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QTL analysis of trichome-mediated insect resistance in potato

M. W. Bonierbale*, R. L. Plaisted, O. Pineda, S. D. Tanksley

Department of Plant Breeding and Biometry, 252 Emerson Hall, Cornell University, Ithaca, NY 14853, USA

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Abstract. Genetic mapping of several components of a complex type of insect resistance has been undertaken as a means toward more efficient use of the valuable characteristics of a wild relative of potato. RFLP maps constructed on interspecific diploid progenies of *Solanum tuberosum* × *S. berthaultii* were used in conjunction with morphological, biochemical and biological phenotyping to identify quantitative trait loci (QTLs) contributing to trichome-mediated insect resistance. By superimposing QTL data for a wide range of phenotypes including biochemical assays, correlative and direct screens for insect resistance, and adaptation to the target environment on the genetic maps, we have addressed the organization, action and interaction of genes controlling the resistance mechanism. The outcome contributes to an understanding of the association between component traits and between desirable and undesirable features of the donor species generated in an applied breeding program. Research is proceeding toward the development of selectable markers for the introgression and transfer of this resistance among potato gene pools.

Key words: Potato – Trichome – Insect resistance – RFLP – QTL

Introduction

A valuable type of insect resistance from the wild Bolivian species *Solanum berthaultii* is associated with the presence of glandular trichomes on the foliage, and some components of this resistance have been introgressed into the cultivated potato (Plaisted 1985; Plaisted et al. 1992).

After Radcliffe and Lauer (1968) recognized aphid and leafhopper resistance in *S. berthaultii*, Gibson (1971) and Tingey and Sinden (1982) attributed differences in levels of resistance among accessions of the species to variability in types and densities of glandular trichomes. In recent years, trichome-mediated resistance to a wide range of insects, including the Colorado potato beetle, aphids, leafhoppers, flea beetles, leaf miner flies and the potato tuber moth, as well as mites, has been confirmed in *S. berthaultii* and its hybrids with the cultivated potato, *S. tuberosum* (reviewed by Gregory et al. 1986; Tingey 1991).

Plants of *S. berthaultii*, and the interspecific populations investigated in the present study, have two types of glandular secretory trichomes, the products of which have been described previously (King et al. 1987; Kowalski 1989; Neal et al. 1990; Steffens et al. 1990). The shorter type-A trichomes have a tetralobulate membrane-bound gland at the tip. When the gland is mechanically broken, component oxidases come into contact with phenolic substrates in a 'browning reaction', and the resulting compound accumulates on the body parts of the predator. The taller type-B trichomes produce droplets of a sucrose ester exudate that are continually renewed. Products of the two types of trichomes act both alone and synergistically to cause physical and chemical deterrence (resulting in host avoidance and restlessness), entrapment, reduced feeding, digestive disorders, and/or reduced reproductive performance. Trichomes of other members of the *Solanaceae*, such as *Solanum neocardenasii* (Dimock et al. 1986; Lapointe and Tingey 1986), *S. polyadenium*

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* *Present address:* CIAT, AA 6713, Cali, Colombia
Correspondence to: R. L. Plaisted

(Tingey and Gibson 1978), *S tarijense* (Gibson 1971), *Lycopersicon pennellii* (Goffreda et al. 1989), and *Nicotiana tabacum* L. (Cutler et al. 1986), also produce exudates that affect insect behavior and biology.

The diverse impacts of this complex resistance on several pests indicate that trichome-mediated host plant resistance is likely to be a stable means of crop protection. Franca (1991) reared Colorado potato beetles (CPB; *Leptinotarsa decemlineata* Say) continuously on *S. berthaultii* for ten generations and did not detect a decrease in the effectiveness of the resistance.

In the Cornell University potato breeding program, hybridizations between potato cultivars and the diploid *S. berthaultii* were initiated 15 years ago. Tetraploid progenies resulted, due to the functioning of unreduced gametes (2n pollen) from the wild parent. The goal was to combine trichome traits from the short-day-requiring donor species with adaptation for tuberization in temperate climates. Inheritance of trichome-mediated insect resistance in potato is quantitative, with each of the several contributing factors appearing to be controlled by a small number of genes (Mehlenbacher et al. 1983). Biochemical assays have been developed for each of the trichome exudates and these are used as correlative screens for insect resistance in several breeding programs.

Major incentives for the introgression of this host plant resistance into cultivated potatos include the cost of alternate control measures, the development of resistance to pesticides, and the environmental hazards associated with chemical control. Resistance to Colorado potato beetle from *S. berthaultii* is primarily associated with the presence of defensively-active type-A trichomes, though the exudate of type-B trichomes increases the expression of resistance (Neal et al. 1989). A very good level of resistance to oviposition and feeding by this pest has been achieved by selecting within segregating populations independently for tuber type and field resistance, in alternating cycles with the evaluation of type-A trichome characteristics (Plaisted et al. 1992). Entomologists are currently researching the nature of the factor(s) responsible for CPB resistance, which are not yet fully understood (Franca 1991; Tingey 1991).

Despite the success in transferring type-A trichome-mediated resistance into advanced selections, it has still not been possible to select clones with acceptable horticultural type and good expression of the type-B trichome properties, which would add resistance to small bodied insects to the adapted material. *S. berthaultii* requires short days to initiate tuberization. Unfortunately, in segregating generations, clones with the best type-B trichome traits have been the most poorly adapted to temperate climates. This association suggests either restricted genetic exchange or linkage between the desirable and undesirable characteristics of the donor species (Kalazich and Plaisted 1991).

We report here the use of restriction fragment length polymorphisms (RFLPs) for genetic mapping of quantitative trait loci (QTLs) controlling the trichome traits and insect resistance from *S. berthaultii*. Diploid backcrosses to both the wild and the cultivated species parents have been evaluated for factors contributing to the resistance mechanism, including trichome density, polyphenol oxidase and sucrose ester production by the type-A and -B trichomes, and the enzymatic browning reaction. Consumption and oviposition assays with the Colorado potato beetle and assays for tuberization under varied daylength regimes have been conducted with the same plant material. Genetic maps have been developed for these progenies, and QTLs for the trichome traits have been identified. Genetic markers for these traits will be useful in the transfer of the effective wild chromosomal segments into and among tetraploid potatoes, and for a better understanding of the resistance mechanism.

Materials and methods

Plant material

Diploid progenies segregating for trichome properties were developed by first crossing a dihaploid (2n = 2x = 24) *Solanum tuberosum* clone (USW 2230) with an accession (PI473331) of *S. berthaultii*. One of the resulting hybrid clones (TB) was then crossed as a female to both *S. berthaultii* (BB) and *S. tuberosum* (II;2x) to generate two backcross families, BCB and BCT, as shown in Fig. 1. Seeds from each of the two backcross families were soaked over night in 2000 ppm of gibberellic acid and sown. Three hundred seedlings of each progeny were later transplanted to 18-cm pots of peat-lite mix under artificial light in the greenhouse.

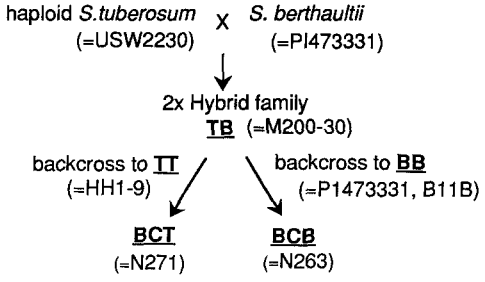

Fig. 1. Scheme of the development of the diploid mapping populations, BCB and BCT. Origin of potato clones involved in the pedigrees are: USW2230 = dihaploid (2x = 2n = 24) derived from tetraploid cv Saco (4x = 2n = 48); P1473331 = USDA accession of *S. berthaultii*; HH1-9 = selected for male fertility from an intermated population of *S. tuberosum* haploids (Sanford and Hanneman 1982); B11B = clone of USDA accession PI473331 selected for type-A and type-B trichome properties

Screening for trichome phenotypes

Foliar samples for the evaluation of each trichome phenotype were taken from approximately the fourth node below the apex of the greenhouse-grown plants just before or at the time of flowering. The average greenhouse temperature during the experiments was 26 °C with a daylength of 16 h.

The densities of type-A and type-B trichomes were determined by counting the number of each type in two 3.2-mm^2 areas of each leaf surface (upper and lower) at 60 x magnification with a stereomicroscope equipped with an ocular grid. Two quantitative biochemical assays were used to determine the type-A trichome properties of each clone:

(1) The modified enzymatic browning assay (MEBA) (Avé et al. 1986) is indicative of the levels of oxidases (primarily polyphenol oxidase) and phenolic substrates in the type-A trichome glands, and of trichome density. Samples containing the products of this oxidative browning reaction are taken from a standard leaf area by pressing a filter paper disk against both leaf surfaces and placing the disk into buffer in a test tube. The result is a spectrophotometric reading of the percent of light transmitted through the samples, measuring the amount of browning that would be caused when an insect ruptures the trichomes. When this assay is used as a correlative screen for insect resistance in the potato variety development program, clones with $\leqq 62\%$ transmission are considered to carry resistance (Kalazich and Plaisted 1991).

(2) Enzyme-linked immunosorbant assays (ELISA) were conducted to determine the concentration of polyphenol oxidase (PPO) in the type-A glands of each clone, according to the methods of Kowalski (1989). For this assay the contents of a standard number of type-A trichome heads are collected from each plant with a capillary tube and placed in buffer in a microtiter plate for quantification by reaction with antiserum to PPO and an enzyme conjugate. The result is a spectrophotometric reading of optical density which can be compared with samples of *S. berthaultii*.

Properties of the type-B trichomes were also evaluated with two assays, one qualitative and the other quantitative:

(1) The presence or absence of sucrose droplets on the type-B trichomes was evaluated visually under the same conditions as the density determinations.

(2) Nelson's (1944) glucose assays, adapted by Goffreda et al. (1989), were used for the quantification of the sucrose esters exuded from the type-B-trichomes as droplets. For this assay, termed FASE for the fatty acids of sucrose esters it measures, methylene chloride leaf rinsates and separate samples of sucrose concentration standards are incubated with copper reagents in a colorimetric reaction. The product is read spectrophotometrically, and corrected for leaf size to determine sucrose exudation (μg) per cm.

Selective genotyping

A process of selecting the most informative individuals of a population (the tails of the phenotypic distribution) for the determination of genotype with RFLPs was presented by Lander and Botstein (1989). A variation of this procedure was used in the present study. Following the evaluation of 300 clcones from BCB for trichome properties, clones at either end of the distribution were selected independently for each characteristic. A subset of 150 clones were thus identified for subsequent genotyping with RFLPs. In separate experiments these 150 selections were evaluated again for their trichome characteristics to obtain replicated results. Since selections included the extremes for several different traits, the distribution of phenotypes of the selected individuals for each trait was not significantly altered from that of the original population.

Simultaneously, 300 clones from BCT were screened for their tuberization response to various daylength treatments (Van den Berg et al. 1992), and 150 clones representing the range of daylength reponses were selected for RFLP genotyping. The 150 selections from BCT were characterized for their type-A trichome properties, including density, browning (MEBA), and PPO concentration, in replicated experiments as described above for BCB. The backcross to *tuberosum* (BCT) does not express type-B droplets, and therefore was only evaluated for type-A trichome characteristics.

Screening for insect resistance and tuberization response to daylength

Resistance to the Colorado potato beetle was assessed in experiments designed to measure oviposition and consumption, in the Department of Entomology Insectary Greenhouse of Cornell University (G.C. Yencho, in preparation). Three male and three female newly-emerged adults from laboratory colonies were caged onto the foliage of established plants of each genotype using sleeve cages of nylon mesh. Approximately 120 clones from each cross, and ten hybrid clones including TB, were included in these evaluations. Data on the time to first egg mass deposition, the number of egg masses laid, total eggs laid, and the number of eggs per mass were collected every 4 days for a period of 3 weeks, with eggs being removed at each count. In a separate experiment using the BCB population, leaf consumption was measured in a leaf-disk assay. Summer generation adults were collected from the field. Four leaf disks from each clone were placed in separate Petri dishes, and a single starved female (24 h) was introduced. The leaf area consumed during 4 h of feeding was determined using a leaf area meter.

In complementary experiments, Van den Berg et al. (1992; and in preparation) used artificially shortened daylengths in the greenhouse to characterize BCB and BCT for tuberization. A range of daylength conditions including the short photoperiod (13 h) required for tuberization of *S. berthaultii* were provided by covering the growing area with black cloth to exclude light. Five plants of each genotype were grown for each light regime in a randomized block design. Data were taken on the number of tubers per pot and on the induction of tuberization as measured by the growth patterns of nodal cuttings under mist.

RFLP analysis and map construction

Foliar tissue samples of the parental clones and the 150 individuals selected from each progeny (BCB and BCT) were harvested over liquid nitrogen for DNA extraction. Restriction digests with *Eco*RI, *Eco*RV, *Hind* III, *Dra*I and *Xba*I were prepared according to manufacturers' instructions. Survey filters containing DNA of the parental clones digested with each restriction enzyme, and progeny filters for each cross with each enzyme were made with Hybond N+™ membranes, following electrophoresis of the respective DNA samples as previously described (Bernatzky and Tanksley 1986). Filters were probed with DNA clones from tomato, based on previous mapping in potato (BCB) (Tanksley et al. 1992). Two framework maps with approximately 80 markers each were constructed with MAPMAKER software (Lander et al. 1987), based on the segregation of RFLPs from the F_1 hybrid parent in each backcross progeny. These framework maps represent a subset of a larger number of markers on the molecular map of potato (Tanksley et al. 1992).

Due to the heterozygous (cross-pollinating) nature of the parental clones used in these experiments, segregating alleles from both parents contribute to the genetic variation of progenies. Thus two maps were constructed for each backcross progeny – one based on the segregation from the hybrid parent

(TB) and the other based on the respective recurrent parents (BB or TT). The framework maps, based on segregation from the hybrid parent, are designated 'F1' maps for BCB and BCT and consist of 80 and 70 loci respectively at average intervals of 10 cM. Segregation from the recurrent parents was limited by their levels of heterozygosity, and therefore maps of BCB and BCT based on recombination in BB and TT consist of only 45 and 35 markers, respectively.

Data transformation

According to the frequency distributions of the phenotypic data of progeny individuals, transformations were performed to approximate normality and equalize variances prior to investigating correlations with RFLP data. The *logit* scale (log p/q, where $p\%t$ and $q\%1 - \%t$) was used to correct data from the MEBA test (% transmission is browning reaction, type-A trichomes). The optical densities (corrected for buffer) from the PPO ELISAs (type-A trichomes) were used directly without transformation or conversion to percent of BB. Transformation to $\log(FASE + 2)$ was used to correct for the highly-skewed distributions of FASE assays (sucrose concentration, type-B droplet); and square roots were taken to equalize variances of the trichome density (count) data and the CPB egg laying data for BCB.

QTL mapping

Correlations between the genotypic data (RFLP classes at mapped loci within each cross) and phenotypic data for trichome characters and insect resistance were determined by interval mapping with MAPMAKER/QTL (Lincoln and Lander 1989) and by analysis of variance using markers as treatments. Interval mapping is an analytical method which localizes the effect of a quantitative trait locus (QTL) between pairs of linked genetic markers. Phenotypic data for each trait from separate (replicated) experiments were analyzed separately in the MAPMAKER/QTL program, and the results compared. A threshold of LOD = 2 was set for declaring an interval significant in a QTL model.

In cases for which LOD scores indicated a maximum likely location within an interval on the framework maps to which additional markers have been mapped in the same cross BCB, regressions were performed to determine which marker on the higher density map of potato is closest to the putative QTL. Genome ratios and genotypic values of QTLs were determined with Hypergene™ and the concept of graphical genotypes (Young and Tanksley 1989).

Results

RFLP maps of the tuberosum-berthaultii hybrids

Monogenic ratios. In the development of each of the F1 maps, two genotypes were identified at each locus (BB, with both alleles of *berthaultii* origin, and TB, the hybrid combination, in BCB; and TT and TB in BCT), and these genotypic differences were used to test the importance of each locus in determining the progenies' phenotypes. For the majority of loci screened in BCB, monogenic ratios fit the 1:1 (BB:TB) ratio expected for a backcross population. Regions of *chromosomes 1, 3* and *8* each exhibited significant ($P < 0.01$) skewing toward BB genotypes (such as 100 BBs and 50 BTs at *TG411* on *chr 3*), and a region of *chromosome 4* was skewed toward the heterozygous class, TB. Areas exhibiting significant skewing are indicated on the chromosome maps of Fig. 2. A greater amount of skewing from the expected 1:1 (TT:BT) ratio was observed throughout the genome of BCT. In this backcross, regions of *chromosomes 1, 2, 3, 5, 6, 7, 8* and *10* were skewed toward the heterozygous class. For example, at *TG306* on *chr 2*, there were 50 TT individuals and 100 TBs). *Chromosomes 3, 4, 11* and *12* included regions skewed in favor of the TT class, and only *chr 9* was free of abnormal monogenic ratios.

Genome ratios. As expected for the first backcross generation after interspecific hybridization, the mean genome ratio of BCB was 75% *berthaultii* 25% *tuberosum*. The genome ratios of individuals in the population were distributed normally about 75% (± 0.07), with extremes ranging from 50% (#168) to 91% (#153) *berthaultii*. The mean genome ratio of BCT was 73% *tuberosum*: The range of ratios for this cross was 50% to 87% *tuberosum*, with some skewing toward the higher percentages of the *tuberosum* genome.

Recombination

BCB vs BCT: recombination frequencies in the (F_1) genetic maps of BCB and BCT were compared using 53 common marker intervals in a paired t-test, and found not to differ significantly from each other ($P = 0.73$). The BCB map consists of 80 markers with an average interval length of 81-cM; and for BCT, 75 markers are spaced at an average of 10-cM intervals. The total length of the framework maps of BCB and BCT (F_1) are 546 and 644 centiMorgans, respectively. These distances are comparable with earlier reported genetic maps for potato (Bonierbale et al. 1988; Gebhardt et al. 1989), but smaller than the more recent report of Gebhardt et al. (1991).

F_1 vs recurrent parents: in BCB 25 map intervals from ten chromosomes were compared for differences in recombination frequencies between the hybrid parent (TB) and the recurrent parent (BB). A paired t-test showed that recombination was significantly greater in BB than in the hybrid ($P < 0.0005$). Average recombination in BB was 1.8-fold greater than in TB, with only one interval constituting an exception.

When recombination frequencies in 20 map intervals from eight chromosomes of the BCT map were compared between the hybrid (TB) and the recurrent parent (TT) the majority showed greater recombination in TT (average of two-fold greater recombination in 13 intervals), but due to several differences in the opposite direction, a paired t-test of all intervals did not reveal highly-significant differences in overall rates

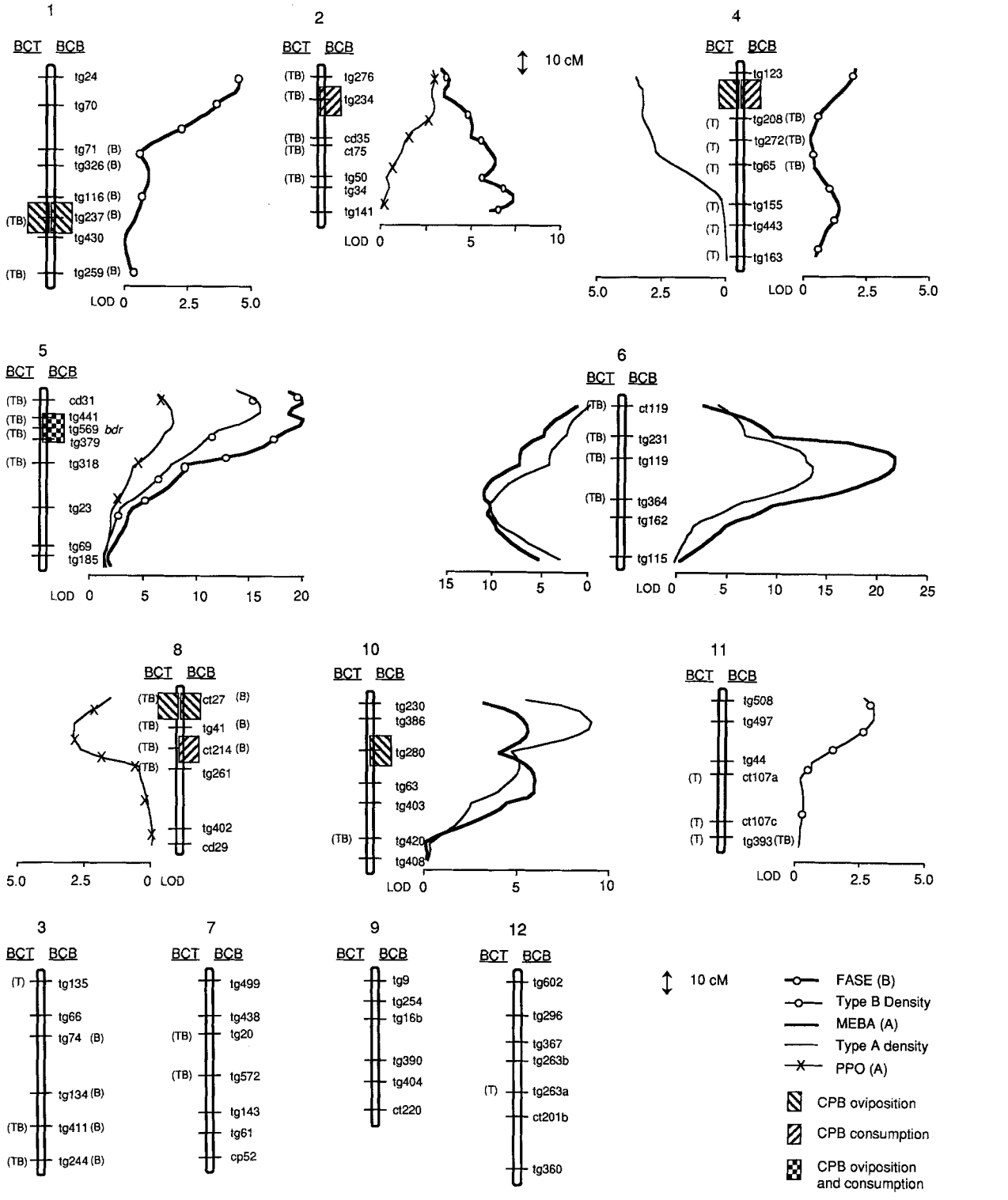

Fig. 2. QTL likelihood maps for trichome characteristics in BCT (left of chromosome) and BCB (right of chromosome). Maps are based on the segregation of alleles from TB (the hybrid parent) in each backcross progeny. Axes below likelihood plots indicate LOD scores. QTLs for insect resistance are shown as *hatched boxes* to the left (BCT) or the right (BCB) of the associated region (see text for LOD scores). Regions of skewed monogenic ratios are indicated near marker names: (*TB*) = skewed toward hybrid class; (*B*) = skewed toward recurrent genome BB; (*T*) = skewed toward recurrent genome TT

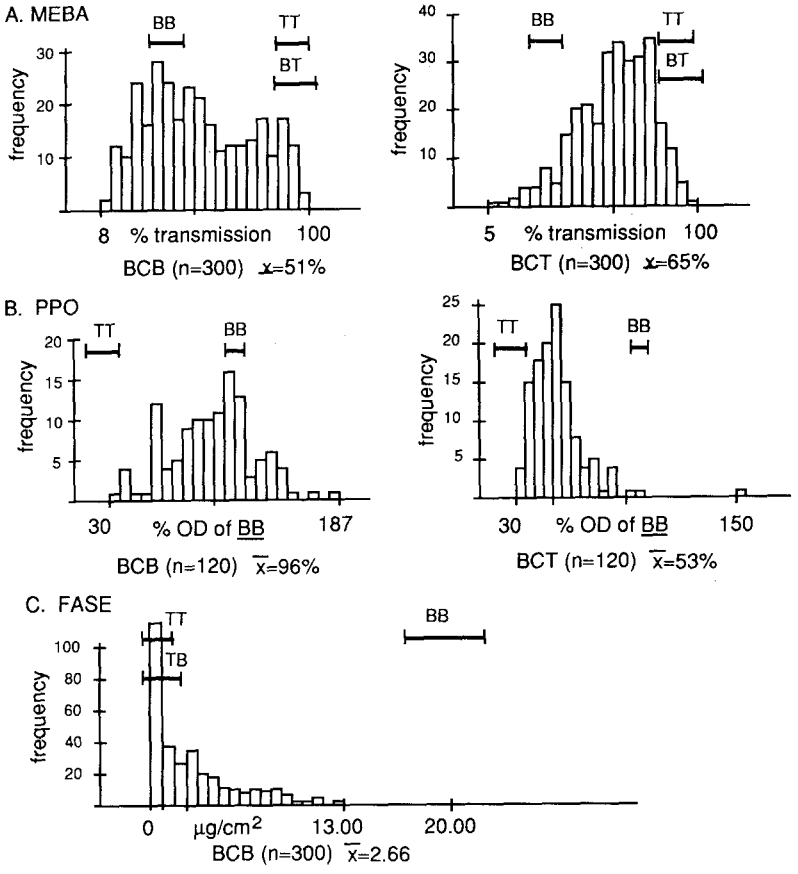

Fig. 3A–C. Frequency distributions of trichome phenotypes among individuals of BCB and BCT. **A** Modified enzymatic browning assay (MEBA) measuring oxidation of type-A trichome products as % transmission. **B** Polyphenol oxidase (PPO) concentration of contents of type-A trichome heads, as percentages of the optical density of BB by ELISA. **C** Fatty acid sucrose ester (FASE) assay quantifying sucrose production in type-B droplets, per cm^2 of leaf area. Means and standard deviations for parents (TT, BB and TB) are indicated with bars according to the Y axis and are averages of ten samples of each clone. Vertical placement of these bars is not associated with the X-axis

of recombination between the two parents of this cross ($P = 0.0547$).

Trichome characteristics of the experimental populations

Frequency distributions of trichome phenotypes in the backcross progenies are presented in Fig. 3. The *S. tuberosum* and *S. berthaultii* accessions used as parents in this study differ significantly in the trichome traits examined, with the wild parent showing higher trichome densities, higher levels of sucrose ester and PPO production, and lower MEBA scores. The F_1 hybrid family of which TB is a representative was not intermediate between the species parents for trichome properties, but more like its *tuberosum* parent for most traits (Fig. 3). TB and its siblings (20 additional clones) show poor type-B trichome properties, with the sucrose droplet being virtually absent. Densities of type-A trichomes and browning reactions (MEBA) in the hybrids were almost as low as in TT.

The two backcross progenies used in these studies differed in their expression of trichome phenotypes. While BCB segregated for all of the trichome traits, BCT did not express type-B trichome properties and could only be evaluated for traits associated with type-A trichomes. It has previously been observed that the sucrose ester droplet of the type-B trichome is not well expressed in backcrosses to the cultivated species (Mehlenbacher et al. 1983; Kalazich and Plaisted 1991).

Type-A trichome traits. BCB expressed the full range of phenotypes for the browning test (MEBA), from as poor as TT (90–100% transmission) to better than the rating of BB. The distribution was distinctly bimodal with a mean of 51% transmission (Fig. 3). BCT had a lower proportion of very good clones for MEBA than did BCB. The population mean of BCT was higher (65%), and the distribution was more skewed toward TT. The range of polyphenol oxidase (PPO) concentrations in the type-A trichomes of the two populations is shown in Fig. 3, in histograms of optical density (OD) relative to BB samples assayed in the same experiment. BCB showed a range from about 0.3- to 1.8-times the PPO production of BB, while BCT showed a narrower

range with greater skewing toward the low end. TT produced about 0.2-times as much PPO as BB.

Type-B trichome traits. The FASE assay of BCB revealed that the level of sucrose ester production of BB ($20 \pm 4.2\,\mu g/cm^2$) was not recovered in the first backcross to BB. All progeny individuals, regardless of whether or not they produced droplets, were included in the assay, resulting in a distribution that was highly skewed toward zero. Again, BCT does not express B droplets and so was not evaluated for FASE. The normality of the phenotypic distributions were significantly improved by the respective data transformations.

Correlations among trichome traits. The browning assay (MEBA) is a function of trichome density as well as the concentration of the oxidases contained in the type-A trichomes. Correlations between upper and lower leaf surface trichome densities were high ($r = 0.82$ for type-A; $r = 0.67$ for type-B in BCB), and densities of type-A trichomes were in turn correlated with the MEBA reaction ($r = -0.78$).

The quantification of PPO is based on individual trichomes, and is therefore independent of trichome density. The results of assays for PPO concentration were poorly correlated with MEBA ($r = -0.189$), but significantly related to FASE ($r = 0.370$; R-square = 15.0, $P < 0.01$), a property of the type-B trichomes. Previous observations in the breeding program have suggested that although PPO concentrations and MEBA are both functions of the type-A trichomes, they are not well correlated, and there does seem to be a positive association between PPO concentrations and the presence of droplets on the type-B trichomes of individual clones. Correlation of these phenotypes with segregating markers from the potato map has identified both coincidence of pleiotropic QTLs and linkages among factors controlling distinct features of the wild phenotype.

Insect resistance. Egg laying by Colorado potato beetles was greatly reduced overall in BCB as compared to BCT. Replicated plants of BB individuals allowed very little egg laying (near zero), while TT averaged about 1,000 eggs per female. When total eggs laid per female on each genotype were analysed, the population distribution of BCB was skewed toward zero (individuals on which no eggs were laid), with a maximum count of about 500 eggs per female per genotype. BCT, on the other hand, showed a more normal distribution with a mean of 1,000 eggs and a range from just under 500 to about 1,500 eggs per female. The hybrid family ranged from plants with no egg laying (0 eggs per female) to the midpoint between BB and TT (500 eggs per female).

QTL mapping results – type-A trichome properties

MEBA and density of type-A trichomes. Figure 2 presents an overview of the QTLs identified for trichome traits. Two quantitative trait loci were found to explain the majority of the phenotypic variation in BCB for the browning reaction of the type-A trichomes (MEBA assay). The allelic configuration along the interval between *TG119* and *TG364* central to *chromosome 6* had the largest effect on MEBA, with BB genotypes yielding significantly lower scores, i.e., more browning, than TB genotypes. This QTL explained 52% of the measured variance, with a LOD-likelihood of 22.08. A second QTL, near *TG63* on *chromosome 10*, also made a large contribution to the variance for browning (20.2% variance explained; LOD 6.45). A two-QTL model comprised of these intervals explained 63.4% of the variation seen in the trait during a given assay. An additional interval on *chromosome 10* is suggested by the data, but is not indicated as significant when the two-QTL model is compared with a three-QTL model in the MAPMAKER/QTL program (LOD 29.61 and

Table 1. Phenotypic effects of the substitution of T alleles for B alleles at two loci in BCB, and of B alleles for T alleles in BCT. MEBA 1 and MEBA 2 represent replicated assays

	Progeny	Genotype			Phenotype		
		Chr 6 TG 119	Chr 10 TG 63	(n)	Percent transmission MEBA 1	Percent transmission MEBA 2	Count in 3.2 mm² A density
1	BCB	BB	BB	(36)	39 ± 12	26 ± 9	34 ± 12
2	BCB	BT	BB	(35)	70 ± 16	60 ± 22	18 ± 14
3	BCB	BB	BT	(32)	46 ± 13	34 ± 13	25 ± 12
4	BCB	BT	BT	(47)	88 ± 13	79 ± 18	8 ± 12
5	BCT	TT	TT	(29)	69 ± 12	72 ± 12	–
6	BCT	BT	TT	(37)	54 ± 14	54 ± 19	–
7	BCT	TT	BT	(33)	74 ± 14	72 ± 18	–
8	BCT	BT	BT	(50)	53 ± 16	60 ± 16	–

LOD 31.44, respectively). When multiple-QTL models are tested, it is generally accepted that a supplemental LOD value of 2.0 is indicative of the significance of an additional locus (Lander and Botstein 1989; Lincoln and Lander 1989; Paterson et al. 1990; Stuber et al. 1992). The direction of the effect of the QTLs for MEBA is an increase in percent transmission (decrease in browning reaction) due to the substitution of a *tuberosum* allele in the background of *berthaultii*. Table 1 illustrates the extent to which T alleles (from TB) in the background of BB influence MEBA and trichome density at each of the QTLs identified. The interactions among significant loci are discussed in the next section.

The two QTL regions identified above were also highly correlated with the density of type-A trichomes (Fig. 2). The area near *TG119* on *chromosome 6* is highly influential, explaining 40% of the variance for density (LOD 14.39), while the interval *TG386-TG280* on *chr 10* also contributes significantly (27.4% variance explained, LOD = 9.69). A two-QTL model involving these regions explains 57.6% of the phenotypic variance for density. The interval just below on *chromosome 10* (see Fig. 2) does not account for additional variation for density but is the maximum likelihood location for the QTL for MEBA.

One of the two QTLs influencing type-A traits in BCB was also found to be effective in BCT (Fig. 2). In this population – the backcross to *tuberosum* – the substitution of a *berthaultii* (B) allele for a *tuberosum* (T) allele at *TG119* (*chr 6*) decreased MEBA (increase in browning), while a substitution at *TG63* (*chr 10*) was not effective. The interval on *chromosome 6* explained 34% of the variance for MEBA (LOD = 11.95), and no other QTLs for MEBA were found segregating from the hybrid parent in this cross. Table 1 also shows the effect on MEBA of allelic substitutions at the loci of interest on *chromosomes 6* and *10* for BCT.

As was the case with BCB, there was close coincidence between the QTL found for MEBA and the density of type-A trichomes in BCT. The same interval on *chromosome 6* responsible for differences in MEBA was also influential for the density in BCT. When upper and lower leaf surface densities were averaged, this was the only interval showing a significant LOD score (11.07) and it explained 32% of the variance in density (Fig. 2). However, when densities of upper leaf surfaces were analysed separately, there was a second QTL contributing to type-A trichome densities. The interval near the top of *chromosome 4* influenced type-A trichome densities on the upper leaf surface (LOD = 3.67; 11.3% of variance explained), but lost significance when upper and lower densities were averaged. The QTL on *chromosomes 6* and *10* appear to function additively in BCB with a significant degree of interaction. Their differential effects in BCT indicate that while the former QTL (*chr 6*) behaves in a dominant fashion, the *chr 10* locus is recessive.

Interaction between QTLs for type-A trichome properties. A two-way analysis of variance (ANOVA) of BCB indicates that there is significant interaction ($P = 0.0001$) between the QTLs on *chromosomes 6* and *10* in their influence on MEBA. This interaction is illustrated in Table 1, which shows that the increase in MEBA (% transmission) occurring when T replaces B at *TG119* alone (line 2 vs line 1) is significant and almost as great as the effect of the substitution at both loci (line 4). However the substitution at *TG63* alone (line 3 vs line 1) is not as effective as it is in the presence of *TG119* (line 2 vs line 4). On the other hand, either substitution seems to be effective on the density of type-A trichomes, with an additive value for both together. In accordance with the data in Table 1, the interaction term from ANOVA of the two QTLs for type-A trichome density is not significant ($P = 0.12$) in *BCB*.

PPO concentration in type-A trichomes. Two QTLs were found in BCB for the concentration of PPO in the type-A trichomes, which together explain 27% of the variance observed. These QTLs are independent of those identified for trichome density and MEBA; however, both of them are linked to segments influencing properties of the type-B trichomes (see Fig. 2 and following section). The more prominent QTL for PPO levels is on *chromosome 5*, in the same area that will be discussed as responsible for variation in type-B trichome density and sucrose ester levels. This region explains 23% of the variance for PPO concentration in BCB and is significant with a LOD of 6.30. The second QTL for PPO levels is on *chromosome 2*, and explains 13.2% of the variance for the trait, with a LOD likelihood of 2.89. PPO production in type-A trichomes is sensitive to environmental conditions in which plants are grown, such as light intensity and temperature (Ganga 1992); due to the nature of the sampling method there is also a noticeable amount of variation for a given plant during the laboratory assay. The large environmental component of the total variation is most likely responsible for the relatively low percentage that can be explained by genetic effects at these loci. A third QTL for PPO production is suggested by the data on *chromosome 11*, near *CT107C*, by a one-way analysis of variance which attributes a significance of $P = 0.007$ and an R-square of 0.06 to this region. This region was not included in the model for explaining variation for PPO because of its sub-threshhold LOD score of 1.6.

As for BCB, above, only a small proportion of the observed variability for PPO levels was explained by genetic variation in BCT. In this cross, one locus was identified which explained 11.1% of the phenotypic

variance, with a LOD likelihood of 2.98. This locus is on *chromosome 8*, precisely at the location to which the structural genes in the PPO gene family have been mapped (Newman et al. 1992; Tanksley et al. 1992). Substitution of a B allele for the T allele in this region of BCT results in a 1.9 fold increase in PPO concentration in the type-A trichome heads.

QTL mapping results – type-B trichome properties

Presence/absence of sucrose droplet. The type-B trichomes contribute to insect resistance by exuding sticky droplets of sucrose esters, which act synergistically with products of the type-A trichomes to entrap and impede the feeding primarily of small-bodied insects. This droplet is poorly expressed in intercrosses of *tuberosum-bethaultii* hybrids (Kalazich and Plaisted 1991) but its presence or absence appears to be simply inherited (Gibson 1979; Mehlenbacher et al. 1983). In the current progenies, the presence of the B droplet behaved as a single recessive gene, which we have mapped to the short arm of *chromosome 5* of potato, in the interval between *TG441* and *TG379* on the BCB/BCT framework maps (Fig. 2). The distribution of droplet phenotypes of individuals in BCB with respect to their genotype at the nearest marker, *TG569* on *chromosome 5*, is shown in Table 2. These data indicate 13% recombination between *TG569* and the gene controlling B droplets (termed here *bdr*). As our data places *bdr* at some distance from *TG569*, and not closer to other markers on the chromosome, we must consider the possibilities of the misclassification of some individuals or else the influence of another gene on expression. An earlier study (Gibson 1979) reported the presence of droplets to be inherited by a single dominant gene accompanied by a recessive gene for droplet size, but this hypothesis was not supported by the experiments of Mehlenbacher et al. (1983). BCB is currently being reevaluated for droplets, to clarify potential misclassifications of phenotype that could have resulted in the lack of closer linkage with markers on the potato map. Precise placement of this gene on *chromosome 5* will be delayed, pending re-evaluation of the phenotype. The recessive nature of this gene is suggested by the failure of TB heterozygotes in BCT to express B droplets.

Table 2. Contingency table showing the distribution of genotypes at *TG569* and the phenotype of presence/absence of the sucrose droplet of type-B trichomes of BCB. (BB = genotype lacking fragment from TT at *TG569*, TB = genotype containing fragment from TT at *TG569*, Present = presence of droplet, Absent = absence of droplet)

Droplet	Genotype at *TG569*		
	BB	TB	Total
Present	77	9	86
Absent	11	54	65
Total	88	63	151

χ^2 (for independent assortment) = 80.27 with 1 df ($P < 0.001$)

Table 3. QTLs for FASE production in BCB

Chromosome	Interval	LOD	Percent variance explained
1	TG24–TG70	4.94	16.8
2	TG276–TG234	3.93	13.3
2	TG34–TG141	7.46	24.9
4	TG123–TG208	2.00	6.1
5	CD31–TG379	19.17	49.4

Sucrose ester levels. In light of the localization of a gene (*bdr*) controlling the exudation of sucrose to the short arm of *chromosome 5*, it is not surprising that this region has a large influence on levels of sucrose ester production. Five QTLs were found in BCB for fatty acid sucrose ester (FASE) levels, the most effective of which is near *bdr*. Together the five QTLs listed in Table 3 account for 67.6% of the phenotypic variance for sucrose ester levels. The QTLs on *chromosomes 1* and *5* are each clearly located to the short arms of the respective linkage groups, but the position of the QTL(s) on *chromosome 2* is less clear. Evidence for two separate QTLs on *chromosome 2* was obtained with MAPMAKER-QTL by searching the genome for variation that could be explained after variation from one interval on the chromosome (*TG34-TG141A*) was accounted for, and by comparing multiple-QTL models. This procedure revealed the significance (increased LOD likelihood of 2.5 in the multiple-QTL model) of a second interval, *TG276-TG234*, at the distal end of *chromosome 2*. In the same analysis, an interval of *chromosome 4* (*TG123-TG208*) was also confirmed to be influential on the trait, effecting a LOD score increase of 2.6 toward explaining the variance. One additional locus on *chromosome 6* (near *TG364*) was suggested as significant (LOD 2.1) during one replication of the experiment with sucrose esters, but was not considered in the final model because of lack of evidence from both tests.

Density of type-B trichomes. Trichome density is likely to constitute an additional factor in the expression of B trichome-mediated insect resistance. To determine density all trichomes of the B-type, with and without droplets, were counted; counts from the upper and lower leaf surfaces were averaged prior to transformation with square roots. Two QTLs were identified

which together explained 38.1% of the variance for the density of type-B trichomes in BCB. The most effective QTL (LOD 14.23) for density, *CD31-TG379* on the short arm of *chromosome 5*, is the same region found to be most strongly associated with sucrose ester levels. Genotypic differences in this region explained 35.4% of the variation measured for density, showing a maximum likely location at the marker *TG441*. The second QTL for density of type-B trichomes was identified on *chromosome 11* in the interval *TG508-TG497*, explaining 8.6% of the variance with a LOD likelihood of 2.90.

Interactions among QTLs for B-trichome properties. When all possible interactions between genotypes at the QTLs described for type-B trichome properties were tested using analysis of variance, each combination involving the QTL on *chromosome 5* showed a significant component due to interaction. This is logical outcome, since levels of sucrose ester production are dependent on the production of the B droplet, which we have shown to be controlled by this region.

There were no significant interactions detected between the unlinked QTLs for FASE (type-B) and MEBA (type-A trichomes).

Segregation from heterozygous loci of the recurrent parents BB and TT

Additional effects on trichome properties due to segregation from the recurrent parent in BCB. When segregation from 45 heterozygous loci of the recurrent parent BB were analysed by one-way analysis of variance, significant effects of loci on *chromosome 2* were found for the densities of both types of trichomes (Table 4). RFLPs representing the different *berthaultii* alleles (say, B and B′) segregating at the heterozygous loci *TG50* and *TG34* showed a significant influence on trichome densities. An additional effect on type-B trichome density and sucrose ester exudation (FASE) due to segregation from BB was identified on *chromosome 9* (*CT220*).

The magnitude of the effects of alleles segregating from the recurrent parent BB were much smaller than the effects due to the segregation of alleles from the hybrid parent. For example, R-square values from the analysis of variance (conducted in parallel to analysis with MAPMAKER/QTL) for trichome density and FASE due to segregation of alleles from the hybrid parent TB were in the range of 0.30 and 0.45, as opposed to maximum R-square values of < 0.10 for the effects of segregation from the recurrent parent BB. Due to the lower levels of heterozygosity (fewer useful polymorphisms), the genome of the recurrent parent was not monitored as well as the hybrid parent's and it is possible that some effects were missed due to incomplete coverage of the chromosomes.

Additional effects of trichome properties due to segregation from the recurrent parent in BCT. When the segregation of 35 loci segregating from TT was evaluated in terms of potential effects on the trichome traits, a correlation was found with the density of type-A trichomes. Segregation from one region, near *TG155* on *chromosome 4*, was found to significantly influence density ($P = 0.0004$; R-square = 0.09). As in the other backcross, the effects of segregation from the recurrent parent of BCT were minor, in terms of R-square values, compared to those associated with the segregation of alleles from the hybrid parent. This type of effect has also been observed in advanced breeding populations among which various *tuberosum* recurrent parents have distinct effects on the expression of traits derived from *tuberosum-berthaultii* hybrids (Ganga 1992).

Resistance to insect feeding and oviposition, and variation in tuberization response to daylength

Preliminary investigation of CPB resistance among the mapping populations shows a significant correlation, but not complete coincidence, between QTLs identified for trichome properties and those for effects on insect behavior and biology. For each measure of resistance, about 20% of the variance measured can be explained by the QTLs identified. The threshold for considering an effect on these traits significant was lowered to just above 1.0 to accomodate the large environmental component of the variance, including

Table 4. Significant effects ($P < 0.005$) of segregation from recurrent parents BB and TT on trichome phenotypes, as determined by ANOVA for single markers

Progeny	Trait	Marker	Chromosomal	Source	*P*-value	R-square
BCB	A density	TG50	2	BB	0.0044	0.06
BCB	B density	TG50	2	BB	0.0048	0.06
BCB	B density	TG34	2	BB	0.0005	0.08
BCB	B density	CT220	9	BB	0.0040	0.05
BCB	FASE	CT220	9	BB	0.0014	0.08
BCT	A density	TG155	4	TT	0.0004	0.09

variability within the pest population itself. At all of the QTLs identified for the resistance measures, alleles from BB decreased feeding and/or oviposition relative to alleles from TT. Locations of putative QTLs for resistance to CPB are indicated in Fig. 2 by hatch-marked boxes.

On *chromosome 1*, near *TG237*, a decrease in oviposition was associated with the presence of B alleles in BCT, and an increase was associated with T alleles in BCB. This locus explained 12.1% and 5.1% of the oviposition variance in BCT and BCB, respectively, with LOD scores of 3.31 and 1.25. This region was not found to be highly significant for any of the trichome traits although sucrose ester levels (FASE) were significantly influenced by another region of the same linkage group. An interval on *chromosome 2*, near *TG234*, was identified as significant for both consumption by CPB and trichome properties in BCB. With a LOD of 1.14, this interval explained 5.3% of the variance for consumption in the leaf-disk assay, and was also significant for both PPO levels and FASE. A third QTL for beetle resistance was located between *TG123* and *TG208* on *chromosome 4*. In BCB this interval explained 12.4% of the variance for leaf consumption (LOD 2.32) and also influenced sucrose ester levels. In BCT, the substitution of *berthaultii* alleles for *tuberosum* decreased oviposition (LOD 1.73;8.1% variance explained) but increased type-A trichome density.

The region of *chromosome 5* found to be most important for the type-B trichome properties, and also responsible for variation in PPO concentration, affected both consumption and oviposition in BCB, with LOD scores of 1.11 and 2.03, respectively. QTLs for both consumption and oviposition were also identified on *chromosome 8*, coincidental with an interval influencing trichome PPO levels. In BCB the interval *CT27-TG41* explained 4.2% of the variance for oviposition (LOD 1.13), and the nearby region *TG41-CT214* influenced consumption (9.2% variance explained, LOD 1.75). A QTL in the same area explained variation for oviposition in BCT (9.1%, LOD 2.13). Further evidence is suggested for CPB response to varying PPO levels by the coincidence of the negative effect of *berthaultii* alleles on oviposition and a sub-threshold effect on PPO at *ct107* on *chromosome 11*. No QTLs were identified for CPB resistance on *chromosome 6*, which is most highly associated with MEBA and the density of Type-A trichomes; but a significant interval was identified in the region of secondary importance for MEBA on *chromosome 10* for oviposition.

Each of the aforementioned effects was associated with the interspecific substitution of alleles segregating from the hybrid parent in the background of the respective recurrent species parent. There was also one incidence, in BCB, in which segregation from the recurrent parent BB influenced CPB oviposition. Analysis of variance of the 45 loci segregating from BB with resistance data showed a significant effect ($P < 0.006$) of particular *berthaultii* alleles at *TG 185* on the long arm of *chromosome 5*. Results of the CPB assays are considered preliminary because they are based on a single experiment for measuring each trait, in which large environmental components of variance were evident. Experiments are planned for re-evaluation of CPB resistance in the mapping populations, with increased replication and more precise techniques, in efforts to minimize the environmental component of these assays and more clearly define genotypic effects. In the assays conducted to-date, the direction of the phenotypic effect of allelic substitutions at the QTLs identified in BCT was always toward reduced oviposition or consumption when *berthaultii* alleles were present; in BCB, the presence of *tuberosum* alleles at the significant loci was correlated with increased oviposition or consumption.

In an upcoming paper (Van den Berg et al., in preparation), we will discuss how QTLs identified through tuberization experiments with the same backcross progenies help to explain the association between the B-trichome traits and the poor adaptation with which the breeding program has been concerned. In brief, it was discovered that QTLs segregating from each of the parental sources (*tuberosum* and *berthaultii*) conferred lateness in the backcross progenies, and in particular, that the locus controlling type-B trichome characters on *chromosome 5* is linked to loci that are associated with late tuberization.

Discussion

Assays for discrete chemicals or biochemical reactions increase the precision of QTL mapping

A very clear correlation can be seen from these results between the precision of the various screening techniques available for phenotypic characterization, and the relative amount of variability explained by genetic effects at RFLP loci by quantitative trait mapping. Accuracy in measurement was certainly gained in using biochemical assays for specific chemical components or reactions, as compared to bioassays measuring behavioral features of genetically-variable populations of insects. Due to sampling and processing techniques and other environmental components of variation, precision of the biochemical assays varies in the order MEBA > FASE > PPO. The effects of genetic variation in specific chromosomal regions, or QTLs, were detected with very high levels of statistical significance for MEBA and FASE and slightly lower

levels for PPO. Relative to those found with biochemical assays, the significance levels of associations found between RFLP classes and direct measures of CPB resistance are low. This tendency stresses the need for establishing very accurate methods of measuring phenotypic differences in quantitative characters as a prerequisite to successful QTL mapping efforts.

Mechanisms of resistance independent of trichomes may be expressed in the hybrids

The incomplete correlation between the trichome phenotypes studied and direct measures of CPB resistance indicates genetic control of resistance that overlaps with, but is not entirely due to, the trichome properties characterized to-date. This may be explained either by our incomplete understanding of the biochemical nature of the resistance mechanism we are studying, or the presence of multiple resistance mechanisms in the same progenies. This incomplete correlation confirms that, however accurate these biochemical screens are in describing trichome phenotypes, they must be combined with the far-less-precise practices of direct resistance screening in the breeding program until more is known about the nature of insect resistance. Toxic glycoalkaloids which affect insect biology and behavior are known to be present in the foliage and tubers of some hybrids of potato and its wild relatives, including the present populations. In routine screening, it is difficult to rule out resistance due to such alternative and less-desirable factors, and years of progress can be lost due to the detection of toxic compounds in the harvested products of advanced selections. The genotypes used in the present studies have been analysed for a wide range of glycoalkaloids and the results will be used to investigate the nature of the observed CPB resistance that is not explained by trichome traits. The development of selectable markers for use against undesirable characteristics of the wild species genome will have practical application in the breeding program.

Genetic mapping explains experiences encountered in breeding for these traits in a tetraploid variety development program

Heretofore, advance has been made in the development of adapted potato clones carrying a part of this resistance machanism through a combination of field assays for CPB resistance and correlative screens for morphological and biochemical traits associated with resistance to a wider range of pests. The practical difficulty of introgressing particular components of the resistance, such as the type-B droplets, into adapted backgrounds had previously been ascribed to either poor pairing and/or restricted recombination between the wild and cultivated genomes or to linkage drag (Kalazich and Plaisted 1991). Genetic linkages, detected in this research, among trichome characters and between trichome characters and lateness (Van den Berg et al. 1992; and in preparation), explain many of the experiences encountered in breeding for trichome traits in a tetraploid population.

(1) The sucrose droplets on type-B trichomes generally are not expressed in populations of tetraploid backcrosses to the cultivated parent, but reappear in selfs or intercrosses of the backcross generations, suggesting recessive control. In most backcross generations only 0–3% of individuals have droplets. Individuals selected on the basis of the presence of droplets are frequently poorly adapted for tuberization during the temperate growing season. [In this research we located a single chromosomal segment on *chromosome 5* largely responsible for the presence or absence of B droplets. The locus identified behaved as a recessive gene, and is on the same chromosome as loci associated with late tuberization (Van den Berg et al. 1992; and ms. in preparation).]
(2) In tetraploid backcrosses, the rare clones with B droplets generally have good PPO scores. (This is consistent with our finding of the same region of *chromosome 5* controlling both the presence of B droplets and PPO levels.)
(3) Breeding for A-trichome traits using the MEBA test as a first screen and PPO activity as the second screen in tetraploid populations has been easier than breeding for B-trichome traits using the presence of droplets as the first screen and the FASE test as the second. (This research indicates that only two loci have a major impact on MEBA scores whereas five loci affect the FASE scores. The single locus identified that determines the presence of B droplets behaves as a recessive. The locus that most influences daylength adaptation is on the opposite end of the same chromosome that has the most influence on all three B-trichome traits, but only the PPO activity of the A trichomes.)
(4) Experience with selection for low MEBA scores in tetraploid populations has shown that of the two factors determining MEBA, A-trichome density has a greater effect than PPO activity. (In this research, the two most influential loci for MEBA scores were the same two which affected A-trichome density.)
(5) It has been easy to produce backcross progeny with MEBA scores equal to those of the donor species. Equality of PPO activity has been somewhat more difficult to achieve, and FASE scores equal to the donor have been very difficult to attain. Some *tuberosum* parents are more effective than others in prescribing MEBA scores in the backcrosses. *Tuberosum* clones have varying densities of A trichomes, but have vestigal B-types without droplets. (The MEBA is used more

routinely than the FASE test in the selection scheme, because it is easier to apply and has greater predictability. In these experiments, the only trait for which mapping indicated that the *tuberosum* parent contributed variation was the density of A trichomes.)
(6) While the nature of resistance to the CPB is still to be determined, it is known that type-B droplets are not essential and that removing the type-A trichome glands makes the resistant plants more susceptible. MEBA scores by themselves do not give an accurate prediction of CPB resistance. In the tetraploid breeding program, there are some indications that PPO activity may be a more effective predictor of CPB resistance than MEBA scores. [This research and other work in progress give an indication that more loci are involved in CPB oviposition and consumption than those that affect A-trichome traits. Of six regions tentatively associated with CPB resistance, five (*chrs 2, 4, 5, 8,* and *10*) are active in the measured A-trichome traits; but the most effective region for both MEBA and A-trichome density does not seem to influence feeding or oviposition.]

One inconsistency was found between the practical experience in breeding for trichome characteristics at the tetraploid level and the present study. In both backcross and intercross progenies, Kalazich and Plaisted (1991) detected significant differences in the proportion of individuals with good browning scores (MEBA < 62% transmission), depending on the presence or absence of the sucrose droplet on type-B trichomes. However, in the present diploid progenies, regions of the genome influencing the droplet and MEBA scores were not linked, and there was no significant interaction between them. The association in tetraploid breeding populations among traits which we have found not to be linked or epistatic to each other could be an indication or restricted genetic exchange between the wild and cultivated genomes at the tetraploid level. For example, tetraploid genotypes composed of *tuberosum* and *berthaultii* chromosomes (TTBB) may experience some degree of preferrential pairing, which could limit interspecific crossing-over to regions of the genome which we assume to be independent based on their distribution in different linkage groups. *S. tuberosum* is generally considered to be an autotetraploid, but interspecific hybrids between *tuberosum* and *berthaultii* have not been examined cytologically to rule out allotetraploid (or segmental allotetraploid) behavior which could influence genetic exchange between *tuberosum* and *bethaultii* chromosomes, and our concept of independent segregation. Alternative explanations might be found in the possibility of slight contamination of the tetraploid progenies with selfed individuals. In the breeding program, emasculation is not performed prior to controlled hybridizations, and our indication of the recessive control of the droplet could not allow for the expression of droplets in backcrosses to the cultivated parent as has been found with low frequency in the breeding populations. Finally, different *berthaultii* and *tubersosum* accessions were used to generate the diploid and the tetraploid experimental materials, and it is possible that intraspecific allelic variation between the sets of parental material could account for the differential phenotypic associations.

Future use of the mapping populations and molecular markers

The results obtained in this research are useful at the advanced tetraploid level to better understand the complex genetic inter-relationships of various traits under selection, and to plan future crossing and selection schemes. These results can also be used to accelerate progress toward the desired genotype in the diploid hybrid populations by selectively choosing parents with loci essential for the resistance mechanisms, and with the least amount of unwanted wild species genome. At the tetraploid level, markers can be used to practice negative selection against the long arm of *berthaultii chromosome 5* (carrying alleles for short-day requirement) while trichome traits (including those on the short arm of *chromosome 5*–top of chromosome in Fig. 2) are introgressed into the *tuberosum* background.

The genetic map and the diploid hybrid populations are currently being used to: (1) test and refine hypotheses generated in the first backcross generation for the genetic control of the traits investigated, (2) superimpose on the current QTL maps genetic effects for other traits segregating in the same progenies, such as glycoalkaloid synthesis and the reported role of secretory trichomes from *S. berthaultii* in resistance to fungal pathogens, and (3) accelerate advance toward the desired genotype for insect resistance and adaptation. These objectives can be accomplished by selecting recombinants of defined genotype for use as parents for advanced generations of segregants. As the mapping populations are vegetatively propagated, selected individuals can also be used in replicated studies to more clearly define the genetic control of traits that are highly influenced by the environment. It will be worthwhile to cross individuals from the first backcross to *tuberosum* that are heterozygous for *bdr* to test the hypothesis of single-gene control of the presence of the B droplet and to confirm the genetic effects defined at the diploid level in tetraploid progenies. The continued recombination that will be experienced in advancing generations, combined with continued genetic mapping, will provide material for fine mapping of the putative QTL effects; and the precision gained in marker-aided selection of experimental material will

help to reduce the complexities of studying insect resistance mechanisms in these populations.

Given the similarity between the potato and tomato genomes (Bonierbale et al. 1988) and commonalities between insect resistance properties among their wild relatives (Goffreda et al. 1989), these results and the detailed linkage map of tomato (Tanksley et al. 1992) may be used to investigate the evolution and divergence of morphological and biochemical properties of agronomic importance in the *Solanaceae*.

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