; in 1988, they were spaced 1.5 × 1.8 m in four completely randomized blocks. Comparisons were made between clonally propagated R_0s and hybrids. In 1988, 1,836 R_1 plants (17 R_1 families) were also examined. Plants were spaced 1.5 × 1.8 m in completely randomized blocks (four to eight blocks per family). Segregation of traits on progeny of the R_1 families was compared with that of the corresponding hybrid.

Ploidy analysis

The ploidy of R_0 plants was determined by scoring the number of chloroplasts in guard cells of at least 50 stomata (L_1 histogenic layer) (Sree Ramulu et al. 1983). For some of the plants, chromosome numbers were estimated in pollen mother cells at diakenesis (L_2 histogenic layer), to confirm the ploidy levels estimated by chloroplast counts. Young flower buds were fixed in ethanol: acetic acid (3:1 v/v) for at least 48 h. One or two crystals of ferric acetate were added to the fixative to aid the stainability of squashed anthers. Fixed anthers about 3 mm long

Table 1. Plant regeneration of *S. tuberosum*, *S. berthaultii*, and interspecific hybrids from short-term, petiole-derived calli[a]

Clone	Petioles	% Petioles w/callus	% Calli w/shoots[b]	Shoots/ callus[c]	Plants/ 10 petioles
S. berthaultii					
PI265858	50	20	0	0	0
PI473331	60	20	8	1	0.2
S. tuberosum					
NY75	90	80	32	20	51
NY76	110	90	27	20	49
S. tuberosum × *S. berthaultii*					
F743-4	98	54	9	10	5
J106-1	60	0	–	0	0
J114-1	125	83	42	16	56
J115-1	50	0	–	0	0
J129-8	129	94	45	14	59
J133-1	116	98	0	0	0
J145-2	136	91	8	1	0.7

[a] Petioles were cultured 2 weeks on callus induction medium and then transferred to shoot regeneration medium
[b] With at least one shoot (regeneration efficiency)
[c] Average number of shoots on calli with shoots

were squashed in 1% acetocarmine. Chromosomes were counted in at least ten well-spread cells. Tetraploid *S. tuberosum* Q155-3 was used as a control.

Results

Plant regeneration

F743-4 hybrid showed 9% regeneration efficiency (percent calli with at least one shoot) (Table 1). Hybrids J106-1 and J115-1 did not initiate calli. J133-1 calli formed no shoots and J145-2 regenerated very poorly. J114-1 and J129-8 showed the highest regeneration efficiency (42% and 45%, respectively) (Table 1). F743-4, J114-1, and J129-8 were selected to generate long-term calli. F743-4 was included in spite of its relatively low regeneration efficiency, because it was the only pathogen-free hybrid available from an early generation of the breeding program not backcrossed to *S. tuberosum*. Comparison of hybrids from different generations may indicate the gain in time possible with an in vitro cycle, as compared to traditional breeding, and therefore help to select the best generations as explant donors for tissue culture. Long-term calli maintained on medium C for more than 14 weeks turned brown, and no plants were regenerated from these older calli. Only healthy-looking R_0 plants from short- or long-term calli were maintained in vitro (40 R_0 plants from F743-4, 151 from J114-1, and 48 from J129-8).

Backcrossed and clonally propagated offspring of F743-4 hybrid and R_0 plants

Seven of the 14 BC-R_1 families evaluated in the greenhouse in 1988 produced from 4- to 15-fold more seedlings with droplets on trichome B than the F743-4 hybrid backcrossed to *S. tuberosum* (Table 2). Progeny of plants regenerated from the same piece of callus showed similar segregation for droplets (Table 2). The highest frequencies of droplets were observed in the BC-R_1 families of

Table 2. Droplets on trichome B and browning in trichome A of progeny from backcross to *S. tuberosum* of F743-4 hybrid and F743-4 R_0 plants in 1988

Hybrid or R_0 no.	Age[a]	Callus	Backcrossed hybrid or BC-R_1		
			% Seedlings w/droplets[b]	% Plants[c]	
				w/droplets	w/browning[d]
F743-4	NA[e]	NA	3	0	18
4001[f]	2	a	44	36	67
4002	2	b	2	–	–
4004	2	b	2	–	–
4009	2	c	2	–	–
4014	2	d	4	–	–
4019	2	e	20	–	–
4026	14	f	11	–	–
4029	14	f	25	24	47
4030	14	f	18	–	–
4033	14	g	5	–	–
4034	14	g	0	–	–
4036	14	h	2	–	–
4038	14	i	33	22	44
4040	14	i	39	29	49

[a] No. of weeks of callus culture before transfer to medium for plant regeneration
[b] Greenhouse
[c] Field
[d] MEBA ≥ 0.21 OD
[e] Not applicable
[f] R_0 plants regenerated from F743-4 calli

the F743-4-R_0 plants 4001 (from a short-term callus), 4029, 4038, and 4040 (from long-term calli) (Table 2). In the field, these BC-R_1 families showed a consistently high percentage of plants with droplets on the B trichomes (Table 2). These families also had a higher number of plants with browning, a measure of the contents of the A trichome. No plants from the backcross of F743-4 hybrid had droplets in the field, and only 18% showed browning (Table 2).

For further analysis, the data were divided into two groups (plants with or without droplets), and the information from the BC-R_1 families of 4001, 4029, 4038, and 4040 was pooled (Table 3). Of the BC-R_1 plants without droplets, 49% had browning (i.e., MEBA $\geq$ 0.21 OD) (Table 3 and Fig. 3), and plants were resistant to leafhoppers (mean score $<2\%$ or $\leq 10\%$ leaf tissue with visible damage) (Table 3). On the other hand, only 18% of the hybrid offspring showed browning (Table 3 and Fig. 3), and the plants were susceptible to leafhoppers (mean score = 3 or > 25% of leaf tissue damaged) (Table 3). About 40% of BC-R_1 plants without droplets and 55% of backcrossed F743-4 progeny had a vine type ≥ 4 (toward *S. tuberosum*) (Fig. 4). Droplets, browning, and vine type did not segregate independently, however. Chi-square analysis of the pooled data from these BC-R_1 families indicated that more plants with droplets had browning ($\chi^2 = 9.38$, $p < 0.005$, 1 *df*) (Table 3). Large numbers of plants with droplets had higher browning (i.e., higher OD) values (Fig. 4) and a vine type more like *S. berthaultii* (score ≤ 2) (Fig. 4). No difference in marketable yield was noted between BC-R_1 plants with droplets and those without droplets (Table 3). It is not clear up to what extent the yield of plants without droplets was more affected by the higher leafhopper damage. Fifteen plants from these BC-R_1 families were selected and are currently being incorporated into breeding lines. These plants all have droplets and browning; some had vine type 4 or a marketable yield up to 3.53 kg/plant.

Clonal progeny of five of the seven R_0 plants whose BC-R_1 offspring included more seedlings with droplets on trichome B in the greenhouse were evaluated in the field. No significant differences in trichome traits,

Table 3. Trichome and horticultural traits of progeny from backcross to *S. tuberosum* of F743-4 hybrid and four BC-R_1 families of F743-4 R_0 plants in the field, 1988[a]

Droplets on trichome B	Family	% Plants w/browning[b]	Leafhopper resistance[c]	Vine type[d]	Yield[e] (kg/plant)
No	BC-F743-4	18	3.0c[f]	3.7b	NE[g]
	BC-R_1[h]	49	1.9b	3.1b	0.99a
Yes	BC-F743-4	–	–	–	–
	BC-R_1	78	0.9a	2.4a	1.11a

[a] Data from the four BC-R_1 families were pooled in one group
[b] Relative percentage within each group (browning = MEBA $\geq$ 0.21 OD)
[c] Scale from 0 (immune) to 5 (highly susceptible or dead)
[d] Scale from 1 (*S. berthaultii* type) to 5 (*S. tuberosum* type)
[e] Marketable, tubers ≥ 5 cm in diameter
[f] Values followed by the same letter are not significantly different (Fisher's protected LSD), $p = 0.01$
[g] Not evaluated, most plants highly damaged by leafhoppers
[h] Pooled data from BC-R_1 families of F743-4 R_0 plants 4001, 4029, 4038, and 4040

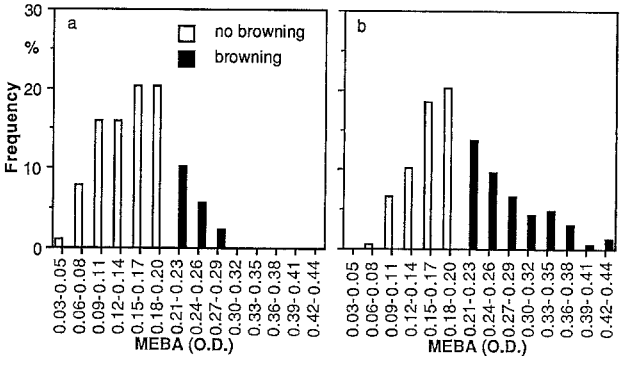

Fig. 3a and b. Frequency distribution of modified enzymic browning assay (MEBA) of progeny without droplets from backcross to *S. tuberosum*, field 1988. **a** Backcrossed F743-4 hybrid; **b** pooled data from BC-R_1 families of F743-4 R_0 plants 4001, 4029, 4038, and 4040

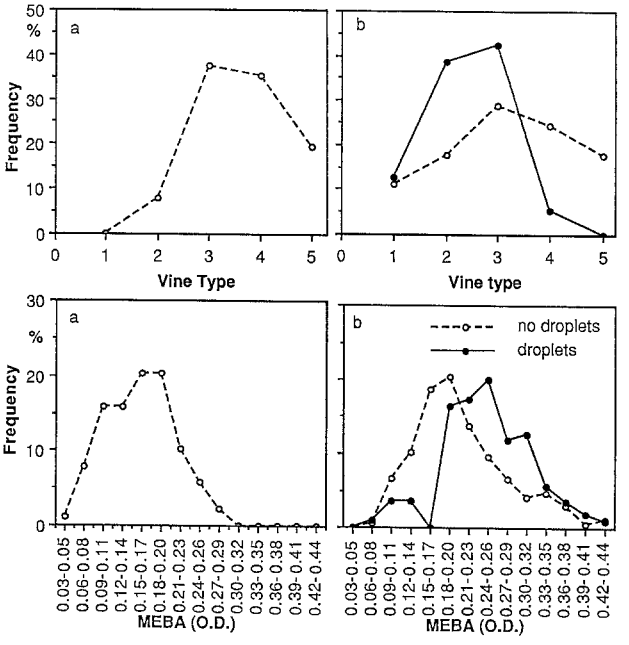

Fig. 4a and b. Frequency distribution of vine type and modified enzymic browning assay (MEBA) of progeny with or without droplets from backcross to *S. tuberosum*, field 1988. **a** Backcrossed F743-4 hybrid; **b** pooled data from BC-R_1 families of F743-4 R_0 plants 4001, 4029, 4038, and 4040

leafhopper resistance, and vine type were seen between these R_0 clones and the F743-4 hybrid.

Clonally propagated and selfed progeny of J114-1 hybrid and R_0 plants

Thirty-three R_0 plants regenerated from short-term calli were evaluated in the field in 1987. Both desirable as well as undesirable changes in trichome traits and horticultural characteristics were recovered in these R_0 plants (data not shown). Of particular relevance to this project was the recovery of plants with improved yield and/or insect resistance traits. Clonal progeny of five R_0 plants showed higher yields than J114-1, but had trichome characteristics comparable or better than the hybrid (Table 4). A second set of new plants obtained through clonal propagation by nodal cuttings of three of these five original R_0 plants, kept as shoot cultures, was examined in the field in 1988. The improved yield seen on R_0 5024 was stable and heritable over the second cycle of evaluation; clonally propagated 5024 showed 2.4-fold and 2.0-fold higher yields than J114-1 in 1987 and 1988, respectively (Table 4). The increase in yield was not associated with a change in vine type toward *S. tuberosum* (data not shown). The improvement in yield observed with the clonally propagated R_0 5013 and 5046 in 1987 was not obtained in the second cycle of evaluation in 1988 (Table 4). R_0 plants 5030 and 5035 showed about three times the yield of J114-1 in 1987, but were not evaluated in 1988.

Table 4. Marketable yield and MEBA of *S. tuberosum* NY 76, *S. berthaultii* PI 473331, J114-1 hybrid, and five J114-1 R_0 plants in the field, 1987 and 1988

Clone or R_0 no.	Marketable yield (kg/plant)		MEBA[a] (OD at 470 nm)	
	'87[b]	'88[c]	'87	'88
NY 76	0.45 d**[d]	NE[e]	0.06 a**	0.04 a**
PI 473331	0.00 a**	0.00 a**	0.33 c	0.31 c
J114-1	0.10 b	1.29 b	0.33 c	0.28 c
5013[f]	0.24 c*	1.56 b	0.25 b	0.29 c
5024	0.28 c*	2.59 c**	0.33 c	0.34 c
5030	0.30 c*	NE[g]	0.28 c	NE
5035	0.32 c**	NE[g]	0.37 d	NE
5046	0.25 c*	1.23 b	0.25 b	0.31 c
5052	0.05 b	–	0.45 d**	0.42 d**

[a] Modified enzymic browning assay. All clones except NY 76 showed droplets both years
[b] Plants spaced 1 × 1 m
[c] Plants spaced 1.5 × 1.8 m
[d] Each value is a mean of four (1987) or eight (1988) plants. Values followed by the same letter are not significantly different (Fisher's protected LSD); ** $p = 0.01$ and * $p = 0.05$ refer to comparison with J114-1
[e] Not evaluated, plants highly damaged by leafhoppers
[f] R_0 plants from J114-1 calli
[g] Not evaluated, not enough material for field trials

One plant regenerated from short-term callus (R_0 5052) showed higher level of phenolic exudate in trichome A (higher OD) than J114-1 both in 1987 and 1988 (Table 4). This increase in browning was not associated with changes in density or head size of trichome A, nor in PPO concentration per A trichome (data not shown). The increase in browning that was stable and transmissible through clonal propagation of R_0 5052 was also heritable through one generation of selfing. The genetic change recovered in 5052 did not have as much effect on the population mean of browning (J114-1 selfed mean = 0.35 OD; 5052 S-R_1 mean = 0.37 OD) as on the frequency distribution. The S-R_1 of 5052 showed about half as many plants in the low range of browning (< 0.30 OD) as the selfs of J114-1, and twice as many plants in the high range of browning (> 0.47 OD). Besides the increased browning, 5052 also showed a mutation in flower color. Flowers of R_0 plant 5052 had flowers that were mainly white with faint blue color around the vascular star of the corolla, whereas J114-1 had solid blue flowers. This change in flower color was heritable through clonal propagation and selfing (Z. Lentini, R. L. Plaisted, E. D. Earle, in preparation).

Clonally propagated progeny of other 25 R_0 plants (eight replicates each) and 6 S-R_1 families of regenerates from short- or long-term calli were evaluated in 1988. No improvement in yield or in insect resistance traits was noted on these plants.

Clonally propagated and selfed progeny from J129-8 and regenerates

Progeny from clonal propagation or selfing of J129-8 hybrids and R_0 plants were highly susceptible to leafhoppers. No reliable scores for trichome and horticultural traits could be taken since the plants were very damaged.

Analysis of ploidy

The genetic changes that resulted in increased yield, increased browning, flower color mutation, and increased frequency of droplets in R_1 progeny from the backcross to *S. tuberosum* appeared not to be due to changes in ploidy in either L_1 or L_2 layers. Chloroplast counts (50 stomates per plant) indicated that R_0 plants as well as hybrids were tetraploids [Q115-3: 23.7 ± 2.3 (mean ± SD) chloroplasts per stomate; F743-4: 23.8 ± 1.6; J114-1: 24.6 ± 2.9; mean of R_0 plants from 22.1 ± 3.0 to 26.0 ± 2.4]. Tetraploidy was confirmed by chromosome counts in pollen mother cells.

Discussion

In the backcross to *S. tuberosum*, some F743-R R_0 plants produced a high percentage of offspring with droplets on

the type B trichomes (up to 44% in the greenhouse), with browning produced by the type A trichomes (up to 67%), and with resistance to leafhoppers. Only 3% of the seedlings from the backcross of F743-4 hybrid had droplets, 18% showed browning, and plants were susceptible to leafhoppers. Unlike the backcrossed progeny of F743-4 hybrid, BC-R_1 plants without droplets were also resistant to leafhoppers and showed an improvement in vine type toward *S. tuberosum* (as compared to BC-R_1 plants with droplets) (Table 3 and Fig. 4). It appears that genetic changes may have been generated during the callus phase of F743-4 petiole cultures, allowing the production of insect-resistant populations in the backcross to *S. tuberosum* of the F743-4 R_0 plants. These genetic changes apparently only had a partial effect, however, since the most resistant BC-R_1 plants (i.e., plants with droplets) showed a vine type toward *S. berthaultii* (Table 3 and Fig. 4).

Kalazich (1989) found that the cultivated phenotype without the desired trichome traits was predominantly recovered in the backcross to *S. tuberosum*; in particular, the droplets on type B trichomes were lost in the backcross. An average of 9% (range 1%–15%) of seedlings in the first backcross to *S. tuberosum* had droplets in the greenhouse (a total of 1,542 seedlings from nine families were evaluated). When the backcross selections were intercrossed, a large number of plants with good trichome traits were recovered, but the best of these were invariably late in maturity with a vine type more like *S. berthaultii*. Trichome traits and vine type appeared to be linked (Kalazich 1989). A segregation that favors the recovery of the cultivated potato phenotype in the backcross to *S. tuberosum* could also be obtained if pairing is preferential between homologues rather than between homeologous chromosomes (partly homologues of the different genomes); this would result in reduced recombination between the cultivated and the *S. berthaultii* genomes (Kalazich 1989).

The PPO found in type A trichomes is encoded by a single dominant gene which is trichome specific (Kowalski 1989). *S. tuberosum* bears trichomes similar in structure to A types, but without PPO. It may be that, to have browning in cultivated types, only the introgression of the PPO gene is needed. To gain high levels of browning, however, it might be necessary to increase the concentration of the substrate, which is likely to be regulated by *S. berthaultii*-specific minor genes. The production of the type B droplet exudate (sucrose esters) also appears to be regulated by *S. berthaultii*-specific minor genes (Kalazich 1989). Perhaps the capability of browning is the first major effect that can be detected phenotypically as a result of enhanced gene recombination. In order to gain high levels of browning and droplets, a large number of minor genes must be accumulated. If some genes for type B droplet were present in those offspring with browning and *S. tuberosum*-like phenotype (as a result of an increased recombination), the expression might be incomplete because the introgression was incomplete. *S. tuberosum* genes might also be affecting the expression of droplets and/or browning genes. There is ample evidence in wheat that the expression of alien genes is greatly affected by the host genome (Kovacs et al. 1986; Multani et al. 1988).

The genetic changes that apparently occurred during callus culture of F743-4 were not visible in the trichome and horticultural traits of the regenerates themselves, but were revealed as altered segregation after meiosis and formation of sexual progeny. Very little is known about the genetic basis of the trichome traits and their close association with the *S. berthaultii* vine type. Segregation studies performed up to now indicate that trichome traits conferring insect resistance from *S. berthaultii* are polygenic (Gibson 1979; Mehlenbacher et al. 1983, 1984; Kalazich 1989). Photoperiodic response, which has a marked effect on the growth and the development of potato and is correlated with time to maturity and tuber initiation, is also under the control of major and minor genes (Mendoza and Haynes 1977; Lazin 1980; Swaminathan and Howard 1953). The location of these genes on the potato genome is not known, nor is the nature of chromosome pairing (conjugation at least by one chiasma) of *S. tuberosum* and *S. berthaultii* chromosomes during meiosis. Thus, at present, it is not possible to draw conclusions about the genetic changes that could have taken place during callus culture of F743-4 to change the segregation of trichome traits in the backcross to *S. tuberosum*.

To elucidate the types of genetic changes that occurred during callus culture of F743-4, it would be desirable to study chiasma formation between *S. tuberosum* and *S. berthaultii* chromosomes of interspecific hybrids and to have molecular genetic markers. It might be possible to associate molecular markers with trichome and horticultural traits by using the restriction fragment length polymorphism (RFLP) potato linkage map (Bonierbale et al. 1988). These molecular genetic markers may be used to quantify the introgression of alien gene(s) into regenerate-backcrossed offspring as compared with the hybrid-backcrossed progeny. RFLP analysis may reveal reassortment of genes indicating increased recombination between the genomes, otherwise masked by the phenotype (Tanskley et al. 1989). This information would help to determine the extent to which tissue culture could assist the breeding program.

Regenerates derived from the same callus showed similar segregation for trichome traits. Such lack of intra callus variation has been interpreted by others as an indication that tissue culture is uncovering pre-existing variation rather than generating variation (Austin and Cassells 1983; Creissen and Karp 1985; Evans and Sharp

1983; Karp and Bright 1985; Scowcroft et al. 1987). Although at present pre-existing variation cannot be ruled out in this work, it is also possible that the changes occurred at very early stages of culture and that the callus used for plant regeneration proliferated from a single altered cell. All calli were initiated from leaf petioles from F743-4 plants clonally propagated in vitro. If callus culture simply revealed pre-existing variation, why did not all calli show the same type of variation? The regenerate with the highest recovery of droplets after backcrossing (4001) was derived from a short-term callus. Therefore, it might be advantageous to use short-term calli to avoid the generation of aberrant plants commonly associated with long-term cultures. Heritable genetic changes occurring 3 or 4 weeks after callus induction have also been reported by others (Evans and Sharp 1983; Sree Ramulu et al. 1985).

It would be interesting to determine: (a) the likelihood of gaining more recombination between the *S. berthaultii* and the cultivated genomes, fixing desirable genes, and/or eliminating undesirable genes by re-culturing those regenerates already showing desirable changes; (b) the progress obtained with the breeding of regenerate-backcrossed offspring in comparison with that of advanced hybrids; and (c) the best generation of hybrids to use as explant donors for the production of regenerates with increased recombination capability in the backcross.

Regenerate 5024, derived from short-term callus of J114-1, showed higher marketable yield (about two fold) than the J114-1 hybrid in two consecutive years in the field. Stable and heritable changes leading to increased yield over three clonal generations have been reported for regenerates of several potato cultivars (Evans et al. 1986; Sree Ramulu et al. 1984). No changes in ploidy or in vine type were noted in 5024. Two of three clonally propagated regenerates with higher yields in 1987 did not show differences in 1988 when compared to J114-1. The variance coefficient of yield in 1988 was an average of 48%, whereas in 1987 it was 81%. A larger number of clones per regenerate (eight versus four) in more plots (four versus two) was tested in 1988. Results suggest that several clones per regenerate (more than four) must be examined for statistical significance, and regenerates must be evaluated more than one season to determine if the variation involved is genetic.

The higher browning recovered in the J114-1 regenerate 5052 was stable over clonal propagation and heritable through one generation of selfing. The increase in browning of 5052 was not associated with a higher density of A trichomes. Thus, the increased browning of 5052 seems to be due to a change related to the chemistry of the browning reaction itself. MEBA detects the level of phenolic exudate in A trichomes (Avé et al. 1986). An increase in the concentration of PPO, in its activity or its substrate, would result in increased browning. The ELISA test indicated no difference in the concentration of PPO per trichome A in J114-1 and 5052. Therefore, the increased browning of 5052 is likely to be associated with a higher PPO activity or higher concentration of the phenolic substrate for the enzyme. Regenerate 5052 also showed a mutation in flower color, from blue to white with faint blue areas. Since anthocyanins are phenolic compounds (Harborne 1973), the two variations recovered in 5052 could be biochemically related.

In this work only healthy-looking R_0 plants that rooted on regular propagation medium without hormones were maintained in vitro. This pre-selection approach was taken to discard the grossly aberrant regenerates that are more likely to offer problems for further breeding because of sterility or low fertility (Creissen and Karp 1985; Hermsen et al. 1981; Gill et al. 1986, 1987; Sree Ramulu et al. 1983), even if they carry desirable genetic changes. Regenerates showed normal development and generally were as vigorous as the controls in the field. Ploidy estimates by chloroplast and chromosome counts suggested that the regenerates were tetraploids.

The fact that several clones per regenerate had to be examined each season for statistical significance greatly restricted the number of regenerated plants that could be evaluated in the field (e.g., to evaluate eight clones each of 63 regenerates in 1988, a total of 504 plants was taken to the field). Regenerates also needed to be evaluated more than one field season to determine whether variation involved true genetic changes rather than environmental and/or physiological effects. In contrast, true variants could be identified with one cycle of sexual crosses. Although selfing was useful to assess the heritability of changes visible on the regenerates, backcrossing seems to be more appropriate to detect changes in culture that affected the crossability barriers between *S. berthaultii* and *S. tuberosum*. By backcrossing to *S. tuberosum*, it was possible to identify those regenerates with apparent increased recombination to cultivated potato in the greenhouse and to produce materials with potential use as breeding lines. A similar approach (callus culture of hybrids, followed by plant regeneration and backcrossing) may aid in the introgression of desirable traits from wild species into other crop plants as well.

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