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UTILIZATION OF SOMACLONAL VARIANTS IN A BACKCROSS PROGRAM FOR GLANDULAR TRICHOMES OF POTATO (*Solanum tuberosum* L.)

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1.1 Introduction

Host plant resistance is a valuable alternative to using pesticides for the control of insects, nematodes and diseases. The use of resistant cultivars will reduce the cost of production and the possibility of adding hazardous pesticide residues in food and the environment. It will also delay the development of populations of insects resistant to pesticides currently being used. The potato breeding program at Cornell University is endeavoring to incorporate a high level of resistance to a broad spectrum of insects into marketable cultivars using attributes of the wild Bolivian species, *Solanum berthaultii* . This diploid potato possesses two types of glandular trichomes; the type A which is short, with a tetralobulate membrane-bound gland at its tip and type B which is longer, with an ovoid gland at its tip that continously discharges a clear, viscous and extremely adhesive exudate (Gregory et al. 1986). These two types of glandular trichomes confer resistance to several insect pests including aphids, leafhoppers, mites, flea beetles, spider mites, the potato tuber moth and the Colorado potato beetle (Gibson 1971, Gibson and Valencia 1978, Tingey and Sinden 1982, Casagrande 1982, Dimock and Tingey 1985, Xia and Tingey 1986, Raman, 1990). In 1977 several tetraploid F1 hybrids were produced using a number of different seedlings of *S.*

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moth and the Colorado potato beetle (Gibson 1971, Gibson and Valencia 1978, Tingey and Sinden 1982, Casagrande 1982, Dimock and Tingey 1985, Xia and Tingey 1986, Raman, 1990). In 1977 several tetraploid F1 hybrids were produced using a number of different seedlings of *S. berthaultii* and a variety of tetraploid clones (Plaisted et al. 1991). After six generations of crossing and selection, clones that are resistant to insects, immune to PVX and PVY and resistant to golden nematodes have been identified. Well-adapted clones with good type A trichomes have been produced but the development of clones with good type B trichomes has been more difficult to obtain (Kalazich 1989, Plaisted et al. 1991). A strong association between the presence of droplets on type B trichomes and undesirable agronomic traits such as late maturity, poor foliage and tuber appearance and low yield in backcrossed generations has been observed. An association between B droplets and type A trichome traits in backcrosses but not in intercrosses had also been found. These strong associations were attributed to linkage or structural genomic differentiation between the genomes of the two species, *S. berthaultii* and *S. tuberosum* (Kalazich and Plaisted 1991).

To overcome the restricted recombination between the two genomes and to produce plants with both good trichome traits and improved agronomic traits somaclonal variation (genetic changes generated during callus culture) (Larkin and Scowcroft 1981) was utilized by Lentini et al. (1991), to generate reassortment of genes already present in hybrids between *S. tuberosum* and *S. berthaultii* . Lentini et al. (1991) reported some desirable changes in the sexual progeny of regenerates. A few regenerated clones had as much as 30% of their progeny with

droplets on type B trichomes when crossed to cv. Atlantic, rather than the usual 0 to 5 % obtained from the conventional breeding methods used earlier (Kalazich and Plaisted 1991).

This research was conducted to determine the stability of the somaclonal variants which convey a high proportion of individuals with droplets on the type B trichomes in backcross generations; to compare the expression of trichome traits and behavior of F_1 hybrid-derived somaclones with the advanced tetraploid hybrid- derived somaclones; and, to generate genetic recombinations in backcrosses to select plants with good agronomic and trichome traits.

1.2 Review of Literature

1.2.1. Glandular trichomes as a mechanism of resistance.

The presence of glandular trichomes on the foliage and stems of *Solanum berthaultii* , a wild diploid Bolivian potato species, confers resistance to several potato pests. Resistance has been associated with the presence of two types of glandular trichomes, type A and type B (Gibson 1971). The type A trichome is short, about 120-210 μm in length, and has a tetralobulate membrane-bound gland at its tip. The contents of the type A trichomes turns dark brown upon rupture, hardens and accumulates on tarsi and mouthparts of small insects, such as aphids. This results in their decreased mobility, exhaustion, starvation and eventual death (Gibson 1971; Gibson et al. 1975; Tingey et al. 1981). The browning reaction is associated with the presence of phenols and the enzyme polyphenol

oxidase (PPO) (Gibson 1971, Ryan et al. 1982, and Kowalski 1989). A phenolic compound tentatively identified as a glucose ester of *p*-hydroxyphenyl-propionic acid has been isolated from type A trichomes by Avé and Tingey (1986). However, it is not established yet whether this compound is the substrate for PPO. Volatile sesquiterpenes, especially E-ß-farnesene associated with aphid repellence is the other group of chemicals found in the glandular type A trichomes of *Solanum berthaultii* (Avé et al. 1986). These compounds are implicated as a possible defense mechanism disturbing feeding, and discouraging settling by the green peach aphids (Lapointe and Tingey 1984; Gibson and Pickett 1983).

Methods to assay for the presence of PPO and its substrates have been developed. The first method, called the modified enzymic browning assay (MEBA) (Avé et al. 1986) , is an improvement of an earlier method called total browning potential (TBP) (Ryan et al. 1982). In the MEBA, the color intensity is indicative of the level of biochemical products in the type A trichomes and trichome density. The biochemical products, a PPO enzyme and phenolic substrates are known to combine naturally into a viscous substance which hardens as a result of the oxidative polymerization (Avé et al. 1986). The other two techniques to test for the PPO enzyme is isoelectric focusing (IEF) gel electrophoresis, which detects the presence of PPO but not its substrates; and the enzyme linked immunosorbent assay (ELISA) - based technique which is able to measure PPO concentration within a single type A trichome (Kowalski 1989).

Both the density of type A trichomes and their exudates have been found to play significant roles in defense mechanism for resistance. An increase in resistance to aphids was associated with higher densities of

type A trichomes (Tingey and Laubengayer 1981). A positive correlation between densities of glandular trichomes on leaves of *S. berthaultii* and *S. polyadenium* and number of immobilized aphids was reported earlier by Gibson (1976).

The type B trichome is longer than the type A trichome, about 600 to 950 µm , with an ovoid gland at its tip which continously discharges a clear, viscous and extremely adhesive exudate (Gregory et al. 1986). The type B trichome exudate in *S. berthaultii* is composed of a complex of 3',3,4,6-tetra-*O*-acyl sucroses containing primarily short-chain branched carboxylic acids (King at al. 1986, Neal et al. 1990). The sticky exudate of type B trichomes has been reported to be very effective in trapping small-bodied insects such as spider mites (Gibson 1976) and aphids (Tingey and Laubengayer 1981, Xia and Tingey 1986). The droplets on type B trichomes have also been found to increase resistance against the Colorado potato beetle, even if entrapment is not involved (Dimock 1985). Tingey and Laubengayer (1981) reported that the size of the droplet on type B is inversely correlated with trichome densities, but the volume of exudate varies independently.

The type A and B glandular trichomes have been found to confer resistance to green peach aphids (*Myzus persicae*), potato leafhopper (*Empoasca fabae*), potato fleabeetle (*Epitrix cucumeris*), spider mites (*Tetranychus urticae*), leaf miner fly (*Liriomyza huidobrensis*) and potato tuber moth (*Phthorimaea operculella*) (Gibson 1971, Gibson and Valencia 1978, Tingey and Sinden 1982, Xia and Tingey 1986, Raman 1990). Populations of these pests on selected advanced trichome materials were reduced more than 85%, compared to those on susceptible commercial

varieties (Tingey et al. 1981). Resistance to the Colorado potato beetle (CPB) has also been reported (Dimock and Tingey 1985; Casagrande 1987). Field experience of Wright et al. (1985) indicated that the present levels of resistance in hybrids can substitute for as much as 40% of the current insecticide usage on susceptible cultivars. A recent clone, L235-4, selected for its trichome traits in the Cornell breeding program was found to be resistant to first brood feeding of Colorado potato beetle, and as good as the standard varieties, Atlantic and Allegany protected with Temik™ (Plaisted et al. 1991). The glandular trichomes have also been reported as a possible source of non-specific resistance to the major potato disease, late blight caused by *Phytophthora infestans* (Mendoza 1979). The exudates present in type B trichomes have been implicated as a possible resistance factor conferring resistance to infection from *P. infestans* (Holley et al. 1987).

The interactions of type A and B trichomes are important in the expression of resistance in some pests. Tingey and Laubengayer (1981) reported that aphids are more likely to rupture type A trichomes if their tarsi are coated with viscous type B exudates. Likewise, the exudates present in type B trichomes were only effective against the Colorado potato beetle in the presence of type A trichomes (Dimock and Tingey 1985). In the field evaluation of accessions of *S. berthaultii, S. tarijense* and their hybrids, those that had both type A and B glandular trichomes were much more resistant to the green peach aphids and potato leafhopper than those bearing type A alone (Tingey and Sinden 1982). Likewise, Rizvi and Raman (1983) studied the effects of glandular trichomes on the occurrence of aphids and on the transmission of viruses. Their findings

demonstrated that the combined pubescences of the A and B types would be more effective in reducing the number of aphids and in preventing PLRV spread than a pubescence of type A alone.

Inheritance studies were conducted on the expression of trichomes. Gibson (1971) reported that the presence or absence of droplets on type B trichomes within the species *Solanum berthaultii* is inherited in a monogenic dominant fashion. In interspecific crosses of *Solanum phureja* and *Solanum berthaultii*, however, at least two genes are necessary to explain the observed segregation. On the other hand, Mehlenbacher et al. (1983, 1984) have reported that the type B trichome is controlled by a few genes with intermediate heritability (32 ± 5%) while the droplet size is highly heritable (64 ± 8%). However, the inheritance of B droplet size cannot be explained by the action of one or two genes. The heritability of type A density within seedlings was low (18 ± 3%) but with field-grown plants the heritability estimate was higher (32 ± 7%).

1.2.2. Wild species as a source of valuable traits in the improvement of commercially cultivated potatoes. Previous reports have shown that wild species are valuable sources of different resistances to diseases and insects, adaptation to stress conditions and important quality characters (Hawkes 1958, Ross 1986, Fold 1987, Hermsen 1987, Iwanaga and Schmiediche 1989). Most successful examples of the use of wild species involve the introgression of simply inherited traits. Complex characters may be introduced but the process is more complicated (Frey et al. 1984, Peloquin 1984). In the germplasm enhancement program at the International Potato Center (CIP) (1985),

various projects are being conducted on the introgression of valuable genes from wild species into cultivated potatoes. Among them are immunity to PVX from *S. demissum* and *S. acaule*, immunity to PVY from *S. stoloniferum*; and resistance to root knot nematodes from *S. sparsipilum* and *S. chacoense* . Genes for resistance to PVX, PVY and golden nematode from *Solanum tuberosum ssp. andigena* have been successfully introgressed into the cultivated potato (Plaisted and Hoopes 1986, Brodie et al. 1991). According to Iwanaga and Schmiediche (1989) a significant number of potato breeders have used wild species successfully as sources of genes for resistance, adaptation and quality in the improvement of the potato crop in recent years however, most wild species possess a low level of agronomic value and are generally more variable than the cultivated potatoes. Chapman (1989) reported that wild species have a larger and often different spectrum of alleles as shown by the comparison of isozymic variation in the cultivated species and their ancestral wild species.

The traditional breeding method used in the introgression of desired traits from wild species is through direct crosses followed by a series of backcrosses to eliminate undesirable wild traits in the following generations (Ochoa and Schmiediche 1983). Backcross breeding for major gene resistance which segregates as a single dominant character has been successfully used in breeding for resistance to golden nematode and immunity to PVX and PVY. More than 60% and 37% of the German and Dutch cultivars respectively, carry the H_1 nematode resistance gene from *S. andigena* ; 19 European cultivars carry genes for resistance to PVY from *S. stoloniferum* ; and 7 cultivars carry the gene for resistance to

PVX from *S. acaule* (Ross 1986). Backcross breeding for polygenically inherited resistance however, has not been very successful. In breeding for field resistance to late blight using *S. demissum, S. stoloniferum and S. verrucosum* as sources of resistance the degree of resistance after four to six backcrosses was very low. This was attributed to the low probability that segments would combine the minor gene complex giving the field resistance to late blight, together with the many genes necessary for yield and quality (Ross 1986). Another reason for the loss of resistance during backcrossing according to Black (1970) is the dispersion of the complex of minor genes responsible for the resistance. Torka (1950) claimed that obtaining a high level of resistance to the Colorado potato beetle in backcrossed clones was very difficult because clones with high level of resistance had low yield and long stolons; and those with good yield often had a low degree of resistance. She recommended intercrossing or selfing the hybrids to maintain the insect resistance. Swaminathan (1953) reported that even with a small differentiation, if the genes of interest are carried in a differentiated segment together with undesirable ones, this combination can be very difficult to break. Structural differences between the series Tuberosa (*Solanum tuberosum ssp. tuberosum and S. chacoense*) genomes makes breeding between the two species unpredictable and difficult. Genome differentiation in potato such as cryptic differentiation was found between Series Tuberosa and Commersoniana (Howard and Swaminathan 1952, Swaminathan 1953, and Matsubayashi 1983). Rammana and Hermsen (1979) reported structural differentiation in interspecific hybrids of diploid species. They also indicated structural differences within the genome of *S. ssp. tuberosum*

through studies with dihaploids of the cv. Gineche. Similar results were reported using monohaploids (Breukelen et al. 1976, Singh et al. 1988) where both univalents and multivalents were observed , suggesting that the non-homologous chromosome in the monohaploids have homologous segments. Genomic differentiation tends to be more evident in polyploids because of competition for pairing among homologous chromosomes (Marks 1965, Hawkes 1972). Two diploid species which have only small structural differences (cryptic in nature), will pair normally at that level, but in the tetraploid derivatives that affinity will necessarily be weaker due to the greater homology within the two genomes (Stebbins 1945, Marks 1965, Hawkes 1972). Dvorak (1983) concluded that heterogenetic pairing is genetically suppressed and that genomic differentiation is non-structural because the non-coding regions between genes are the ones differentiated. Non-conventional breeding methods such as the use of somaclonal variants through tissue culture have been used to overcome crossability barriers.

1.2.3. Somaclonal variation in potato varietal improvement. Somaclonal variation arising through tissue culture has been used to generate new variability to complement conventional breeding in potato improvement. It could be a means of introgressing an alien gene of interest from a wild relative species such as insect resistance from *Solanum berthaultii* into cultivated potato (Lentini et al. 1990, Serraf 1991).

Most of the studies of potato somaclones have tried to identify within a named variety clones with better yield or resistance to pests and

diseases (Secor et al. 1981, Gunn et al. 1985, Evans et al. 1986, Shepard et al. 1986, Thompson 1987). Two aspects of somaclonal variation that could be of importance to plant breeding are the facts that changes involve quantitative as well as qualitative traits and may involve homozygous as well as heterozygous changes (Karp et al.1989). A wide range of variation in phenotypes, ploidy levels and important traits such as pest and disease resistance have been reported among plants regenerated through protoplast or tissue culture of commercial potato cultivars and breeding clones (Shepard et al. 1980, Karp et al. 1982, Webb et al. 1983, Thomas et al. 1983, and Wheeler et al. 1985). Tissue culture-derived variation includes alteration in both major and polygenic characters (Larkin and Scowcroft 1981, Secor and Shepard 1981, van Harten 1981, Cassels et al. 1983, Sree Ramulu et al. 1983, Wheeler et al. 1985). Reported causes of somaclonal variation are change in ploidy level (Karp et al. 1982) chromosomal rearrangement (Larkin 1987) and point mutation, methylation changes or transposon activation (Orton 1984, Karp and Bright 1985, Evans 1989, Muller et al. 1990). The most frequently reported cause of somaclonal variation is chromosomal rearrangement. Larkin (1987) documented numerous examples of recorded evidence from deletions, chromosome fusion and interchanges, the consequences of which may result in mutation that affect the phenotypic expression of one or more genes. Karp et al. (1989) however, reported that structural chromosome changes have more subtle effects that may only be detectable at the DNA or protein level. Morphological variations observed in regenerated potato plants were attributed to structural changes such as deletions, translocations, duplications and

centric fusion; changes in ploidy level or chromosome number, and changes in nucleotide sequence (cryptic changes) (Hermsen et al. 1981, Karp et al. 1982, Carlberg et al. 1984, Sree Ramulu 1984 and 1986, Creissen et al. 1985, Landsman and Uhrig 1985, Evans et al. 1986, Gill et al. 1986, 1987, Pijnacker and Ferweda 1987, Scowcroft et al. 1987). The genetic changes occurring in tissue culture may be stable after repeated cycles of vegetative propagation and may be heritable via sexual crosses (Sibi et al. 1984, Bright et al. 1985, Evans et al. 1986, Barwale and Widholm 1987).

The use of somaclonal variation has both advantages and disadvantages. According to Karp et al. (1989), not all desired character changes may occur but a change in the trait of interest is possible. Novel changes are possible and in some cases, somaclonal variants that are better in one or few traits than the parents may be recovered. However, two of the major drawbacks are that many changes that arise through somaclonal variation are undesirable and changes that occur may not be stable. Nelson (1983) reported that plants regenerated from a protoplast of cv. Fortyfold had changed tuber color into white instead of the usual purple-splashed color; however, this change was not stable over subsequent generations. It is therefore important that the stability of somaclones be tested over time.

1.3 Materials and Methods

1.3.1. Somaclones from a tetraploid hybrid. Two sets of families were selected for this study; the first set (I) includes four families which exhibited a high (≥ 20%) percentage of plants with B droplets while the second set (II) had a low (≤ 3%) percentage. The unregenerated tetraploid hybrid, F743-4 was included as a control (Table 1.1). The tetraploid hybrid F743-4 is two generations removed from the initial cross between *S. tuberosum* x *S. berthaultii* (Figure1.1). Seeds of the first-backcrossed somaclones to the cv. Atlantic were produced by Lentini (1989), who regenerated the somaclones from leaf petiole-calli using tissue culture techniques (callus culture).

This research was started in the spring of 1989 by sowing true seeds from each BC_1 family in Quadripacks™ with Cornell soil mix (Appendix 1). At about the 4 to 5 leaf stage, seedlings were transplanted into 18-hole speedling trays (first evaluation) or to 6-inch plastic pots (all subsequent evaluations) in the greenhouse with temperature maintained at 20°C to 28°C and relative humidity ranging from 60 to 90%. Natural lighting was supplemented with illumination from Sylvania Metalarc MP lamp with the photoperiod programmed at 16 light : 8 dark hours.

Each plant progeny was evaluated in the glasshouse for the presence of droplets on type B trichomes (i.e. production of sticky exudate) at 4 to 5 weeks after transplanting. This was done by examining the adaxial and abaxial surfaces of at least two fully expanded leaflets of the fourth or fifth node leaf (proximal to the plant apex) using a Panasonic™

Table 1.1. Hybrid F743-4 and its somaclones regenerated from petiole calli and backcrossed (BC_1) to cv. Atlantic.

Pedigree	Family Code
I. BC_1 of Somaclones with Many B Droplets*	
4001 x Atlantic	M502
4029 x Atlantic	M509
4038 x Atlantic	M514
4040 x Atlantic	M515
II. BC_1 of Somaclones with Few B Droplets**	
4002 x Atlantic	M503
4004 x Atlantic	M504
4009 x Atlantic	M505
III. BC_1 of Unregenerated Hybrid	
F743-4 x Atlantic	M501

* ≥ 16% of progenies with B droplets

** < 16% of progenies with B droplets

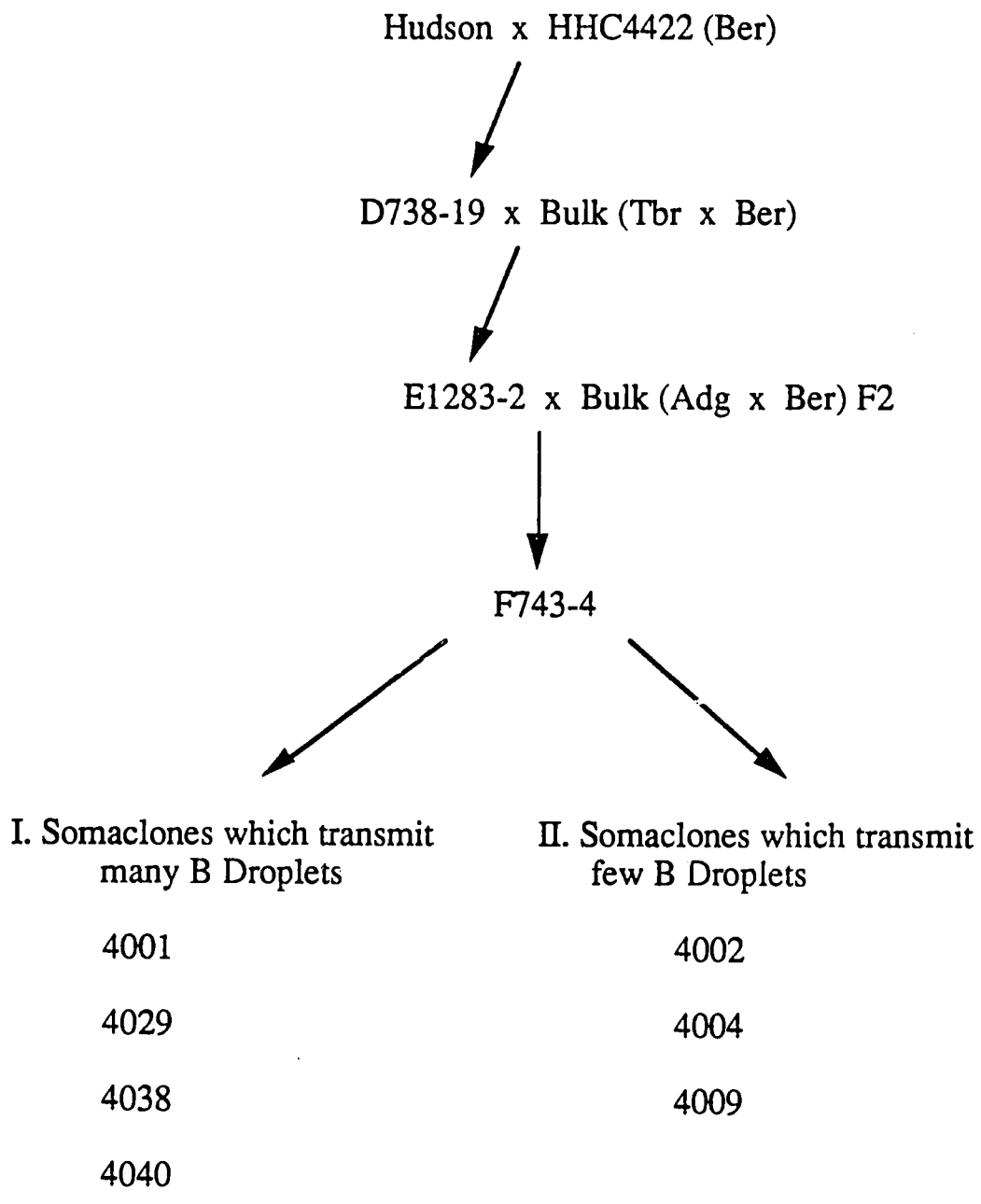


Figure 1.1 Pedigree of F743-4 hybrid and its somaclones.

lightscope at 30X magnification. The frequency of plants with droplets on type B trichomes was noted for each family.

All progenies were also evaluated for the production of phenolic exudate in the head of type A trichomes and trichomes density using the modified enzymic browning assay (MEBA) (Ryan et al. 1982, Avé et al. 1986). The MEBA test is an assay which measures the activity (i.e.) oxidative polymerization) of polyhenol oxidase (PPO) and its substrates. Results of the MEBA test are expressed in percent transmittance where the lower the value, the higher is the concentration of the compounds and trichome density. Plants with MEBA value of ≤ 62% were considered to have significant browning activity (Kalazich, 1989). The *Solanum berthaultii* check usually had a score between 30% to 45% and the *Solanum tuberosum* check had a score of 70% to 80%.

Selected clones from each family (with and without droplets on type B trichomes) were backcrossed to *S. tuberosum* cultivar Atlantic or the clone Q155-3. These recurrent parents were selected because of their good tuber qualities, vine type and good crossing abilities. Selfing was also done for each clone. Backcrossing was done up to three generations for some selected clones. Crosses were made during the winter months of 1989, 1990 and 1991 and the evaluations were conducted either during the spring or fall of the same years. In some cases, fruit setting in backcrosses and selfings was not successful because of unfavorable environmental conditions or genetic incompatibility. Vine score rating was done at 45 days after transplanting using the rating scale 1 to 5, where 1 refers to wild looking *Berthaultii*-type, and 5 refers to the cultivated *Tuberosum*-type. Clones were selected for further selection or

breeding purposes on the basis of their trichomes and agronomic traits. The tuber glycoalkaloid content of some BC_1 clones were analyzed by De Rodney Bushway, Orono, Maine.

1.3.2. Somaclones from diploid hybrids. To determine how an F_1 diploid hybrid would respond to culture in contrast to the more advanced tetraploid lines, Hopper (1989) regenerated plants from F_1 hybrids produced from a cross between a dihaploid *S. tuberosum* (HH1-9) and *S. berthaultii* (PI 473331). About 150 regenerated plants were made available for this particular study. The tissue culture laboratory of the Department of Plant Breeding and Biometry had earlier determined the ploidy level of the regenerated plants by analyzing their DNA content using flow cytometry (Arumuganathan and Earle 1991).

Cuttings from regenerated plantlets produced from in vitro were rooted in mist chambers and later transplanted to 8-inch pots with Cornell soil mix (Appendix 1) supplemented with the fertilizer Osmocote™ in the greenhouse with temperature maintained at 20 to 28°C and relative humidity ranging from 60% to 90%. Natural lighting was supplemented with illumination from Sylvania™ Metalarc MP lamps to provide a photoperiod of 16 hrs light. Each somaclone was evaluated for its vine type, browning potential of type A trichomes and the presence of droplets on type B trichomes during the fall of 1990. The evaluation was conducted at 4-5 weeks after transplanting. Thirty five diploid and tetraploid somaclones were selected and backcrossed to *S. tuberosum* (cv. Atlantic or the clone Q155-3). Pollen viability during crossing was determined by a simple staining test using acetocarmine solution. Selfing

and reciprocal crosses were also done. Progeny evaluation for trichomes and agronomic traits from these crosses were conducted in the fall of 1991.

1.4 Results

1.4.1. The presence of droplets on type B trichomes of backcrossed and selfed progenies of F743-4 hybrid and its somaclones. The progenies of seven somaclones of F743-4 plus the unregenerated hybrid backcrossed to the cv. Atlantic were evaluated for their trichome traits under greenhouse conditions. The results of the evaluation for the presence of B droplets in the first backcross progenies corroborate the results reported by Lentini (1989). The same set (I) of families (M502, M509, M514 and M515) consistently gave a high percentage of plants with B droplets in the evaluations conducted in 1989 and 1990 (Table 1.2). The percentage of plants with droplets on type B trichomes among the progenies of these four families (set I) were 10-20x higher than the unregenerated F743-4 hybrid (set III) and the three control families with few B droplets (set II). The percentage of progenies with B droplets of families in set I ranged from 22% to 40% while set II and set III had only 0% to 5%.

Clones from the three sets of families were selected based on their trichome traits and vine type and backcrossed for the second time (BC_2) to the cv. Atlantic or to the clone Q155-3. Selfing was also done. From the set I families which previously exhibited a high percentage of

Table 1.2. Presence of droplets on type B trichomes of F743-4 hybrid and its somaclones backcrossed (BC_1) to cv. Atlantic.

Female Parent	Family Code	% of Plants with B Droplets 1989	1990	Mean
I. Special B-type Somaclone Lines				
4001	M502	40	25	32.5
4029	M509	22	35	28.5
4038	M514	32	36	34.0
4040	M515	40	27	33.5
II. Regular Somaclone Lines				
4002	M503	0	1	0.5
4004	M504	3	0	1.5
4009	M505	0	2	1.0
III. Unregenerated Hybrid				
F743-4	M501	0	5	2.5

progenies with B droplets, only the clones M502-6 and M514-1 backcrossed to Q155-3 showed a high frequency (28% and 37%, respectively) of their offspring with B droplets; the rest had none or only a few (0 to 17%) (Table 1.3). Selfing however, resulted in a very high percentage of plants with the B droplets (50% to 100%). In set II families (somaclones which transmit few B droplets), only a few (1% to 6 %) plants with B droplets were found in backcrossed and selfed clones of M503-2, M504-1 and M504-5, while the rest did not have any. No plants with the B droplets were found from the backcrossed and selfed families of the hybrid F743-4 except for one, M501-110 selfed which had 5% of its progenies bearing the droplets on type B trichomes.

Selected clones from the second backcrossed and selfed families were further backcrossed for the third time (BC_3) to the same recurrent parents, cv. Atlantic or the clone Q155-3. An attempt was made to produce backcrossed and selfed seeds from each clone selected but fruit setting was very poor, especially in selfed clones where none or only a few seeds were successfully produced. No seeds were produced from selfed clones in set I in spite of the large number of flowers pollinated. Some third backcrossed clones which came from set I (somaclones which transmit many B droplets) exhibited a high percentage of B droplets on their progenies (Table 1.4). The progenies of two clones (N308-1 and N308-5) backcrossed to Q155-3 (BC_3) had over 20% of their progenies with B droplets. These two clones were previously selected from the cross M509-4 x Q155-3 which had only 2% of its progenies with the B droplets. In the previous generation, the backcross of M514-1 produced the highest percentage of clones with B droplets.

Table 1.3. Presence of droplets on type B trichomes of BC_2 and selfed progenies of F743-4 hybrid and its somaclones.

Female Parents (BC_1)		% of Plants w/ B Droplets		
Clone No.	Presence of B Droplets*	Atlantic	Q155-3	Selfed
I. Somaclones which transmit many B droplets				
M502-1	-	- **	0	-
M502-2	+	-	0	99
M502-3	+	-	-	84
M502-5	-	-	-	95
M502-6	+	-	28	92
M502-104	+	5	-	-
M502-105	-	0	0	-
M502-107	-	0	-	-
M509-2	-	-	0	-
M509-3	-	-	-	92
M509-4	+	-	2	91
M509-5	+	-	-	94
M509-103	+	-	10	-
M509-105	+	-	0	-
M509-109	-	-	0	-
M514-1	+	-	37	73
M514-2	-	-	1	85
M514-106	+	-	0	72
M514-111	-	5	17	50
M515-1	-	-	0	77
M515-3	+	-	-	88
M515-4	+	-	-	100
M515-5	+	-	3	-
M515-101	+	0	5	100
M515-105	-	0	0	-
II. Somaclones which transmit few B droplets				
M503-1	-	-	-	0
M503-2	+	-	4	4
M503-4	-	-	0	-

* (+) = with B droplets, (-) without B droplets
** (-) = no test/data

Table 1.3. (Continued...)

Female Parents (BC_1)		% of Plants w/ B Droplets		
Clone No.	Presence of B Droplets*	Atlantic	Q155-3	Selfed
II. Somaclones which transmit few B droplets				
M504-1	+ *	- **	1	6
M504-3	-	-	0	0
M504-4	-	-	0	0
M504-5	+	-	1	0
M505-1	+	-	-	0
M505-3	-	-	-	0
M505-4	-	-	-	0
III. Unregenerated Hybrid				
M501-2	-	-	-	0
M501-3	-	-	-	0
M501-4	+	-	-	0
M501-109	+	0	0	-
M501-110	-			

* (+) = with B droplets, (-) without B droplets
** (-) = no test/data

Table 1.4. Presence of droplets on type B trichomes of BC_3 and selfed progenies of F743-4 hybrid and its somaclones.

Pedigree (BC_2)	Female Parents	Percentage of Plants w/ B Droplets		
		Atlantic	Q155-3	Selfed
I. Somaclones which transmit many B droplets				
M502-2 selfed	N303-4	0	0	- *
	N303-5	0	-	-
	N303-8	0	-	-
M502-3 selfed	N304-1	-	9	-
	N304-4	0	-	-
	N304-6	-	0	-
	N304-10	-	2	-
M502-6 selfed	N306-1	2	-	-
	N306-4	5	-	-
M509-4 x Q155-3	N308-1	-	47	-
	N308-2	-	0	-
	N308-5	-	29	-
	N308-7	-	3	-
M514-1 x Q155-3	N312-12	0	-	-
	N312-14	-	6	-
	N312-32	-	0	-
	N312-37	0	10	-
	N312-38	-	6	-
	N312-45	-	12	-
	N312-46	-	1	-
	N312-50	0	-	-

* (-) No Data

Table 1.4. (Continued...)

Pedigree (BC2)	Female Parents	Percentage of Plants w/ B Droplets Atlantic	Q155-3	Selfed
I. Somaclones which transmit many B droplets				
M514-1 selfed	N314-5	0	- *	-
	N314-16	0	-	-
	N314-17	-	14	-
M515-5 x Q155-3	N317-6	-	19	-
II. Somaclones which transmit few B droplets				
M503-2 x Q155-3	N324-1	0	-	-
	N324-3	-	0	0
M503-4 x Q155-3	N325-6	0	-	-
M503-1 selfed	N326-4	0	-	0
M503-2 selfed	N327-2	0	-	0
M504-1 x Q155-3	N328-5	-	0	-
M504-3 x Q155-3	N329-3	-	0	-
M504-4 x Q155-3	N330-1	-	0	-
M504-5 x Q155-3	N331-1	-	0	-

* (-) No Data

Table 1.4. (Continued...)

Pedigree (BC2)	Female Parents	Percentage of Plants w/ B Droplets Atlantic	Q155-3	Selfed
II. Somaclones which transmit few B droplets				
M504-1 selfed	N332-1	- *	0	-
	N332-2	-	0	-
	N332-5	-	-	0
M504-4 selfed	N334-3	-	0	-
M504-5 selfed	N335-1	-	0	-
M505-1 selfed	N336-2	-	0	-
M505-3 selfed	N337-5	0	-	-
III. Unregenerated Hybrid				
M501-2 selfed	N321-1	0	-	0
	N321-3	0	-	-
	N321-4	0	-	-
M501-4 selfed	N323-2	0	-	-
	N323-7	0	-	0

* (-) No Data

Five clones from M514-1 x Q155-3, the family which previously produced progenies with a high percentage of B droplets in the second backcross generation, had some progenies with B droplets when backcrossed to Q155-3 but in a lower frequency (1% to 12%). The B droplets completely disappeared in the progenies of either backcrossed or selfed progenies of families in set II and set III (somaclones which transmitted few B droplets and the unregenerated F743-4 hybrid, respectively).

Visual comparison showed that the genotype of the recurrent parent used may have an effect in the expression of droplets on type B trichomes. In general, backcrossing to the clone Q155-3 resulted to a higher percentage of progenies bearing the B droplets as compared to backcrossing to the cv. Atlantic. In the second backcross, 47% of the families in set I had B droplets, 18% of which had greater than 16% of their progenies with B droplets when backcrossed to the clone Q155-3 (Table 1.5). Backcrossing to the cv. Atlantic resulted in 23% of the families with B droplets and 33% with only 1 to 15% of their progenies with B droplets. In set II, backcrossing to Q155-3 resulted in either a low frequency or absence of B droplets. In set III, backcrossing the clone from the unregenerated hybrid resulted in 100% of the progenies without B droplets. All selfed families of set I had ≥16% of their progenies with B droplets.However, 22% to 25% of the families of set II and III had progenies with the B droplets. In the third backcross, the same case was observed where 75% of the set I backcrossed-families to Q155-3 exhibited B droplets and 19% of these had greater than 16% of their progenies with B droplets (Table 1.6). Backcrossing to the cv. Atlantic resulted in only 18% of the families

Table 1.5. Frequency distribution of BC_2 families of F743-4 and its somaclones with B droplets.

Cross/Female Parent	Family Set Number	Percent Family/Group* 0%	1-15%	≥16%
Backcross				
Atlantic	I	67	33	0
	II	- **	-	-
	III	100	0	0
	Mean	83.5	16.5	0
Q155-3	I	53	29	18
	II	50	50	0
	III	100	0	0
	Mean	67.7	26.3	6.0
Mean (Backcross)		**74.0**	**22.4**	**3.6**
Selfed	I	0	0	100
	II	78	22	0
	III	75	25	0
Mean (Selfed)		**51.0**	**15.7**	**33.3**

* Percent of progenies with B droplets

** (-) No data

Table 1.6. Frequency distribution of BC_3 families of F743-4 and its somaclones with B droplets.

Cross/Female Parent	Family Set Number	Percent Family/Group* 0%	1-15%	≥16%
Backcros				
Atlantic	I	82	18	0
	II	100	0	0
	III	100	0	0
	Mean	94.0	6.0	0
Q155-3	I	25	56	19
	II	100	0	0
	III	- **	-	-
	Mean	62.5	28.0	9.5
Mean (Backcross)		**81.4**	**14.8**	**3.8**
Selfed	I	-	-	-
	II	100	0	0
	III	100	0	0

* Percentage of progenies with B droplets

** (-) No data

with progenies having B droplets with only 1 to 5% frequency while the majority (82%) had no B droplets at all. Backcrossing or selfing clones from from set II and III did not produce progenies with B droplets. Unfortunately, no selfed seed was successfully produced from set I for comparison.

It appears that using plants with B droplets from the families of set I (somaclones which transmit many B droplets) as progenitors will conserve the trait (the presence of droplets on type B trichomes) during backcrossing in either high or low frequency. This was evident in most first, second and third backcrosses of somaclones from set I. Figures 1.2, 1.3, 1.4 and 1.5 show the expression of the trait (i.e. the presence of the droplets on type B trichomes) in four somaclones which transmit many B droplets as it is passed on from one generation to the next through backcrossing or selfing. In BC_2 and BC_3, using clones with the B droplets as the donor parent resulted to either high or low percent of progenies with the B droplets while using clones without the B droplets resulted in the disappearance of the B droplets in the progeny. Selfing of the clones which either have or do not have B droplets however, resulted in a higher frequency of progenies with B droplets. Using plants without the B droplets resulted in progenies which had only a few droplets or without droplets on type B trichomes in the subsequent generations, as seen from the results of backcrossing or selfing somaclones of set II or set III families (Figure 1.6 and Figure 1.7).

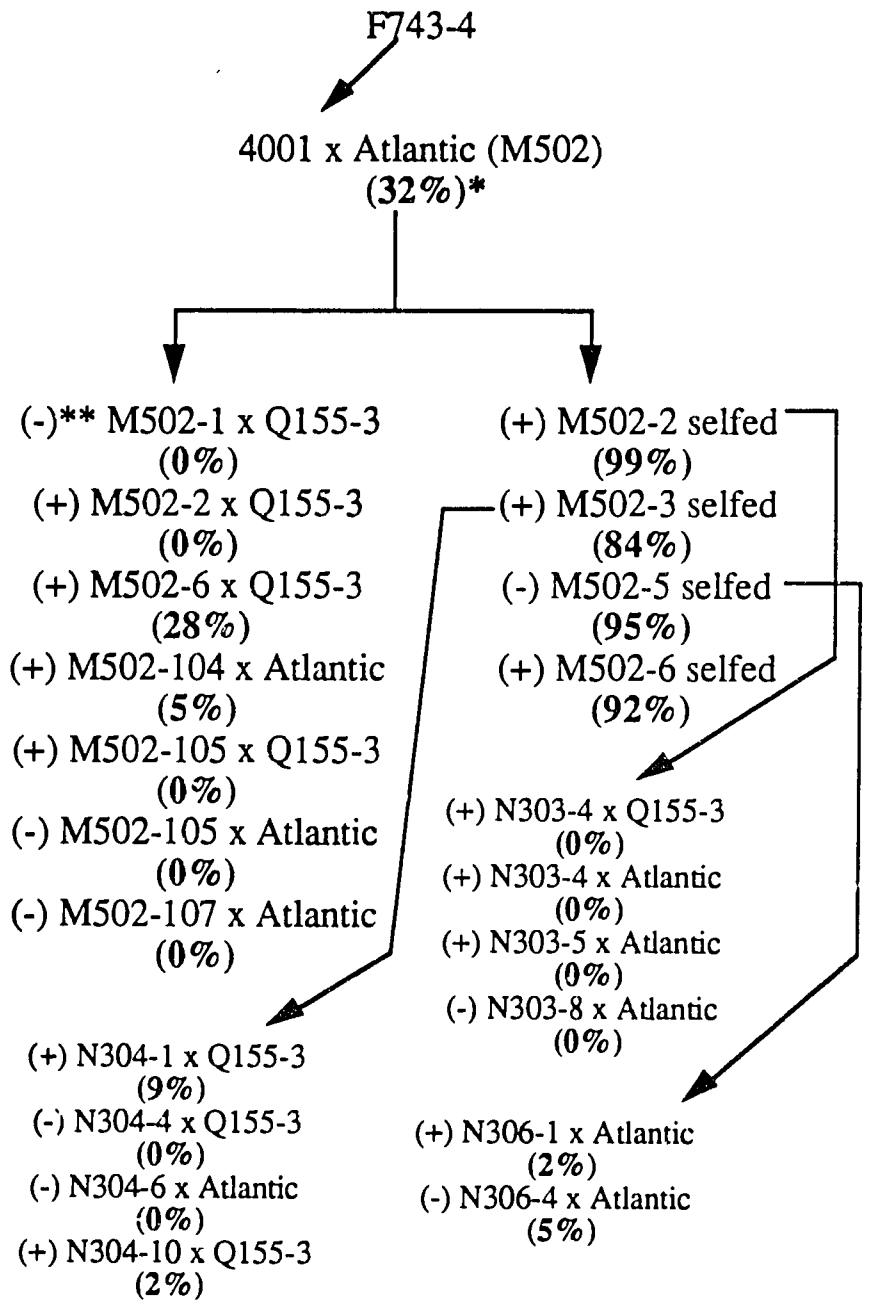


Figure 1.2. The presence of droplets on type B trichomes of somaclone 4001 *Tuberosum*- backcrossed and selfed progenies.

* Percentage of plants with B droplets

** Clone does (+) or does not (-) display B droplets

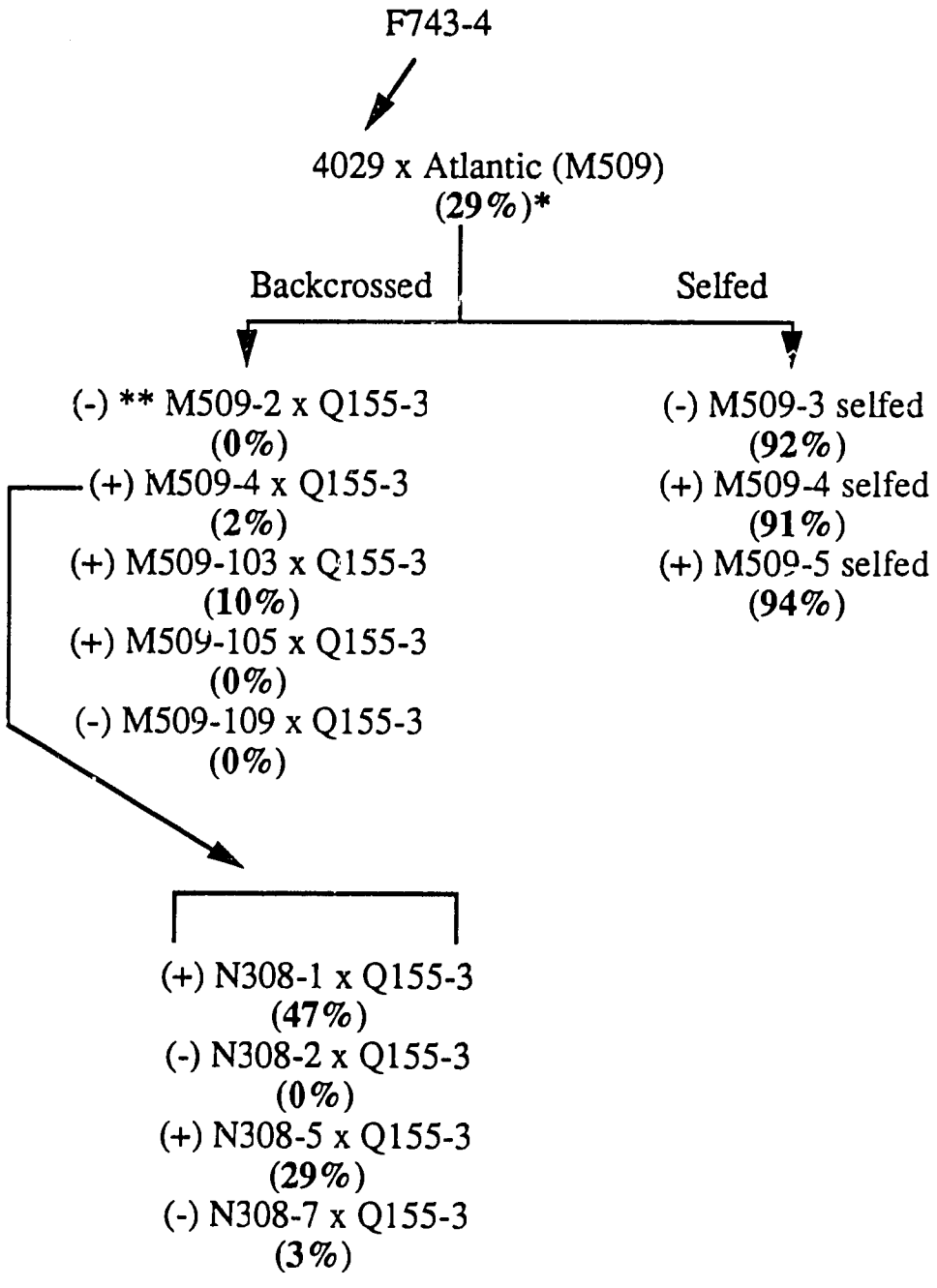


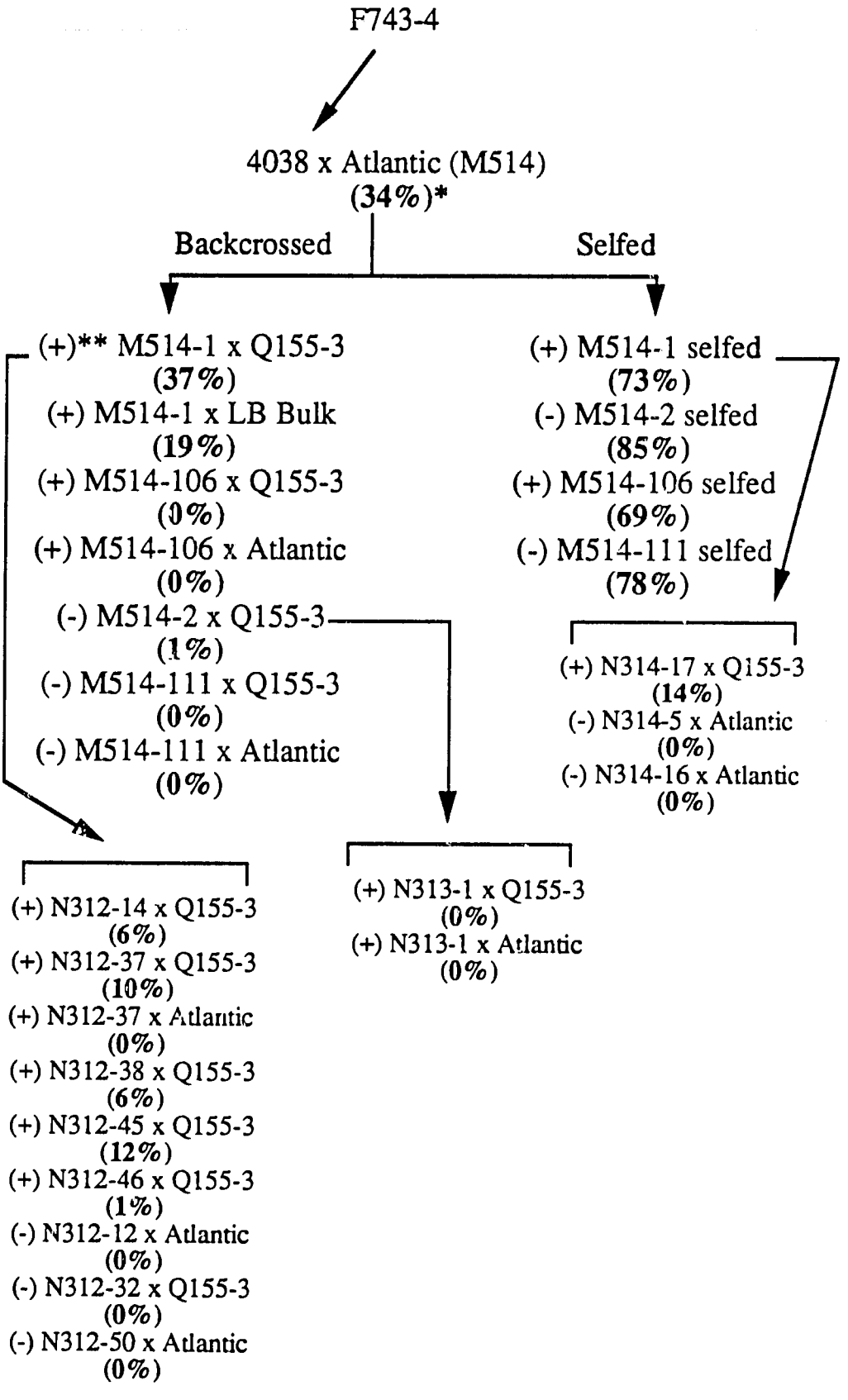
Figure 1.3. The presence of droplets on type B trichomes of somaclone 4029*Tuberosum*-backcrossed and selfed progenies.

* Percentage of plants with B droplets
** Clones does (+) or does not (-) display B droplets.

Figure 1.4. The percentage of droplets on type B trichomes of somaclone 4038 Tuberosum-backcrossed and selfed progenies.

* Percentage of plants with B droplets

** Clone does (+) or does not (-) display B droplets


F743-4
4038 x Atlantic (M514)
(34%)*
Backcrossed
Selfed
(+)** M514-1 x Q155-3
(37%)
(+) M514-1 x LB Bulk
(19%)
(+) M514-106 x Q155-3
(0%)
(+) M514-106 x Atlantic
(0%)
(-) M514-2 x Q155-3
(1%)
(-) M514-111 x Q155-3
(0%)
(-) M514-111 x Atlantic
(0%)
(+) M514-1 selfed
(73%)
(-) M514-2 selfed
(85%)
(+) M514-106 selfed
(69%)
(-) M514-111 selfed
(78%)
(+) N314-17 x Q155-3
(14%)
(-) N314-5 x Atlantic
(0%)
(-) N314-16 x Atlantic
(0%)
(+) N312-14 x Q155-3
(6%)
(+) N312-37 x Q155-3
(10%)
(+) N312-37 x Atlantic
(0%)
(+) N312-38 x Q155-3
(6%)
(+) N312-45 x Q155-3
(12%)
(+) N312-46 x Q155-3
(1%)
(-) N312-12 x Atlantic
(0%)
(-) N312-32 x Q155-3
(0%)
(-) N312-50 x Atlantic
(0%)
(+) N313-1 x Q155-3
(0%)
(+) N313-1 x Atlantic
(0%)

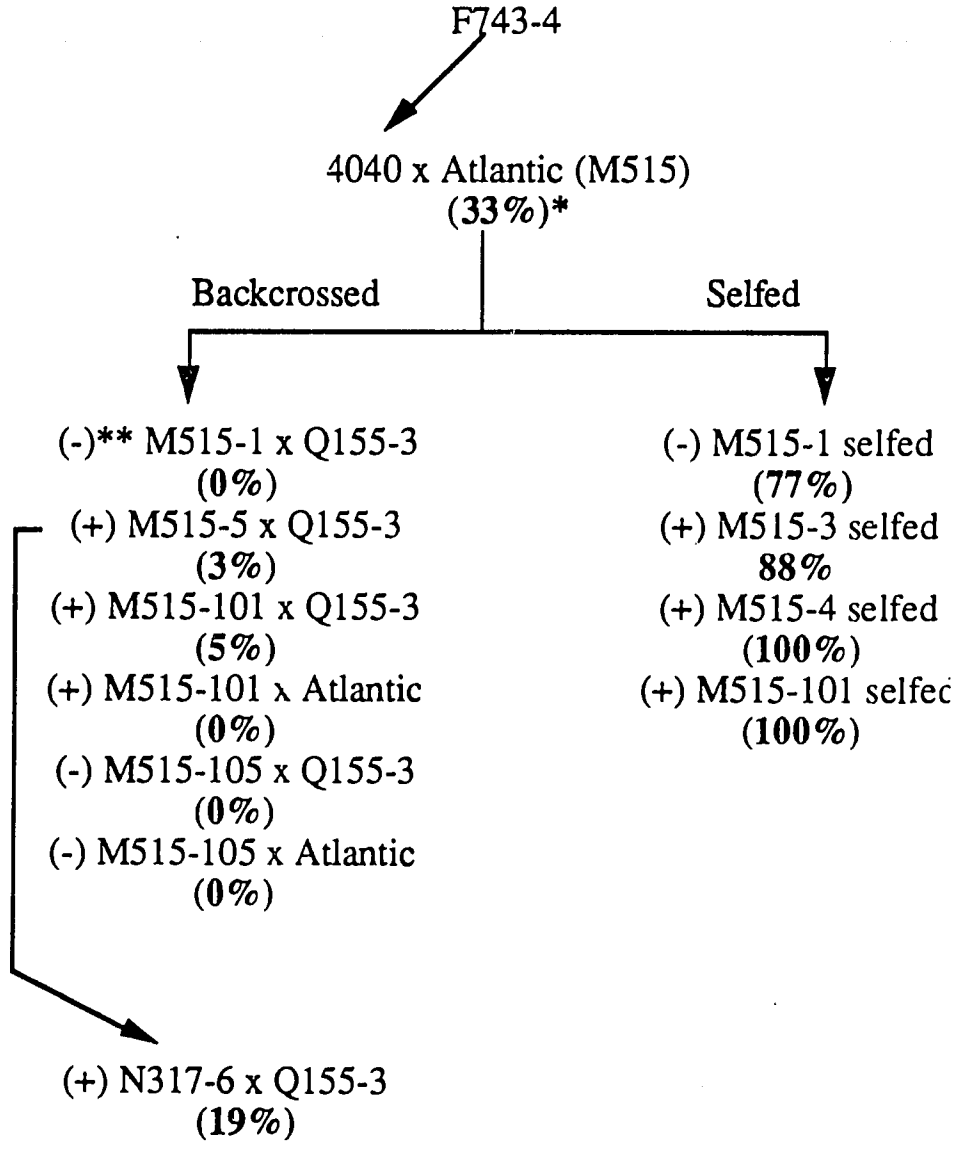


Figure 1.5. The presence of droplets on type B trichomes of somaclone 4040 *Tuberosum*-backcrossed and selfed progenies.

* Percentage of plants with B droplets
** Clone does (+) or does not (-) display B droplets

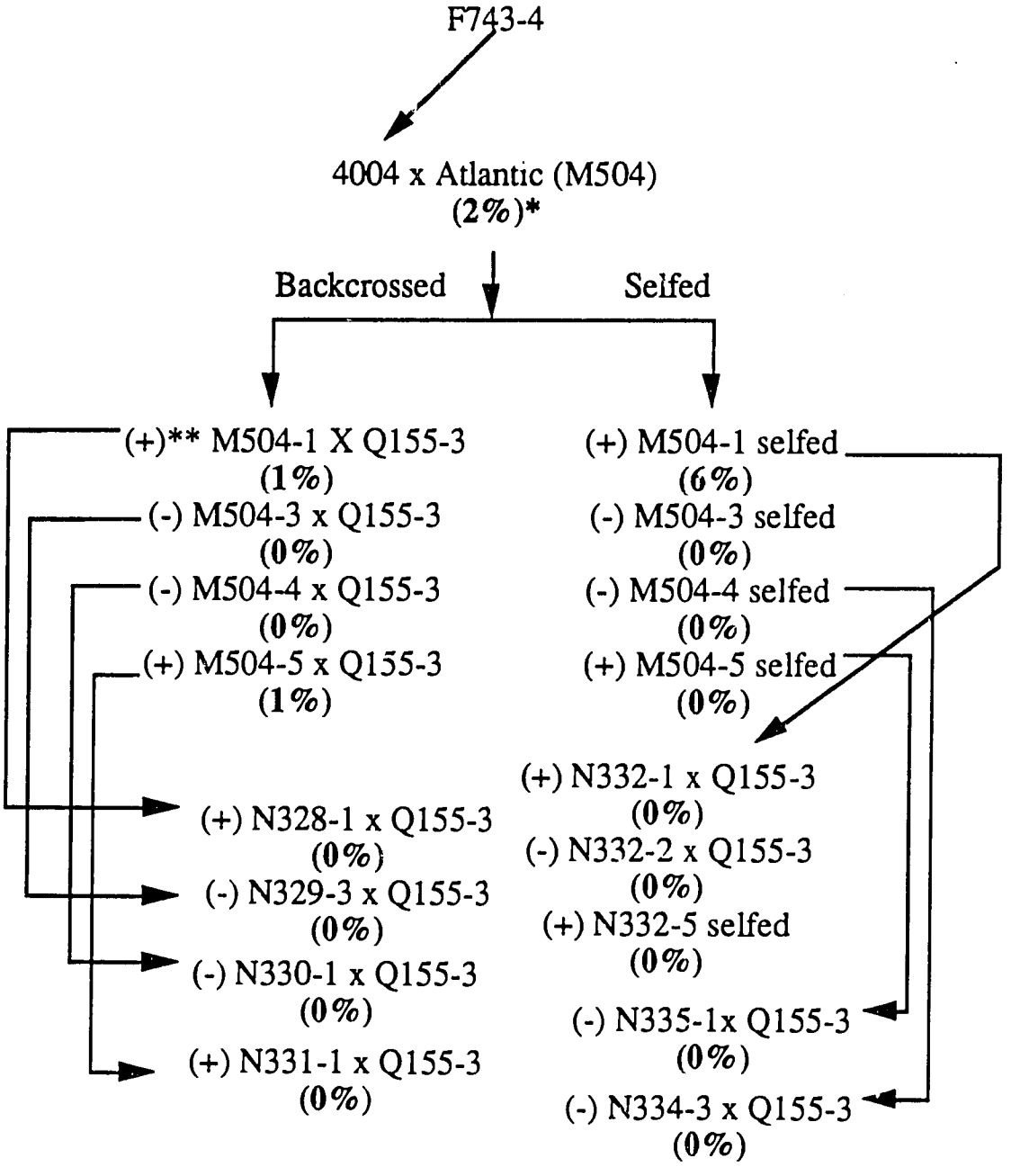


Figure 1.6. The presence of droplets on type B trichomes (somaclone 4004*Tuberosum*-backcrossed and selfed progenies.

* Percentage of plants with B droplets

** Clone does (+) or does not (-) display B droplets

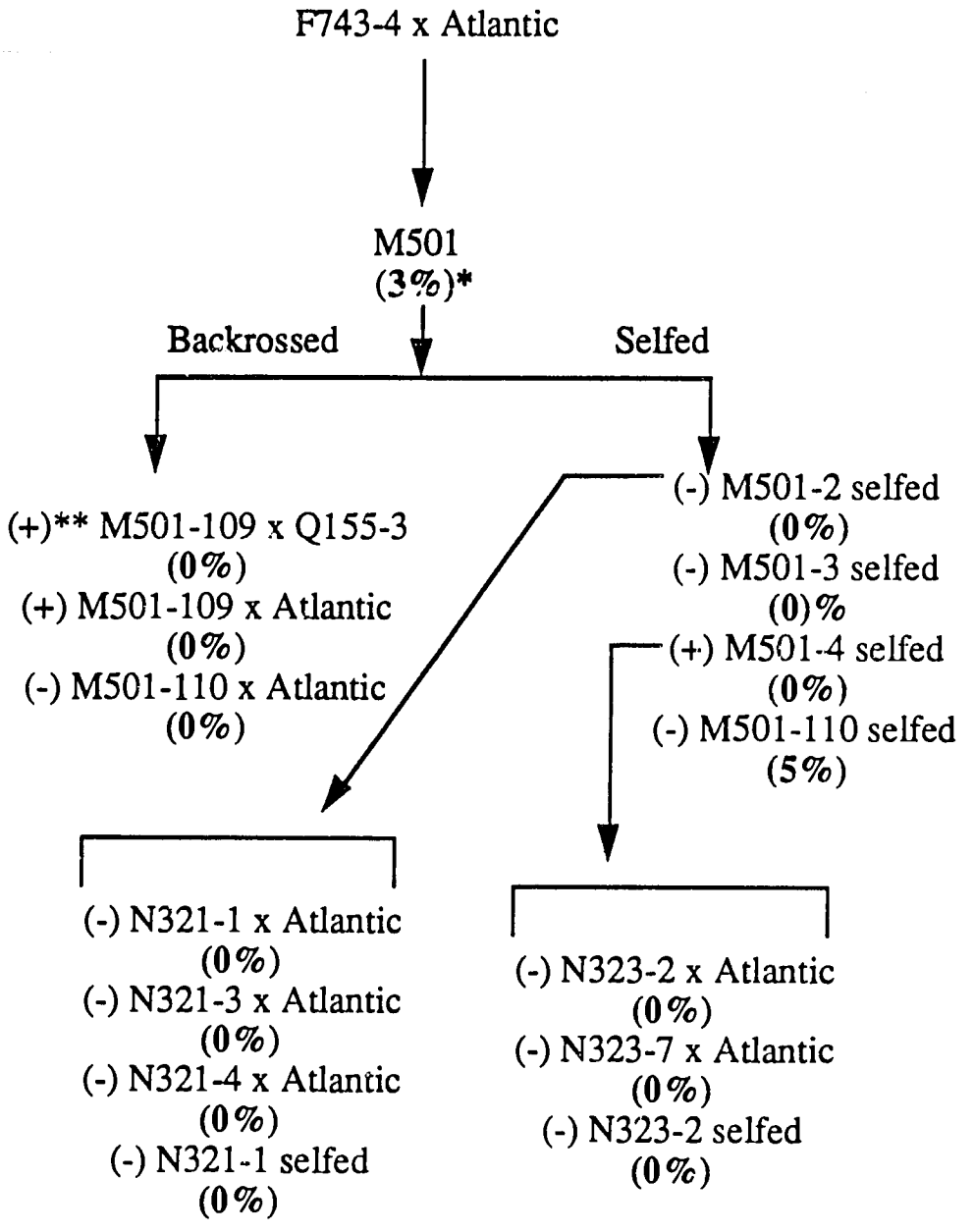


Figure 1.7. The presence of droplets on type B trichomes of backcrossed and selfed progenies of the unregenerate hybrid F743-4.

* Percentage of plants with B droplets
** Clone does (+) or does not (-) display B droplets

1.4.2 Browning activity of backcrossed and selfed progenies of F743-4 hybrid and its somaclones. The percentage of plants with browning activity (i.e. MEBA ≤ 62%) was generally higher in somaclone-backcrossed families which transmit many B droplets (set I). In the first backcross (BC_1), the mean percentage of plants with browning activity in somaclones which transmit many B droplets (set I) was 77.5% as compared to the progenies of set II families which had only 18.8% (Table 1.7). The percentage of progenies with browning activity of backcrossed-families from the unregenerated F743-4 hybrid had the least, with an average of only 10% of the progenies showing browning activity. The same trend was observed in the second and third backcrosses, where families of set I generally had a higher percentage of their progenies with browning activity as compared to the backcrossed-somaclones of set II and the unregenerated F743-4 hybrid (set III) (Table 1.8 and Table 1.9). Selfing resulted in a higher percentage of plants with browning as compared to backcrossing. In the second backcross, the Q155-3-backcrossed families of set I had a mean of 77.7% progenies with good browning while the Atlantic-backcrossed families had 65.8%. Selfed families of set I had a mean of 90% progenies with browning activity while set II selfed families had 6[illegible]%. The third-backcrossed (BC_3) families of set I had 64.4% and 54.2% of their progenies with browning activity in the Atlantic and Q155-3-backcrossed clones, respectively. Set II families had only 19.8% and 16.3% in the Atlantic- and Q155-3-backcrossed clones, respectively. In general, backcrossed progenies of families from set I (somaclones which transmit many B droplets) had

Table 1.7. Browning activity of F743-4 and its somaclones backcrossed (BC_1) to cv. Atlantic evaluated under greenhouse conditions.

Family Code	% of Plants with Browning Activity* 1989	1990	Mean
I. Somaclones which transmit many B droplets			
M502	71	77	74.0
M509	67	86	76.5
M514	75	81	78.0
M515	83	80	81.5
Mean ± Std. Dev.	74 ± 5.9	81 ± 3.2	78 ± 2.7
II. Somaclones which transmit few B droplets			
M503	18	15	16.5
M504	13	25	19.0
M505	22	20	21.0
Mean ± Std. Dev.	18 ± 3.7	20 ± 4.1	19 ± 1.8
III. Unregenerated Hybrid			
M501	9	11	10.0

* MEBA ≤ 62% transmittance at OD 470

Table 1.8. Browning activity of type A trichomes of BC_2 and selfed progenies of F743-4 hybrid and its somaclones evaluated under greenhouse conditions.

Female Parent	Male Parents: Atlantic	Q155-3	Selfed
	(% of Plants w/ Browning)*		
I. Somaclones which transmit many B droplets			
M502-1	- **	76	-
M502-2	-	88	90
M502-3	-	-	100
M502-5	-	-	98
M502-6	-	-	85
M502-104	69	-	-
M502-105	70	73	-
M502-107	56	-	-
M509-2	-	80	-
M509-3	-	-	88
M509-4	-	64	79
M509-5	-	-	95
M509-103	-	71	-
M509-105	-	83	-
M509-109	-	67	-
M514-1	-	70	95
M514-2	-	84	89
M514-106	-	85	-
M514-111	62	74	-
M515-1	-	89	96
M515-3	-	-	92
M515-4	-	-	78
M515-5	-	79	-
M515-101	72	83	-
M515-105	66	77	-
Mean ± Std. Dev.	65.8 ± 5.4	77.7 ± 7.2	90.4± 6.7
II. Somaclones which transmit few B droplets			
M503-1	-	-	55
M503-2	-	45	61
M503-4	-	29	-

* MEBA ≤ 62% transmittance at OD 470

** (-) No Data

Table 1.8. (Continued...)

Female Parent	Male Parents Atlantic	Q155-3	Selfed
	(% of Plants w/ Browning)*		
II. Somaclones which transmit few B droplets			
M504-1	- **	39	63
M504-3	-	42	72
M504-4	-	33	58
M504-5	-	51	72
M505-1	-	-	73
M505-3	-	-	57
M505-4	-	-	65
Mean ± Std. Dev.	-	39.8 ± 7.3	64.0 ± 6.5
III. Unregenerated Hybrid (F743-4)			
M501-2	-	-	65
M501-3	-	-	78
M501-4	-	-	59
M501-109	49	36	-
M501-110	-	-	62
Mean ± Std. Dev.	-	-	66.0 ± 7.2

* MEBA ≤ 62% transmittance at OD 470

** (-) No Data

Table 1.9. Browning activity in type A trichomes of BC_3 and selfed progenies of F743-4 hybrid and its somaclones evaluated under greenhouse conditions.

Pedigree (BC_2)	Female Parents	Male Parents Atlantic	Q155-3	Selfed
		(% of Plants w/ Browning)*		
I. Somaclones which transmit high B droplets				
M502-2 selfed	N303-4	95	65	-
	N303-5	100	-	-
	N303-8	51	-	-
M502-3 selfed	N304-1	-	45	-
	N304-4	50	-	-
	N304-6	-	80	-
	N304-10	-	57	-
M502-6 selfed	N306-1	9	-	-
	N306-4	18	-	-
M509-4 x Q155-3	N308-1	-	63	-
	N308-2	-	31	-
	N308-5	-	40	-
	N308-7	-	50	-
M514-1 x Q155-3	N312-3	-	35	-
	N312-12	77	-	-
M514-1 x Q155-3	N312-14	-	68	-
	N312-32	-	70	-
	N312-37	86	34	-
	N312-38	-	90	-

* MEBA ≤ 62% transmittance at OD 470

** (-) No Data

Table 1.9. (Continued...)

Pedigree (BC2)	Female Parents	Male Parents Atlantic	Q155-3	Selfed
		(% of Plants w/ Browning)*		
I. Somaclones which transmit high B droplets				
M514-2 x Q155-3	N312-45	- **	67	-
	N312-46	-	35	-
	N312-50	88	-	-
	N313-1	43	14	-
M514-1 selfed	N314-5	78	-	-
	N314-16	78	-	-
	N314-17	-	67	-
M515-5 x Q155-3	N317-6	-	65	-
Mean ± Std. Dev.		64.4 ± 28.6	54.2 ± 19.2	-
II. Somaclones which transmit few B droplets				
M503-4 x Q155-3	N324-1	24	-	-
	N324-3	-	15	57
M503-4 x Q155-3	N325-6	11	-	-
M503-1 selfed	N326-4	24	-	51
M503-2 selfed	N327-2	17	-	32
M504-1 x Q155-3	N328-5	-	8	-
M504-3 x Q155-3	N329-3	-	19	-
M504-1 selfed	N332-1	-	24	-
	N332-2	-	21	-
	N334-3	-	16	-

* MEBA ≤ 62% transmittance at OD 470

** (-) No Data

Table 1.9. (Continued...)

Pedigree (BC2)	Female Parents	Male Parents Atlantic	Q155-3	Selfed
		(% of Plants w/ Browning)*		
II. Somaclones which transmit few B droplets				
M505-1 selfed	N336-2	- **	11	-
M505-3 selfed	N337-5	23	-	-
Mean ± Std. Dev.		19.8 ± 5.11	16.3 ± 5.2	-
III. Unregenerated Hybrid (F743-4)				
M501-2 selfed	N321-1	46	-	73
	N321-3	28	-	-
	N321-4	39	-	-
M501-4 selfed	N323-2	16	-	-
	N323-7	22	-	41
Mean ± Std. Dev.		30.2 ± 11.0	-	57.0 ± 16.0

* MEBA ≤ 62% transmittance at OD 470

** (-) No Data

more plants with good browning activity than the families of set II (somaclones which transmit few B droplets).

Results of the study also show that plants with the B droplets generally had better browning activity, although a few plants bearing the B droplets in some families did not have good browning activity. Plants with good browning but without B droplets have also been recovered, especially in the third backcrosses.

1.4.3 <u>Association of trichome traits and vine type.</u> To look at the association between trichome traits and vine type, the progenies of the backcrossed and selfed families were evaluated for their vine type and trichome traits. Vine scores were from 1= *Berthaultii*-type to 5 = *Tuberosum*-type. Each clone was classified into four groups: 1) with B droplets and good browning, 2) without B droplets and good browning, 3) with B droplets and poor browning, and 4) without B droplets and poor browning (Appendix 2, 3, and 4). The different classes were analyzed using the chi-square test. Table 1.10 shows the mean vine type scores and MEBA values of families in each group. The chi-square test shows that *Berthaultii* -vine type is related to trichome traits (X^2 = 0.189, P = 0.750 1df). Progenies with good browning activity and with B droplets had the poorest vine type (i.e., resemble the wild *S. berthaultii* parent). This is especially true for backcrossed progenies of somaclones which transmit many B droplets (set I). Progenies which did not have good browning activity and did not have B droplets had vine types that were closer to the *Tuberosum*-type. Progenies with B droplets and poor browning activity, and those without B droplets but with good browning

Table 1.10. Mean and std. dev. of vine scores of backcrossed families of F743-4 and its somaclones.

Backcross/ Set		Presence of Droplets			
		With B Droplets		Without B Droplets	
		Browning Activity			
		≤ 62%	≥ 63%	≤ 62%	≥ 63%
BC1					
	I	3.28 ± 0.2*	3.98 ± 0.4	3.98 ± 0.3	4.90 ± 0.1
	II	3.55 ± 0.2	4.60**	4.40 ± 0.4	5.00 ± 0
	III	- ***	-	4.50	5.00
BC2					
	I	3.46 ± 0.3	4.30 ± 0.3	4.09 ± 0.3	4.84 ± 0.2
	II	4.22 ± 0.2	4.48 ± 0.2	4.52 ± 0.3	4.96 ± 0.1
	III	4.20	4.50	4.70	5.00
BC3					
	I	3.49 ± 0.5	4.24 ± 0.6	4.45 ± 0.4	4.83 ± 0.2

* Vine scores: 1 = *Berthaultii*-type, 5 = *Tuberosum*-type

** One Family sample

*** (-) = No Data

had intermediate vine type scores. The mean vine scores of the different family sets over backcross generations are shown in Table 1.11. Backcross progenies in set I with B droplets and good browning had the poorest vine type while those in set II and III had better vine scores. Backcrossed progenies without B droplets and poor browning activity had the best vine type scores. Mean vine scores of the backcrossed progenies over family sets are shown in Table 1.12. In all the backcross generations, progenies with B droplets and good browning always had the poorest vine types while those without B droplets and poor browning were closest to the desired *Tuberosum* vine type. It appears that better expression of both type A and type B trichome traits is still associated with wild or poor vine type in the backcrossed progenies of the somaclones, though the BC_2 population was an improvement over the BC_1.

1.4.4 Total glycoalkaloid (TGA) content of selected progenies of F743-4 hybrid and its *S. tuberosum*,-backcrossed somaclones. Some clones selected for their trichome traits from set I and a few clones from set II and III were evaluated for their total glycoalkaloid content and the result is shown in Table 1.13. Most of the clones were found to have solasonine and solamargine, two glycoalkaloids not commonly found in cultivated commercial varieties. Two of these clones (M502-106 and M509-102) have significantly high amounts of these two gylcoalkaloids but with very low amounts of the other two glycoalkaloids, chaconine and solanine. Generally higher amounts of chaconine and solanine were found in somaclones from the the set of

Table 1.11. Mean vine scores and std. dev. of the different set of families over backcross generations.

Backcross/Set	Presence of B Droplets			
	With B Droplets		Without B Droplets	
	Browning Activity			
	≤ 62%	≥ 63%	≤ 62%	≥ 63%
I (BC_1, BC_2, BC_3)	3.44* ± 0.4	4.17 ± 0.5	4.21 ± 0.4	4.84 ± 0.2
II (BC_1, BC_2)	4.03 ± 0.4	4.50 ± 0.2	4.48 ± 0.3	4.98 ± 0.1
III (BC_1, BC_2)	4.20**	4.50	4.47 ± 0.2	5.00 ± 0

* Vine scores: 1 = *Berthaultii*-type, 5 = *Tuberosum*-type

** One Family sample

Table 1.12. Mean vine scores and std dev. of the different backcrossed families of F743-4 and its somaclones.

Set/ Backcross		Presence of B Droplets			
		With B Droplets		Without B Droplets	
		Browning Activity			
		≤ 62%	≥ 63%	≤ 62%	≥ 63%
(I, II, III)	BC_1	3.37*± 0.2	4.10 ± 0.4	4.20 ± 0.4	4.96 ± 0.1
(I, II, III)	BC_2	3.66 ± 0.5	4.38 ±0.3	4.22 ± 0.4	4.87 ± 0.2
(I)	BC_3	3.49 ± 0.5	4.24 ±0.7	4.45 ± 0.4	4.83 ± 0.2

* Vine scores: 1 = *Berthaultii*-type, 5 = *Tuberosum*-type

Table 1.13. Tuber glycoalkaloid (TGA) content of selected progenies from BC_1 of F743-4 hybrid and its somaclones.

Family Set/ Clone No.	Tuber Glycoalkaloid (mg/100g FW)			
	Solasonine	Solamargine	Chaconine	Solanine
I. Somaclones which transmit many B droplets				
M502-101	1.3	1.6	20.8	33.4
M502-102	4.4	4.0	35.7	25.3
M502-103	1.3	0.4	20.0	23.9
M502-104	0.0	0.0	31.0	30.8
M502-105	1.8	0.0	5.3	4.4
M502-106	47.0	18.9	0.0	0.0
M502-107	1.6	1.5	2.8	2.5
M502-108	0.0	0.0	19.1	16.9
M509-101	0.0	0.0	22.5	25.6
M509-102	24.8	20.70	0.8	0.0
M509-103	3.4	3.2	21.2	17.9
M509-104	2.5	0.7	18.0	18.2
M509-105	1.6	1.1	33.2	24.6
M509-106	0.0	0.0	22.9	17.8
M509-107	0.0	0.0	17.7	19.1
M509-108	0.0	0.0	21.5	24.5
M509-109	1.4	2.4	29.1	24.3
M509-110	0.0	0.0	4.6	6.9
M514-101	0.0	0.0	17.2	17.5
M514-102	0.0	0.0	39.7	29.2
M514-103	5.2	4.2	46.1	36.8
M514-104	4.3	3.4	53.6	36.2
M514-105	4.6	0.0	31.3	20.3
M514-106	2.9	3.2	21.1	22.2
M514-107	1.1	0.4	11.4	9.3
M514-108	3.5	3.2	29.7	28.6
M514-109	2.5	2.2	17.9	19.9
M514-110	0.0	0.0	32.3	29.7
M514-111	0.0	0.0	12.1	10.0
M515-101	2.2	1.0	39.7	24.5
M515-102	2.1	0.7	26.3	25.3
M515-103	3.0	1.5	34.5	29.5
M515-104	0.0	0.0	11.5	16.4
M515-105	7.7	6.7	47.8	34.6
M515-106	0.0	0.0	32.1	29.6
M515-107	0.0	0.0	12.9	0.0

Table 1.13. (Continued...)

Family Set/ Clone No.	Tuber Glycoalkaloid (mg/100g FW) Solasonine	Solamargine	Chaconine	Solanine
II. Somaclones which transmit few B droplets				
M503-4	0.8	1.4	8.7	15.6
M504-1	0.0	0.0	3.6	6.7
M504-2	0.0	0.0	3.3	4.0
M504-3	0.0	0.0	8.2	5.1
M504-4	0.6	0.4	11.5	11.0
M504-5	0.0	0.0	19.6	23.2
III. Unregenerated Hybrid (F743-4)				
M501-4	2.0	1.5	38.8	20.2
M501-5	0.0	0.0	21.2	22.5
IV. Standard Cultivars *				
Atlantic	-	-	4.0	2.4
	-	-	4.9	5.0
	-	-	4.2	4.7
Monona	-	-	5.1	3.7
	-	-	6.6	5.3

* No test made on solasonine and solamargine

families with high B droplets (set I), as compared to the ones from families with low B droplets (II), although only a few samples were evaluated from the latter.The clones from the unregenerated hybrid, F743-4 had high amounts of chaconine and solanine but low amounts of the solasonine and solamargine glycoalkaloids. One clone in set I and three clones in set II were comparable to the standard varieties.

1.4.5 <u>**Clonal selection.**</u> Several clones were selected from BC_1, BC_2 and BC_3, mainly for their good trichome traits and fairly acceptable vine types (Table 1.14). In selecting these clones, the presence of B droplets was the first consideration but clones without droplets on type B trichomes but with good browning activity have also been selected. In BC_1, a total of 36 progenies were selected some of which have been used for further crossing work in the potato program. From the first backcrossed progenies selected, two clones, M514-6 and M504-2 have been found to be resistant to the Colorado potato beetle in a field evaluation conducted by the Entomology group of the potato program and these clones have been used as progenitors for further breeding . From BC_2 and BC_3, a total of 88 and 270 clones, respectively, have been selected because they either have droplets on their type B trichomes, have good browning, or have fairly acceptable vine type. These clones will be evaluated further for their trichome traits and other agronomic traits by the potato program.

Table 1.14. Selected clones from backcrossed and selfed progenies of hybrid F743-4 and its somaclones.

Cross	Family Set			
	I	II	III	Total
BC_1	19	13	4	36
BC_2	57	15	10	82
BC_3	270	0	0	270
Total	**346**	**28**	**14**	**388**

1.4.6 Result of the evaluation for trichomes and morphological traits of somaclones derived from F_1 diploid hybrids. Initially, a total of 145 somaclones (89 diploids and 56 tetraploids) were evaluated under greenhouse conditions from October to December,1990. These somaclones were regenerated from F_1 diploid hybrids of *S. tuberosum* x *S. berthaultii*. The F_1 hybrids used as parents in the regeneration process together with the original parental clones have been included in the evaluation (Table 1.15). The clone HH1-9 is a diploid *S. tuberosum* while B11-A is a *S. berthaultii* clone that possesses both type A and type B trichomes, selected from the accession PI 473331. All the F_1 parents used in the regeneration were diploid but somaclones produced from them were either diploid or tetraploid. In the evaluation for the presence of droplets in type B trichomes, only the diploid hybrid

Table 1.15. Somaclones from diploid hybrids and their parents evaluated for trichome traits under greenhouse conditions.

Plant Code	Ploidy Level	Number of Regenerates
I. Controls		
HH1-9	2n	
B11-A (PI 473331)	2n	
cv. Norland	4n	
II. F_1 Parents		
2	2n	
3	2n	
11	2n	
16	2n	
III. Somaclones		
2's	2n	26
	4n	31
3's	2n	2
	4n	0
4's	2n	7
	4n	4
11's	2n	21
	4n	11
16's	2n	33
	4n	10
Total (Somaclones)	**2n**	**89**
	4n	**56**

#11 was found to possess the B droplets; however, the droplet size observed was much smaller and the trichomes less dense than the usual type B trichomes density and droplet size in the wild *Solanum berthaultii* plants. This hybrid also had the best browning activity among the four F_1 hybrids. Among the somaclones, a number of plants were found to possess the B droplets with the #11 somaclone's family having the highest percent of plants with the B droplets (except #3's, which had only two somaclones, both of which have the B droplets) (Table 1.16). A number of clones had been observed to have browning activity, especially in F_1 backcrossed somaclones that were tetraploids. In terms of vine type, the somaclones and their parents were generally wild looking, with not much difference between the diploid and the tetraploid regenerates (Table 1.17). Other traits such as flower color, tuberization and vigor have also been observed. However these traits did not vary significantly between the parents and the regenerates. In some clones, the shade of their flower color was darker or lighter than the parents. A few (3 out of 11) abnormal looking plants were observed in #4 somaclones which had stunted growth, chlorotic leaves and distorted leaf shapes.

Thirty eight somaclones selected for their trichome traits and vine types were selfed or backcrossed to either cv. Atlantic or the clone Q155-3 to produce the first-backcrossed somaclone progenies. The pollination was conducted from January to May, 1991. The success of pollination was very low especially with the diploid somaclones. The very low percentage of fruit setting could either be due to the warmer than usual condition in the greenhouse especially during the later part of hybridization or could be due to genetic incompatibility. In spite of the numerous amount of

Table 1.16. Presence of droplet on type B trichomes and browning activity in type A trichomes of diploid F1 hybrid-derived somaclones evaluated under greenhouse condition.

Family Code #	Ploidy Level	Percentage of Plants with B droplets	Percentage of Plants with browning*
Somaclones			
2's	2n	0	4
	4n	13	74
3's	2n**	50	100
4's	2n	0	100
	4n	0	33
11's	2n	14	82
	4n	45	85
16's	2n	11	50
	4n**	0	100

* MEBA value ≤ 62% transmittance at OD 470

** Only 2 somaclones evaluated in the group

Table 1.17. Browning activity and vine type score of F_1 diploid hybrids and their somaclones.

Family/Cultivar Code	Ploidy Level	MEBA* (Mean Value)	Vine Type** Rating
A. Control			
HH1-9	2n	70	4
B11-A	2n	19	3
Norland	2n	92	5
B. F1 Parents			
2	2n	87	2
3	2n	58	3
11	2n	51	2
16	2n	55	1
C. Somaclones			
2's	2n	55.1	1.9
	4n	78.6	2.9
3's	2n	48.0	2.5
11's	2n	45.3	2.9
	4n	46.1	3.3
16's	2n	31.7	2.8
	4n	61.3	3.0

* MEBA = % transmittance at OD 470

** 1= *Berthaultii*-type, 5= *Tuberosum*-type

flowers of the unregenerated diploid F1 hybrid that were backcrossed or selfed, fruit setting was very poor producing no seeds for comparison (Table1.18). Backcrossed or selfed seeds were obtained from only a few tetraploid somaclones but none from the diploid somaclones. Reciprocal crosses were also made and a number of them have successfully produced seeds.

Progenies of selected backcrossed and selfed families were evaluated for their trichome traits under greenhouse conditions. Table 1.19 shows the results of the evaluation for the presence of droplets on type B trichomes. The total percentage of progenies with B droplets in backcross was generally lower (0% to 16%) than what were obtained in the tetraploid-derived somaclones presented earlier. Progenies of selfed families produced a higher percentage of plants with B droplets as compared to the backcrossed families. The same trend was observed earlier with the tetraploid hybrid-derived somaclones.

The browning activity of the different crosses is shown in Table 1.20 and 1.21. The percent of progenies with browning is generally higher in selfed as compared to the backcrossed progenies. A number of clones with browning activity have been observed in reciprocal crosses but at a lower frequency.

Table 1.18. Result of the hybridization work done from March to July, 1991 using diploid hybrids (*S. tuberosum* x *S. berthaultii*) and their somaclones.

Clone Number	Number of Flowers			Percent Fruit Set		
	Backcrossed		Selfed	Backcrossed		Selfed
	Q155-3	Atlantic		Q155-3	Atlantic	
F1 Parents*						
2	71	35	38	0	0	0
3	32	8	29	0	0	0
11	19	14	44	0	0	0
16	78	21	54	0	0	0
Somaclones*						
2-01	**13**	**0**	**37**	**0**	**0**	**0**
2-15	**54**	**23**	**33**	**0**	**0**	**0**
2-17	**26**	**21**	**20**	**30**	**14**	**30**
2-32	**0**	**35**	**0**	**0**	**25**	**0**
2-40	**73**	**14**	**16**	**3**	**57**	**44**
2-41	**56**	**24**	**25**	**13**	**25**	**16**
2-42	**55**	**38**	**49**	**0**	**0**	**0**
2-43	**49**	**45**	**19**	**4**	**16**	**11**
2-52	**17**	**40**	**16**	**0**	**0**	**70**
2-55	**64**	**18**	**20**	**0**	**0**	**10**
2-60	**100**	**35**	**28**	**0**	**0**	**18**
Mean	**50.7**	**29.3**	**26.2**	**5**	**13.7**	**21.7**
2-09	39	36	42	0	0	0
2-67	82	25	35	0	0	0
Mean	60.5	30.5	38.5	0	0	0
3-01	39	56	76	0	0	1
3-02	31	9	47	0	0	0
Mean	35.1	32.5	61.5	0	0	0.5
11-16	**17**	**0**	**23**	**0**	**0**	**0**
11-17	**16**	**0**	**42**	**50**	**0**	**11**
11-27	**0**	**0**	**22**	**0**	**0**	**0**
11-36	**0**	**0**	**23**	**0**	**0**	**0**
11-37	**2**	**0**	**26**	**0**	**0**	**0**
Mean	**7**	**0**	**27.2**	**10**	**0**	**2.2**

*All tetraploid (2x=4n) somaclones are printed bold, the rest are diploid (2x=2n)

Table 1.18. (Continued...)

Clone Number	Number of Flowers			Percent Fruit Set		
	Backcrossed		Selfed	Backcrossed		Selfed
	Q155-3	Atlantic		Q155-3	Atlantic	
11-05	50	5	58	0	0	0
11-10	6	0	5	33	0	0
11-18	38	24	19	0	0	0
11-24	3	0	23	0	0	4
11-25	15	0	0	13	0	0
11-30	23	6	41	0	0	0
11-35	0	0	28	0	0	0
11-40	48	0	24	6	0	0
11-41	47	16	57	0	0	0
11-43	22	12	40	0	0	0
11-44	40	0	33	0	0	0
Mean	26.5	5.7	29.8	4.7	0	0.4
16-28	**10**	**4**	**5**	**0**	**0**	**0**
16-29	**81**	**10**	**27**	**0**	**0**	**4**
Mean	**45.5**	**7.0**	**16.0**	**0**	**0**	**2**
16-06	94	8	38	0	0	0
16-08	164	32	53	0	0	0
16-16	54	10	30	0	0	0
Mean	104.0	16.7	40.3	0	0	0

*All tetraploid (2x=4n) somaclones are printed bold, the rest are diploid (2x=2n).

Table 1.19. Result of the evaluation for the presence of droplets on type B trichomes of diploid hybrid-derived somaclones.

Female Parent	Ploidy Level	% of Plants w/ B droplets		
		Q155-3	Atlantic	Selfed
2-17	4n	10	0	30
2-32	4n	- *	10	-
2-40	4n	8	5	15
2-41	4n	0	0	15
2-43	4n	5	-	28
2-60	4n	-	-	15
11-10	4n	5	-	-
11-17	4n	0	-	60
11-24	4n	-	16	-
11-25	4n	0	-	-
16-29	4n	-	-	41

* (-) No Data

Table 1.20. Percentage of plants with browning activity in reciprocal crosses of somaclones derived from diploid hybrids of *S. tuberosum* x *S. berthaultii*.

Family Code	Pedigree	Percent of Plantsw/ browning*
Q443	Atlantic x 2-01	48
Q444	Atlantic x 2-17	64
Q446	Q155-3 x 2-60	6
Q449	Atlantic x 11-30	34
Q448	Atlantic x 11-40	44
Q447	Atlantic x 16-06	8
Q450	Atlantic x 16-08	29
Q445	Atlantic x 16-29	52

Table 1.21. Result of the evaluation for browning activity in type A trichomes of diploid hybrid-derived somaclones.

Female Parent	Ploidy Level	% of Plants w/ Browning*		
		Q155-3	Atlantic	Selfed
2-17	4n	25	9	85
2-32	4n	- **	52	-
2-40	4n	52	17	62
2-41	4n	56	24	58
2-43	4n	-	33	72
2-60	4n	-	-	17
11-10	4n	-	33	-
11-17	4n	-	17	92
11-24	4n	-	-	68
11-25	4n	-	31	-
16-29	4n	-	-	88

* MEBA ≤ 62% transmittance at OD 470

** No Data

1.5 Summary and Discussion

The four somaclones of set I backcrossed to the cv. Atlantic (M502, M509, M514 and M515) produced a high percentage of progenies with B droplets in 1990, confirming the 1989 data reported by Lentini et al. (1991). The percentage of plants with B droplets in these backcrossed-somaclones was 10 to 20 times more (22% to 44%) than the backcrossed somaclones from set II (M503, M504 and M505) and the unregenerated F743-4 hybrid (M501) of set III, which had only 0 to 5% of their progenies with the B droplets. Previous work done by Kalazich (1989) using conventional breeding methods to introgress trichome traits into the cultivated potato resulted in a range of only 1 to 15% with an average of 9% of the progenies with B droplets in the *Tuberosum*-backcrossed hybrids.

The ability to transmit a high percentage of backcross offspring with B droplets was conserved in BC_2 in only four clones. All of these were in the lineage of the somaclones which transmitted a high percentage of B droplets in BC_1 (Set I). In the BC_3, the ability to transmit a high percentage (29% and 47%) of droplets was conserved in only two progenies and at intermediate levels (6% to 19%) in seven more. All nine were derived from the four somaclones in set I. None of the backcrosses related to the somaclones which did not transmit a high frequency of B droplets (Set II), nor to the original clone, F743-4, produced progenies with many B droplets.

The somaclone 4038 was the most productive of the four original selections. One desirable clone which transmits high frequency of B

dsroplets to its progenies was recovered in the first backcross (M514-1). When the second backcross was made using it as the parent, four clones were identified which possessed the ability to transmit an elevated percentage of offspring with B droplets. One of the selfed seedlings (N314-17) of M514-1 also produced the desired trait when it was backcrossed. One desirable clone was recovered in the first backcross of 4020. It did not produce any BC_2 offspring. One of its siblings which produced only a low frequency of backcross offspring with droplets, produced both of the BC_2 parents which expressed the highest frequency of offspring with droplets in the BC_3. This was also true of N317-6 which was recovered from a BC_2 progeny which was not notable in its expression of this trait. The BC_2 seedling, N304-1, produced an intermediate level of expression. It came from a selfed seedling which did not produce any offspring with droplets in BC_2. A sibling of it did produce a high frequency of BC_2 seedlings with droplets.

In all cases, the backcrosses which produced the higher frequency of droplets were made using clones as nonrecurrent parents which had droplets. Even within set I, 17 of the 20 clones without droplets that were used in further backcrosses failed to produce any progeny with B droplets. Of the 32 clones with droplets used in backcrossing, only 13 failed to produce some droplets in the offspring.

Kalazich et al. (1991) showed that intercrosses among siblings without droplets would recover a high frequency of droplets in the progeny. The same result is evident in the selfed progenies of this study. The mean percentage of plants with droplets in selfed progenies in set I in which the parent plant had droplets was 89%. When the parent plant did

not have droplets, the percentage of selfed offspring with droplets was 85%.

These results confirm that genetic changes generated during callus culture in the four somaclones (4001, 4029, 4038 and 4040) which resulted in increased frequency of the transmission of droplets on type B trichomes in backcrosses is conserved, after three generations of backcrossing to *S. tuberosum*. The pattern of inheritance does not give a clear explanation of the expression of the trait. It is possible to make the following conclusions: a) The trait exists only in the progeny of the four selected somaclones. It does not exist in other somaclones which did not initially express the trait, nor in the parent hybrid clone; b) the trait appears to exist only in those backcross clones which have droplets. Siblings without droplets did not conserve the trait; and c) the trait does not appear to influence the expression of B droplets in selfed progeny, but it is conserved and can be detected in backcrosses of selfed seedlings.

Bonierbale et. al.(1991, unpublished report) at Cornell University using restriction fragment length polymorphisms (RFLP) for genetic mapping of the factors controlling the trichome traits from *S. berthaultii* , identified four quantitative trait loci (QTL) on chromosomes 1, 2, 5 and 6 for sucrose ester levels from the droplet of type B trichomes. One of these QTL's controls the presence or absence of B droplets. Mehlenbacher et al. (1983, 1984) reported that the expression of type B trichomes is controlled by a few genes while Gibson (1971) claimed that at least two genes are necessary to explain the segregation observed for the presence of droplets on type B trichomes in interspecific crosses between *S. phureja* x *S. berthaultii* . It is possible then that the presence of B

droplets is governed by one or a few recessive genes and during the regeneration of the four somaclones, a structural change might have taken place which resulted in gene duplication or in a chromosomal rearrangement that favored recombination resulting in higher frequency of progenies with the B droplets.

Mean comparisons show a difference in the observed expression of droplets on type B trichomes as determined by the recurrent *Tuberosum* parent used. Backcrossing to the clone Q155-3 resulted in a higher percentage of progenies with the B droplets as compared to backrossing to the cv. Atlantic. Lentini (1989) reported that *Tuberosum* genes might have some effect on the expression of droplets and/or browning genes. Kalazich (1989) also reported that the choice of *Tuberosum* parent is important since he claimed that H266-2 was chosen as a backcross parent for its trichome traits, rather than its yielding ability. He further stated that "the negative results for agronomic characters and the positive results for trichome characters obtained with this backcross parent have clearly indicated the great importance of choosing the right parents for both group of characters". In wheat, Kovacs et al. (1986) and Multani et al. (1988) found ample evidence that the expression of an alien gene is greatly affected by the host genome.

In contrast, the expression of good browning on type A trichomes seems to be less complicated and simpler than the B droplets. In general, the percentage of progeny with good browning activity was higher in clones from set I (somaclones which transmit many B droplets). This result corroborates previous results of Kalazich (1989) in which he found a significant association between the presence of type B droplets and

segregation of the PPO enzyme in type A trichomes in backcross generations. Furthermore, Lentini(1989) reported a similar pattern, when she observed that the backcrossed families (M502, M514 and M515) with the highest droplet frequencies in the greenhouse produced a larger number of plants with browning. Just recently, Bonierbale et al. (1991, unpublished report) in their genetic mapping using RFLP's found a possible linkage between a locus associated with the type A trichomes and another locus associated with levels of sucrose esters exudate on the type B trichomes. This might explain previous observed associations between these two trichome traits. The good browning in type A trichomes is conserved in backcrossing, provided the B droplets were present.

Results from the study also show that backcrossed progenies of the somaclones which have good browning activity and possess B droplets have poor vine type. In contrast, progenies without the B droplets and having poor browning activity had the most desirable *Tuberosum* vine type. Some clones which have fairly acceptable vine type (i.e. intermediate between the *Tuberosum* and *Berthaultii* or better) had good browning but do not have the B droplets. These results could mean that gene(s) responsible for the wild or poor vine type are still linked with the gene(s) that control the presence of droplets on type B trichomes and the browning reaction even after the regeneration process. This is also supported by the fact that better browning was observed in the progenies of set I (somaclones which transmit many B droplets). Plants without the B droplets and having poor browning were almost always of the *Tuberosum*-vine type. Kalazich (1989) established that there is a strong association between the presence of the type B trichomes exudate

(droplet) and undesirable agronomic characteristics (including vine type) of the wild species parent. This was evident in backcrosses where plants bearing the B droplets had a wilder foliage, later maturity and poorer tuber appearance than their sibs without the B droplets. He attributed these to linkage or to a structural genomic differentiation between the genomes involved. Lentini (1989) also reported that the genetic change that might have occurred during the callus phase of F743-4 which enhanced the recombination between the cultivated *Tuberosum* and the *Berthaultii* had only a partial effect because she found plants with better trichome traits and insect resistance but still had a Berthaultii-like vine type. The objective of developing clones which possess good trichome traits for both type A and type B, with acceptable agronomic traits, was not fully resolved with three generations of backcrossing using these special somaclones which transmit high frequency of B droplets in their backcrossed progenies. However, some clones which have very good browning activity and good vine type but do not have the B droplets were identified. Further studies such as genetic mapping based on molecular markers (RFLP's) being done by Bonierbale et al. (1991) are necessary to better understand the strong association between type B trichome traits and agronomic traits (such as wild vine type) and for more effective introgression of these trichome-mediated resistant traits into the cultivated potato.

Two glycoalkaloids, solamargine and solasonine not commmonly found in cultivated commercial varieties have been detected in most clones from the progenies of backcrossed-somaclones. The amount of the other two glycoalkaloids, chaconine and solasonine were found to be

generally higher in clones from the families of backcrossed-somaclones which exhibited high B droplets than the clones in set II and III.

To determine whether diploid F_1 hybrids between *S. berthaultii* and haploid *S. tuberosum* are better starting materials for the generation of somaclones with increased recombination, some diploid F1 hybrid-derived somaclones were evaluated for their trichome traits and the best clones were selected and backcrossed to *Tuberosum*. Except for #11, all the diploid F1 hybrid-derived somaclones evaluated did not show any good trichome traits. However, when some tetraploids from the same source were successfully backcrossed to *Tuberosum*, their progenies segregated for both trichome traits (browning potential and the presence of B droplets). The same result was found by Lentini (1989) in her studies where she observed better trichome expression in the sexual progeny of somaclones but not in the somaclones themselves.

The production of backcross progenies of F1 hybrid somaclones was successful in only a few tetraploids. Fruit setting was very poor or impossible in most diploid somaclones in spite of their good pollen production and viability as determined from a simple pollen viability staining test done in some entries. Reciprocal crosses using the cv. Atlantic or the clone Q155-3 were then conducted in some clones which had good pollen production. Fruit setting was only successful in tetraploid somaclones. All crosses in which the *Tuberosum* clones were used as female resulted in complete disappearance of the B droplets in the backcrossed progenies. A few plants had good browning potential. In the first backcrossed generation of diploid F_1 hybrid-derived somaclones, the percentage of progenies with B droplets and with good browning activity

was generally lower as compared to the tetraploid advanced hybrid-derived somaclones presented earlier. Selfing produced a higher percentage of progenies with the B droplets as compared to the backcrosses; a similar pattern was observed in the tetraploid hybrid-derived somaclones. In general, better trichome traits expression and better success in pollination were obtained from the tetraploid advanced hybrid-derived somaclones than the diploid F_1 hybrid-derived somaclones. In this case, the difference in ploidy level of the progenitors is a factor in the success of pollination. Using the diploids as recurrent male parent, was not a good strategy since it resulted in loss of the trichome traits. Another negative aspect of using diploid F_1 hybrids was their poor vine type. In general, first backcrossed progenies regardless of the ploidy level of their somaclone parents, looked wilder than the progenies of the F743-4 hybrid-derived somaclones.

General Conclusions

The use of somaclones in a backcross program.

1) The rare ability of the four selected somaclones - M502, M509, M514 and M515 - to pass on the trait for the presence of droplets on type B trichomes to their backcrossed progenies in a high frequency is conserved after three generations of backcrossing.

2) The trait (high frequency of B droplets) does not exist in other somaclones which did not initially express the trait, nor in the unregenerated parent hybrid clone, F743-4.

3) The trait appears to exist only in those backcross clones which have B droplets. Siblings that do not possess B droplets did not conserve the trait.

4) The genotype of the recurrent parent (i.e. Q155-3 or cv. Atlantic) had an effect in the expression of droplets on type B trichomes during backcrossing. Backcrossing to the clone Q155-3 resulted in higher percentage of progenies with B droplets than backcrossing to the cv. Atlantic.

5) Backcrossed families of the four somaclones which transmit high frequency of B droplets had a higher percentage of progenies with good browning activity than the backcrossed families of somaclones which transmit a few B droplets.

6) Wild vine type (*Berthaultii*-type) is associated with the expression of both type A and type B trichome traits (good browning activity and the presence of B droplets, respectively). Backcrossed clones with B droplets and good browning activity had poorer vine type than clones without B droplets and poor browning activity. Clones with good

browning and without the B droplets had good vine type, closer to the desired *Tuberosum*-type.

7) It appears that the association between the presence of B droplets and poor vine type has not been broken by the process of regeneration nor the continous backcrossing of the somaclones to *Solanum tuberosum*. A better understanding of the association or the linkage that exists between these traits is necessary to be able to come up with the desired recombinations.

8) Several clones with good trichome traits and intermediate vine types have been selected for further evaluation and use in the potato breeding program.

Influence of temperature on the expression of trichome traits.

1) Higher temperature (32°C) significantly increased the density of type A and type B trichomes both on the adaxial and abaxial leaf surfaces of most genotypes evaluated. The higher density observed was attributed to the decrease in leaf area of the plants grown at higher temperature condition.

2) The browning activity of type A trichomes which was correlated with the total trichome density was significantly increased in the higher temperature condition. The better browning activity observed in plants at higher temperature could be the result of higher trichome density per given area and not necessarily caused by increased production of trichome exudates.

3) Significant genotype differences were observed in all the parameters evaluated with the hybrids showing wide variations in terms of their trichome traits, some performing better than their wild parent, *Solanum berthaultii.*

Influence of light intensity on the expression of trichome traits

1) Light intensity significantly affected the expression of both type A and type B trichomes as well as plant growth.

2) The adaxial and abaxial densities of type A and type B trichomes were significantly decreased with lower irradiance (higher degree of shading). Significant differences were observed among the genotypes especially on the expression of type B trichome traits, where some hybrids lost their B droplets at the highest extent of shading.

3) Light intensity also affected the MEBA values. The browning activity and PPO level of plants grown at high light intensity (unshaded) were superior to the same measurements of the shaded plants. The amount of sucrose esters secreted by type B trichomes, however, was not significantly affected.

4) The significant effects of shading on the expression of trichome traits could be attributed to the low level of irradiance that affected plant growth and physiological processes in the plants such as photosynthesis, which altered the expression or the development of the glandular trichomes.

5) The significant effects of environmental factors such as temperature and light intensity, on the expression of trichome traits, make

it important that right conditions are observed during selection and evaluation for trichome traits.

Appendix 1. Composition of the Cornell mix used in the study.

3 bales	Peatmoss (4 cu. ft. bale)
4 bags #2	Vermiculite (6 cu. ft.)
14 lbs	Dolomitic lime
3 lbs	0-20-0
3/4 lbs	calcium nitrate
5 oz.	FTE (trace elements)
1 lb	Iron sulphate
3 oz.	wetting agent
20 lbs.	Osmocote (14-14-14)

Appendix 2. Browning activity and vine type of BC_1 families of F743-4 and its somaclones .

Code Pedigree	Group	Mean/Group MEBA*	Vine Type**
I. Somaclones which transmit many B droplets			
M502 (4001 x Atlantic)	+ B, good MEBA	34.3	3.2
	- B, good MEBA	42.9	4.1
	+ B, poor MEBA	78.6	4.0
	- B, poor MEBA	81.2	4.9
M509 (4029 x Atlantic)			
	+ B, good MEBA	28.4	3.6
	- B, good MEBA	37.5	4.4
	+ B, poor MEBA	69.7	4.6
	- B, poor MEBA	74.2	5.0
M514 (4038 x Atlantic)			
	+ B, good MEBA	29.7	3.1
	- B, good MEBA	35.1	3.9
	+ B, poor MEBA	66.2	3.6
	- B, poor MEBA	79.3	5.0
M515 (4040 x Atlantic)			
	+ B, good MEBA	28.9	3.2
	- B, good MEBA	29.5	3.5
	+ B, poor MEBA	82.1	3.7
	- B, poor MEBA	89.8	4.8
II. Somaclones which transmit few B droplets			
M503 (4002 x Atlantic)			
	+ B, good MEBA	- ***	-
	- B, good MEBA	59.7	4.6
	+ B, poor MEBA	68.3	4.6
	- B, poor MEBA	85.1	5.0
M504 (4004 x Atlantic)			
	+ B, good MEBA	53.4	3.7
	- B, good MEBA	57.3	4.7
	+ B, poor MEBA	-	-
	- B, poor MEBA	91.5	5
M505 (4009 x Atlantic)			
	+ B, good MEBA	52.7	3.4
	- B, good MEBA	59.3	3.9
	+ B, poor MEBA	-	-
	- B, poor MEBA	83.9	5.0

Appendix 2. (Continued...)

Code Pedigree	Group	Mean/Group MEBA*	Vine Type**
III. Unregenerated Hybrid			
M501 (F743-4 x Atlantic)			
	+ B, good MEBA	-	-
	- B, good MEBA	49.6	4.5
	+ B, poor MEBA	-	-
	- B, poor MEBA	77.2	5.0

* MEBA ≤ 62 % Transmission at OD 470

* * Vine Type : 1= *Berthaultii*-type, 5 = *Tuberosum*-type

*** (-) No clone classified under the group

Appendix 3. Browning activity and vine type of some BC_2 families of F743-4 and its somaclones.

Code Pedigree	Group	Mean/Group MEBA*	Vine Type**
I. Somaclones which transmit many B droplets			
M502-6 x Q155-3	+ B, good MEBA	37.5	3.5
	- B, good MEBA	49.3	4.2
	+ B, poor MEBA	- ***	-
	- B, poor MEBA	79.6	4.8
M502-6 selfed			
	+ B, good MEBA	32.4	3.3
	- B, good MEBA	38.1	4.3
	+ B, poor MEBA	65.2	3.8
	- B, poor MEBA	80.8	5.0
M502-104 x Q155-3			
	+ B, good MEBA	45.2	3.8
	- B, good MEBA	39.6	4.5
	+ B, poor MEBA	74.7	4.6
	- B, poor MEBA	86.4	5.0
M509-4 x Q155-3			
	+ B, good MEBA	30.4	3.1
	- B, good MEBA	46.1	4.5
	+ B, poor MEBA	75.9	4.7
	- B, poor MEBA	84.3	5.0
M509-4 selfed			
	+ B, good MEBA	34.0	3.3
	- B, good MEBA	40.3	3.9
	+ B, poor MEBA	-	-
	- B, poor MEBA	70.5	4.7
M509-103 x Q155-3			
	+ B, good MEBA	41.9	4.1
	- B, good MEBA	44.1	3.8
	+ B, poor MEBA	-	-
	- B, poor MEBA	78.2	4.8
M514-1 x Q155-3			
	+ B, good MEBA	32.7	3.2
	- B, good MEBA	48.2	3.6
	+ B, poor MEBA	63.5	4.3
	- B, poor MEBA	77.8	5.0
M514-1 selfed			
	+ B, good MEBA	34.1	3.1
	- B, good MEBA	46.5	3.9
	+ B, poor MEBA	-	-
	- B, poor MEBA	68.3	4.2

Appendix 3. (Continued...)

Code Pedigree	Group	Mean/Group MEBA*	Vine Type**
M514-2 x Q155-3			
	+ B, good MEBA	42.2	3.9
	- B, good MEBA	44.6	4.2
	+ B, poor MEBA	67.4	3.9
	- B, poor MEBA	79.3	4.7
M514-2 selfed			
	+ B, good MEBA	33.5	3.6
	- B, good MEBA	45.1	4.0
	+ B, poor MEBA	-	-
	- B, poor MEBA	72.8	4.8
M514-111 x Q155-3			
	+ B, good MEBA	36.4	3.4
	- B, good MEBA	48.2	4.5
	+ B, poor MEBA	74.3	4.5
	- B, poor MEBA	85.5	5.0
M514-111 x Atlantic			
	+ B, good MEBA	48.3	4.1
	- B, good MEBA	42.7	4.4
	+ B, poor MEBA	-	-
	- B, poor MEBA	88.1	5.0
M514-111 selfed			
	+ B, good MEBA	30.6	3.1
	- B, good MEBA	48.8	3.8
	+ B, poor MEBA	-	-
	- B, poor MEBA	77.0	4.8
M515-5 x Q155-3			
	+ B, good MEBA	40.4	3.3
	- B, good MEBA	43.7	4.1
	+ B, poor MEBA	-	-
	- B, poor MEBA	82.5	5.0
M515-101 x Q155-3			
	+ B, good MEBA	39.7	3.2
	- B, good MEBA	40.8	4.2
	+ B, poor MEBA	-	-
	- B, poor MEBA	81.5	5.0
M515-101 selfed			
	+ B, good MEBA	30.8	3.3
	- B, good MEBA	42.7	3.6
	+ B, poor MEBA	-	-
	- B, poor MEBA	77.8	4.6

Appendix 3. (Continued...)

Code Pedigree	Group	Mean/Group MEBA*	Vine Type**
II. Somaclones which transmit few B droplets			
M503-2 x Q155-3			
	+ B, good MEBA	49.5	4.6
	- B, good MEBA	59.6	4.5
	+ B, poor MEBA	73.4	4.5
	- B, poor MEBA	88.4	5.0
M503-2 selfed			
	+ B, good MEBA	39.7	4.1
	- B, good MEBA	51.3	4.1
	+ B, poor MEBA	79.5	4.2
	- B, poor MEBA	84.0	4.8
M504-1 x Q155-3			
	+ B, good MEBA	45.1	4.2
	- B, good MEBA	45.8	4.8
	+ B, poor MEBA	74.0	4.4
	- B, poor MEBA	89.2	5.0
M504-1 selfed			
	+ B, good MEBA	41.7	4.3
	- B, good MEBA	56.2	4.8
	+ B, poor MEBA	81.2	4.8
	- B, poor MEBA	90.4	5.0
M504-5 x Q155-3			
	+ B, good MEBA	36.9	3.9
	- B, good MEBA	59.2	4.4
	+ B, poor MEBA	-	-
	- B, poor MEBA	87.4	5.0
III. Unregenerated Hybrid			
M501-110 selfed			
	+ B, good MEBA	46.3	4.2
	- B, good MEBA	53.2	4.7
	+ B, poor MEBA	89.1	5.0
	- B, poor MEBA	93.2	5.0

* MEBA ≤ 62 % Transmission at OD 470

** Vine Type : 1= *Berthaultii*-type, 5 = *Tuberosum*-type

*** (-) No clones classified under the group

Appendix 4. Browning activity and vine type of some selected BC3 families of F743-4 and its somaclones from set I.

Pedigree	Group	Mean/Category	
		MEBA*	Vine Type**
N304-6 x Q155-3	+ B, good MEBA	- ***	-
	- B, good MEBA	34.7	5.0
	+ B, poor MEBA	63.0	4.0
	- B, poor MEBA	64.0	5.0
N306-4 x Q155-3			
	+ B, good MEBA	29.5	3.5
	- B, good MEBA	45.3	4.7
	+ B, poor MEBA	74.5	5.0
	- B, poor MEBA	95.2	5.0
N308-1 x Q155-3			
	+ B, good MEBA	12.7	4.0
	- B, good MEBA	17.0	5.0
	+ B, poor MEBA	-	-
	- B, poor MEBA	83.3	5.0
N308-5 x Q155-3			
	+ B, good MEBA	31.4	3.8
	- B, good MEBA	47.4	3.9
	+ B, poor MEBA	82.6	4.7
	- B, poor MEBA	85.5	5.0
N308-7 x Q155-3			
	+ B, good MEBA	50.0	3.5
	- B, good MEBA	29.5	4.5
	+ B, poor MEBA	-	-
	- B, poor MEBA	78.2	4.8
N312-3 x Q155-3			
	+ B, good MEBA	-	-
	- B, good MEBA	46.5	4.5
	+ B, poor MEBA	91.0	5.0
	- B, poor MEBA	-	-
N312-14 x Q155-3			
	+ B, good MEBA	24.2	3.4
	- B, good MEBA	32.6	4.0
	+ B, poor MEBA	-	-
	- B, poor MEBA	78.7	4.8
N312-32 x Q155-3			
	+ B, good MEBA	-	-
	- B, good MEBA	44.2	4.8
	+ B, poor MEBA	-	-
	- B, poor MEBA	70.0	4.3
N312-38 x Q155-3			
	+ B, good MEBA	34.0	4.0
	- B, good MEBA	36.8	4.5
	+ B, poor MEBA	-	-
	- B, poor MEBA	74.0	5.0

Appendix 4. (Continued...)

Pedigree	Group	Mean/Category	
		MEBA*	Vine Type**
N312-45 x Q155-3			
	+ B, good MEBA	10.1	2.5
	- B, good MEBA	26.4	4.6
	+ B, poor MEBA	-	-
	- B, poor MEBA	-	-
N312-46 x Q155-3			
	+ B, good MEBA	-	-
	- B, good MEBA	33.9	4.0
	+ B, poor MEBA	81.0	4.0
	- B, poor MEBA	85.0	4.9
N314-5 x Q155-3			
	+ B, good MEBA	-	-
	- B, good MEBA	24.9	4.1
	+ B, poor MEBA	-	-
	- B, poor MEBA	81.8	4.8
N314-17 x Q155-3			
	+ B, good MEBA	-	-
	- B, good MEBA	35.1	4.4
	+ B, poor MEBA	78.0	3.0
	- B poor MEBA	87.5	4.6
N317-6 x Q155-3			
	+ B, good MEBA	19.4	3.2
	- B, good MEBA	38.3	4.3
	+ B, poor MEBA	71.7	4.0
	- B, poor MEBA	79.8	4.7

* MEBA ≤ 62 % Transmission at OD 470

** Vine Type : 1= *Berthaultii*-type, 5 = *Tuberosum*-type

*** (-) No clones classified under the group

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