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QTL mapping of foliar glycoalkaloid aglycones in *Solanum tuberosum* × *S. berthaultii* potato progenies: quantitative variation and plant secondary metabolism

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Abstract Glycoalkaloids are quantitatively inherited in *Solanum*, and in high concentrations they can be toxic to humans. The increased use of wild potato germplasm to improve the pest resistance, yield, and quality characteristics of cultivated potato may elevate or introduce new, more toxic glycoalkaloids into the cultivated gene pool. Therefore, it is important to increase our understanding of their inheritance, accumulation, and biosynthesis. Glycoalkaloids have two basic constituents – a glycosidic grouping and a steroid alkaloid skeleton. Steroid alkaloids are classified as solanidanes and spirosolanes, of which solanidine and solasodine are, respectively, representatives. RFLP-mapped, diploid, reciprocal backcross potato progenies involving the parents *S. tuberosum* and *S. berthaultii*, which produce solanidine and solasodine, respectively, were analyzed for segregation of the glycoalkaloids solanine, chaconine, solasodine and solamargine to identify quantitative trait loci (QTLs) for the production of the aglycones solanidine and solasodine. The F_1 clone M200-30 exhibited low to nondetectable levels of solasodine and solanidine, suggesting that expression was controlled by recessive genes. In a backcross to *berthaultii* (BCB) and backcross to *tuberosum* (BCT), several QTLs for the accumulation of solasodine and solanidine were identified. Three QTLs explaining approximately 20% of the variation in solasodine were identified in BCB on chromosomes 4, 6, and 12. Similarly, three QTLs were identified in BCT on chromosomes 4, 8 and 11, but these accounted for only 10% of the variation observed in solasodine accumulation. Two QTLs for solanidine were identified in BCT on chromosomes 1 and 4. The QTL located on chromosome 1 was highly significant, accounting for 17% and 22% of the variation in solanidine accumulation in 1994 and 1995, respectively. This same QTL was also detected in BCB. The QTLs detected in this study probably represent structural and/or regulatory genes controlling the accumulation of solasodine and solanidine. Results are discussed in the context of steroid alkaloid accumulation and biosynthesis.

Key words Solasodine · Solanidine · Steroid alkaloid · Restriction fragment length polymorphisms (RFLPs) · Plant breeding

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Introduction

Glycoalkaloids are found throughout the Solanaceae. In cultivated species, they occur in *Solanum* (potato and eggplant) and *Lycopersicon* (tomato) (Maga 1994). They are found in every plant organ (roots, tubers, stolons, stems, foliage, flowers, and fruits) with fresh weight concentrations in potato plants ranging from 10 mg/kg (fresh weight) in the tubers to 5,000 mg/kg (fresh weight) in the flowers (Smith et al. 1996). In potatoes, they have been a particular concern, due to

their toxicity to humans (Maga 1994; Smith et al. 1996; Valkonen et al. 1996; Friedman and McDonald 1997). The increased use of wild potato germplasm to improve the pest resistance, yield, quality, and processing characteristics of cultivated potato presents the possibility that glycoakaloid levels may increase to unsafe levels or that new, more toxic glycoalkaloids might be introduced into cultivated potato (Valkonen et al. 1996; Friedman and McDonald 1997). Breeders typically assay breeding materials and advanced clones resulting from crosses with wild species to ensure that tuber glycoalkaloid levels are within accepted safety levels. Increasing our understanding of glycoalkaloid inheritance, accumulation, and biosynthesis would benefit breeding efforts and provide information useful for the future manipulation of these important compounds.

Structurally, glycoalkaloids are steroidal glycosides. Their chemistry and biosynthesis have recently been reviewed (Maga 1994; Friedman and McDonald 1997). Solanine and chaconine, derived from the aglycone solanidine, are the most prevalent glycoalkaloids found in cultivated potato, while solasonine and solamargine, derived from the aglycone solasodine, are found in the wild potato species *Solanum berthaultii* as well as other wild species (Deahl et al. 1993). Solanine and solasonine have a common sugar moiety (solatriose), while chaconine and solamargine have chacotriose in common (Sinden et al. 1991) (Fig. 1a). The alkamine steroidal skeletons (aglycones) of the glycoalkaloids are classified into two groups: the spirosolanes and solanidanes, of which solasodine and solanidine are representatives, respectively (Goodwin and Mercer 1983). These compounds are derived from mevalonic acid (Goodwin and Mercer 1983), sharing a common biosynthetic pathway to cholesterol (Osman 1984). Cholesterol is a pivotal precursor in various biosynthetic pathways in plants (Goodwin and Mercer 1983; Tetenyi, 1987). In the biosynthetic scheme presented (Fig. 1b), the committed step to either solanidine or solasodine biosynthesis is the stereospecific hydroxylation of cholesterol. Uptake studies using labeled precursors with the subsequent isolation and identification of metabolic intermediates in *Veratrum grandiflorum* (solanidine producer) (Kaneko et al. 1976, 1977) and *S. laciniatum* (solasodine producer) (Tschesche and Spindler 1978) have indicated the biosynthetic steps from cholesterol to these aglycones (Fig. 1b). Such biosynthetic schemes can be used as models to investigate analogous pathways in other species within the Solanaceae. The 3-*O*-glycosylation of the aglycones to form glycoalkaloids probably occurs following their synthesis (Maga 1994; Ehmke et al. 1995).

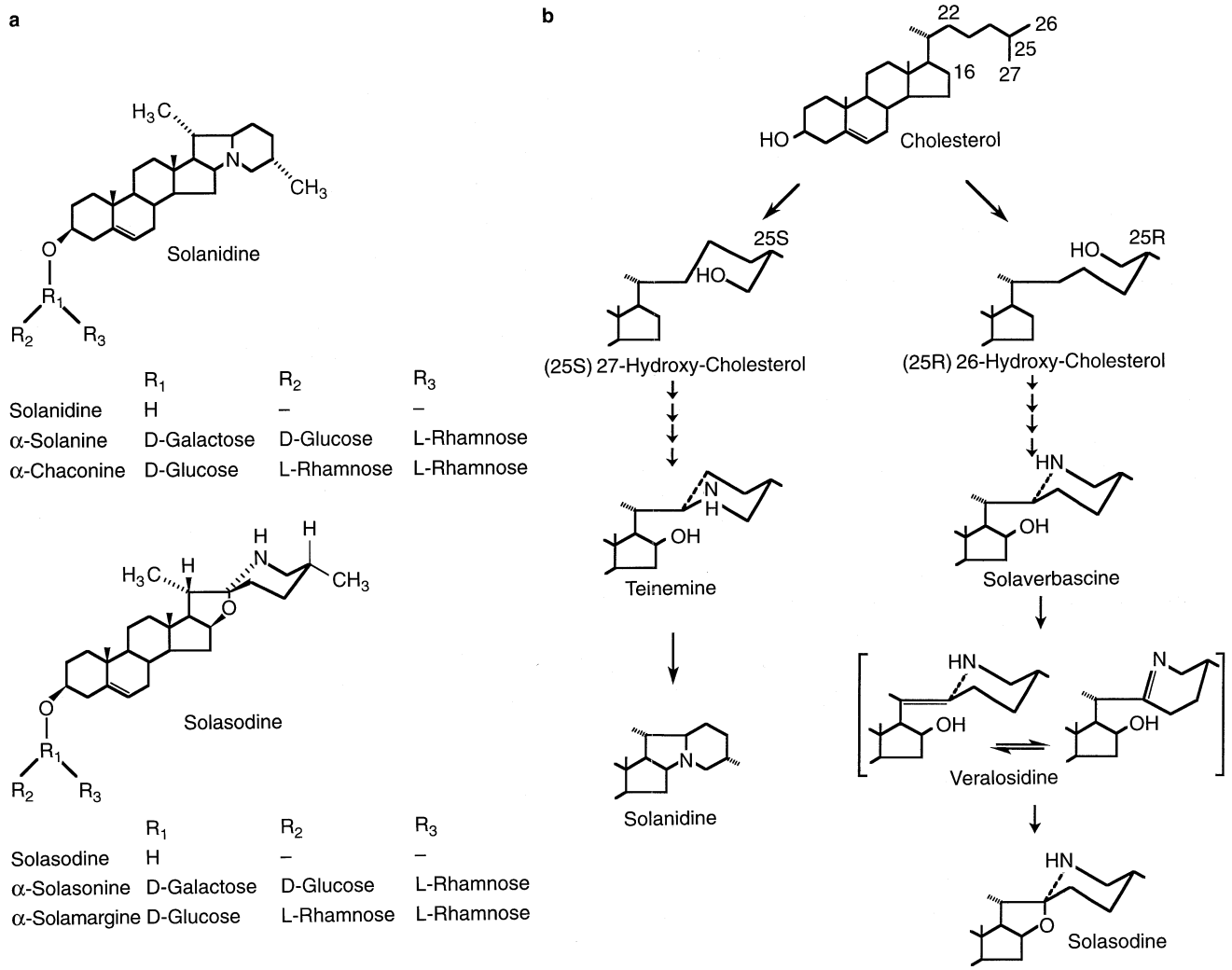
In *S. tuberosum*, glycoalkaloids are quantitatively inherited with broad-sense heritability ranging from 86–89% (Sinden et al. 1984). In segregating offspring (F_2 or backcross), variation in the level of glycoalkaloids is continuous, indicating polygenic inheritance (Sanford and Sinden 1972). Although the heritability of glycoalkaloids appears to be high, several environmental factors, such as temperature, light, and even mechanical stress, will affect levels of glycoalkaloids (Maga 1994; Valkonen et al. 1996). Foliar and tuber glycoalkaloid levels have been reported to be moderately correlated (Sanford et al. 1996a). In interspecific crosses between *S. tuberosum* and various wild *Solanum* species, F_1 progenies had low levels of glycoalkaloids (Ross 1966). In a cross between *S. chacoense* and *S. tuberosum*, genes from *S. tuberosum* were dominant for lower levels of glycoalkaloids (Sanford et al. 1994). In intraspecific crosses between *S. chacoense* parents with high and low glycoalkaloid levels, the levels of glycoalkaloids in the F1 were closer to that of the low parent (McCollum and Sinden 1979; Sanford et al. 1994, 1996a). These observations suggest that suppression of glycoalkaloid expression is probably dominant and that multiple recessive alleles are required for the expression of elevated glycoalkaloid levels. A similar mode of gene action has been reported for maysin, a flavonoid derivative mediating resistance to corn earworm in maize (Byrne et al. 1996).

Herein, we report on quantitative trait loci (QTLs) analyses of steroid alkaloid aglycones in segregating reciprocal backcross populations between *S. tuberosum* (solanidine producer) (Gregory et al. 1981; Morgan et al. 1985; Sanford et al. 1994) and *S. berthaultii* (solasodine producer) (Gregory et al. 1981; Deahl et al. 1993). The populations used in these studies are well characterized having been used to determine the genetic basis of: (1) trichome-mediated insect resistance (Bonierbale et al. 1994; Yencho et al. 1996); (2) potato tuberization (Van den Berg et al. 1996a); and (3) tuber dormancy (Van den Berg et al. 1996b). The results presented here indicate that in these progenies several major and numerous minor QTLs affect the accumulation of glycoalkaloids.

Materials and methods

Plant populations and restriction fragment length polymorphism (RFLP) maps

The progenies used in these studies were previously developed, described, and mapped by Bonierbale et al. (1994). They have been maintained vegetatively as *in vitro* tissue culture plantlets and tubers since their first propagation as seedlings and are available as tissue culture plantlets from the USDA/ARS Potato Introduction Station, Sturgeon Bay, Wisconsin. A brief description of their development and RFLP genotyping is as follows. The dihaploid ($2n = 2x = 24$) *Solanum tuberosum* (*tbr*) clone (USW 2230) (female) was crossed with *S. berthaultii* (*ber*) accession PI 473331a (male). One of the resulting hybrids, M200–30, was selected and crossed as a female to *S. berthaultii* PI 473331b (a sib of PI 473331a) and *S. tuberosum* (HH1-9) (Sanford and Hanneman 1982) to generate two reciprocal backcross families termed backcross to *ber* (BCB) and backcross to *tbr* (BCT), respectively (Fig. 2). RFLP linkage maps for BCB and BCT were based on the segregation of 150 clones from each population. Because the parental clones were heterozygous and segregating alleles from each parent contributed to the genetic variation observed in



	R_1	R_2	R_3
Solanidine	H	–	–
α-Solanine	D-Galactose	D-Glucose	L-Rhamnose
α-Chaconine	D-Glucose	L-Rhamnose	L-Rhamnose

	R_1	R_2	R_3
Solasodine	H	–	–
α-Solasonine	D-Galactose	D-Glucose	L-Rhamnose
α-Solamargine	D-Glucose	L-Rhamnose	L-Rhamnose

Fig. 1 a Structures of *Solanum* steroid alkaloids, solasodine and solanidine, and the glycoalkaloids solanine, chaconine, solasonine, and solamargine. **b** Proposed biosynthetic pathways for the *Solanum* steroid alkaloids solanidine and solasodine (Osman 1984; Tschesche and Spindler 1977; Ripperger and Schreiber 1981). C-25 is a prochiral center. Stereospecific hydroxylation of cholesterol at C-26, or C-27, results in either equatorial (25α) or axial (25β) orientation (Heftmann 1983). *Arrows* indicate steps with likely stereoisomeric intermediates

BCB and BCT, four RFLP maps (one each based on segregation from the hybrid parent, and one each based on the segregation of the recurrent *ber* and *tbr* parents) were constructed. The genetic maps based on segregation from the hybrid for BCB and BCT consisted of 80 and 70 markers, respectively, with an average interval of 10 cM. Fewer polymorphic loci were observed in the recurrent parents; therefore, these maps were based on 45 and 35 markers, respectively.

Plant growth conditions

The BCB and BCT progenies were grown in the greenhouse at Cornell University, Ithaca, N.Y. In 1994, 112 and 114, and in 1995, 125 and 121, BCB and BCT clones, respectively, and their parents were planted. Tubers of each clone were planted in 25-cm diameter × 19-cm deep plastic pots filled with Cornell peat-lite soil mix A (Boodley and Sheldrake 1982) and placed in a greenhouse with a 16-h photoperiod and mean daily temperature of approximately 22.5°C. Natural lighting of the greenhouse was supplemented by 1000-W metal halide multivapor growth lights. All plants were fertilized weekly with 20-10-20 liquid fertilizer (Peters Peat-Lite Professional, W. R. Grace & Co., Fogelsville, Pa.).

Extraction and analysis of foliar glycoalkaloids

When the plants were still in the pre- to early-bloom stage, one sample of three fully expanded leaves was harvested from the upper third of each clone, placed in Whirlpak™ bags (Nasco, Fort Atkinson, Wis.) and immediately placed in a cooler containing liquid nitrogen and later transferred to a − 72°C freezer. The samples were then dried in a Labconco Model 75015 lyophilizer (Labconco Corp., Kansas City, Mich.) for approximately 72 h and ground to a uniform consistency using No. 14 (1.4 mm) and No. 20 (0.85 mm) USA Standard Testing Sieves (W.S. Tyler, Mentor, Ohio).

The leaf powder was sent to the USDA/ARS/PSI Vegetable Laboratory, Beltsville Agricultural Research Center, Beltsville, Md. for quantification. For each clone, 0.25 g of leaf powder were extracted in 100 ml of hot, acidified ethanol (Sanford et al. 1996a, b). The concentrated extract (20 ml) was filtered (Whatman No. 4 filter paper), further concentrated under steam (Arnold steamer) to 1 ml,

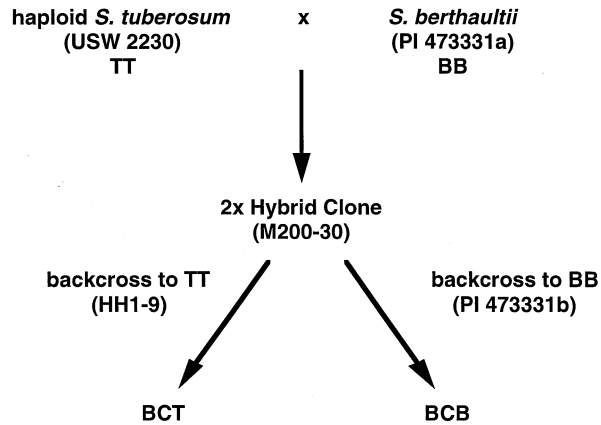


Fig. 2 Pedigree of the diploid mapping populations. The clones involved in the crosses were: the dihaploid USW2230 (2n = 2x = 24), which was derived from *S. tuberosum* cv 'Saco'; two sibs of *S. berthaultii*, PI 473331a and PI 373331b; and HH1-9, a diploid *S. tuberosum* clone selected for its male fertility (Bonierbale et al. 1994)

and cooled on wet ice. Five milliliters of aqueous heptanesulfonic acid (Carman et al. 1986) were added to the concentrated extract and thoroughly mixed; a precipitate formed after 90 s. This was filtered (Whatman No. 4 filter paper), the beakers and filter paper extensively washed with aqueous heptanesulfonic acid, and 40 ml was recovered. Ten milliliters (62.5 mg) was preparatory-cleaned using a reverse-phase C-18 Sep-Pak cartridge (Waters, Milford, Mass.) (Sanford et al. 1996a, b). The sample was eluted from the column with 5 ml of methanol. A second preparatory-cleaning, using C-18 Sep-Pak cartridges, was then performed (Saito et al.1990). The eluate was dried, redissolved in 2 ml of methanol and analyzed by HPLC (Sanford et al. 1996b).

The entire experiment was repeated over 2 years (1994 and 1995). In each year, the same BCB and BCT progenies were analyzed. Because of poor growth and/or tuberization in the previous year we were not able to screen exactly the same set of clones in each experiment, but the correspondence between clones for each year was greater than 90%. HPLC analyses of glycoalkaloids were, generally, not duplicated per clone per year. However, in the initial set-up of the analytical procedure, a subset of clones were subjected to replicated extraction and analyses to determine if experimental variability would be an issue. Variability between extractions and analyses of the same clones were found to be negligible. Therefore, individual analysis was performed per clone per year. Nonetheless, for some clones, if the analysis appeared unusual or peculiar, a second extraction/HPLC was conducted. Additionally, for the few individuals that had no detectable glycoalkaloids by HPLC, this was confirmed by reanalyzing these samples using gas-liquid chromatography as described in Deahl et al. (1993).

Statistical analysis

To study the inheritance of their aglycone precursors, we pooled separately the data from the HPLC analyses of the glycoalkaloids solanine and chaconine (solanidine aglycone) and of solasonine and solamargine (solasodine aglycone) (Sinden et al. 1991). Concentrations were expressed as milligrams per gram dry weight glycoalkaloid and interpreted to represent the foliar accumulation of the aglycones solanidine and solasodine. Putative QTLs for each trait were identified using one-way ANOVA with phenotypic data as the independent variable and marker genotype as a dependent class variable. The progenies were analyzed as two separate experiments over two years (1994 and 1995). In order to protect against Type-I errors we adopted a conservative QTL threshold. If a QTL did not exceed a threshold of $P = 0.0001$ three additional criteria had to be met before we determined that a QTL was present at a given locus. First, the putative QTL had to be significant at $P = 0.05$ by one-way ANOVA. Second, it had to be detected at the same or an adjacent locus in both years. Third, the direction of the effect had to be the same for the putative QTL in each of the 2 years. QTLs identified in this manner were further tested and confirmed by multiple regression methods using techniques described by Knapp et al. (1992). All analyses were conducted using the genetic analysis program, QGENE 2.27S, developed by Dr. J.C. Nelson, Cornell University (Nelson 1997). Missing genotypic data in the multiple regression analyses were estimated by inferring the most likely genotypes from flanking-markers and estimated distances. Epistatic interactions between loci were determined using the same software.

Results

Accumulation of glycoalkaloids in BCB and BCT

Analysis of the glycoalkaloids present in the parents of BCB and BCT indicated that *ber* produced only solasonine and solamargine (aglycone = solasodine), while *tbr* produced solanine and chaconine (aglycone = solanidine) (Table 1). Foliar glycoalkaloid levels in the F_1 hybrid, M200-30 were low to nondetectable (Table 1). Pearson product-moment correlations (r) between total glycoalkaloids present in the foliage of the same greenhouse-grown clones in 1994 and 1995 were $r = 0.461$ ($n = 100$) for BCB, and $r = 0.814$ ($n = 97$) for BCT respectively. Figure 3 shows the distribution of phenotypes in the BCB and BCT progenies for both years. Based on the levels of foliar glycoalkaloids present in both populations solasodine was the predominant aglycone, averaging 15.6 mg/g dry weight in BCB and 2.0 mg/g dry weight in BCT over both

Table 1 Concentration of foliar glycoalkaloids (mg/g dry weight) present in the parents of BCB and BCT (*nd* none detected)

Parent	Solanine + Chaconine (Solanidine Aglycone)		Solasonine + Solamargine (Solasodine Aglycone)		Total glycoalkaloids	
	1994	1995	1994	1995	1994	1995
USW2230	–	6.5	nd	nd	–	6.5
HH1-9	0.1	2.2	nd	nd	0.1	2.2
PI 473331a	nd	nd	25.1	29.9	25.1	29.9
PI 473331b	nd	nd	14.0	20.7	14.0	20.7
M200-30	0.1	nd	0.3	nd	0.4	nd

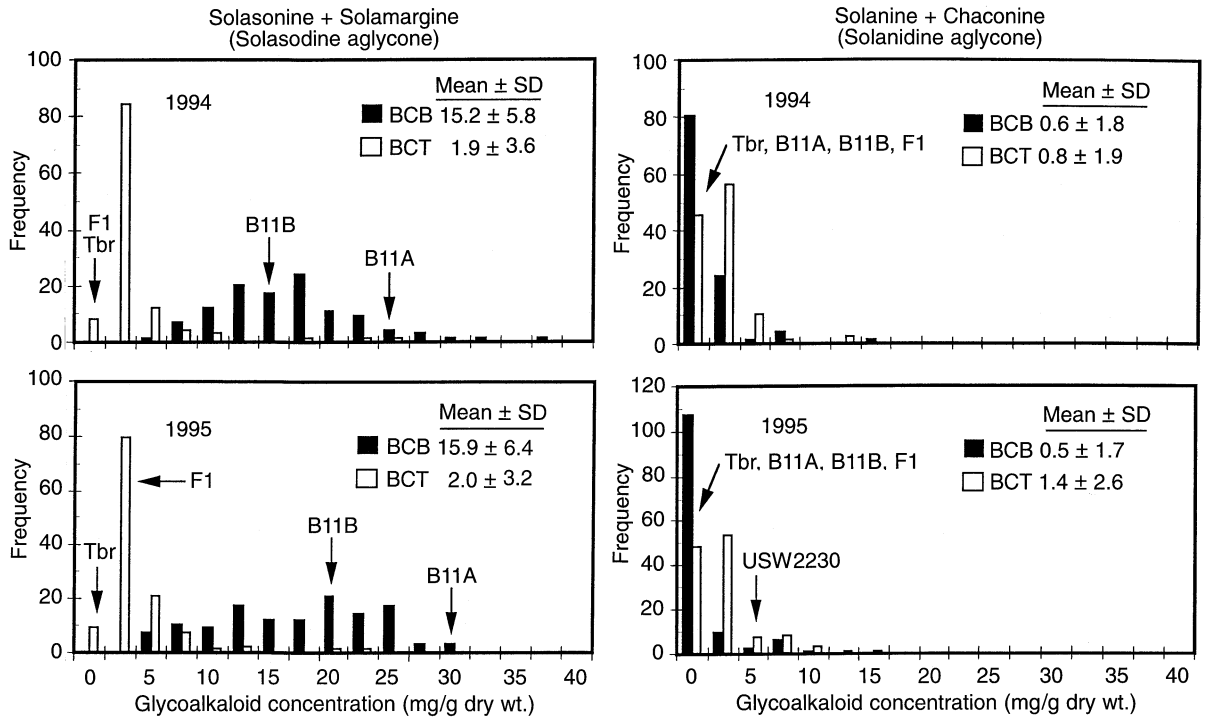


Fig. 3 Frequency distributions of solasodine and solanidine for 1994 and 1995 in BCB and BCT. Means for the parental *S. tuberosum* (*Tbr*) HH1-9 and USW2230, *S. berthaultii* PI473331a (B11A) and PI473331b (B11B), and F_1 hybrid are indicated by *arrows*

years, compared with solanidine aglycone levels of 0.6 and 1.1 mg/g dry weight in BCB and BCT, respectively (Fig. 3). Whereas solasodine was the major aglycone present in both populations, solanidine comprised approximately 30–40% of the total aglycone present in BCT; this in contrast to BCB, where solanidine only comprised approximately 3–4% (Fig. 3).

QTLs for solasodine in BCB

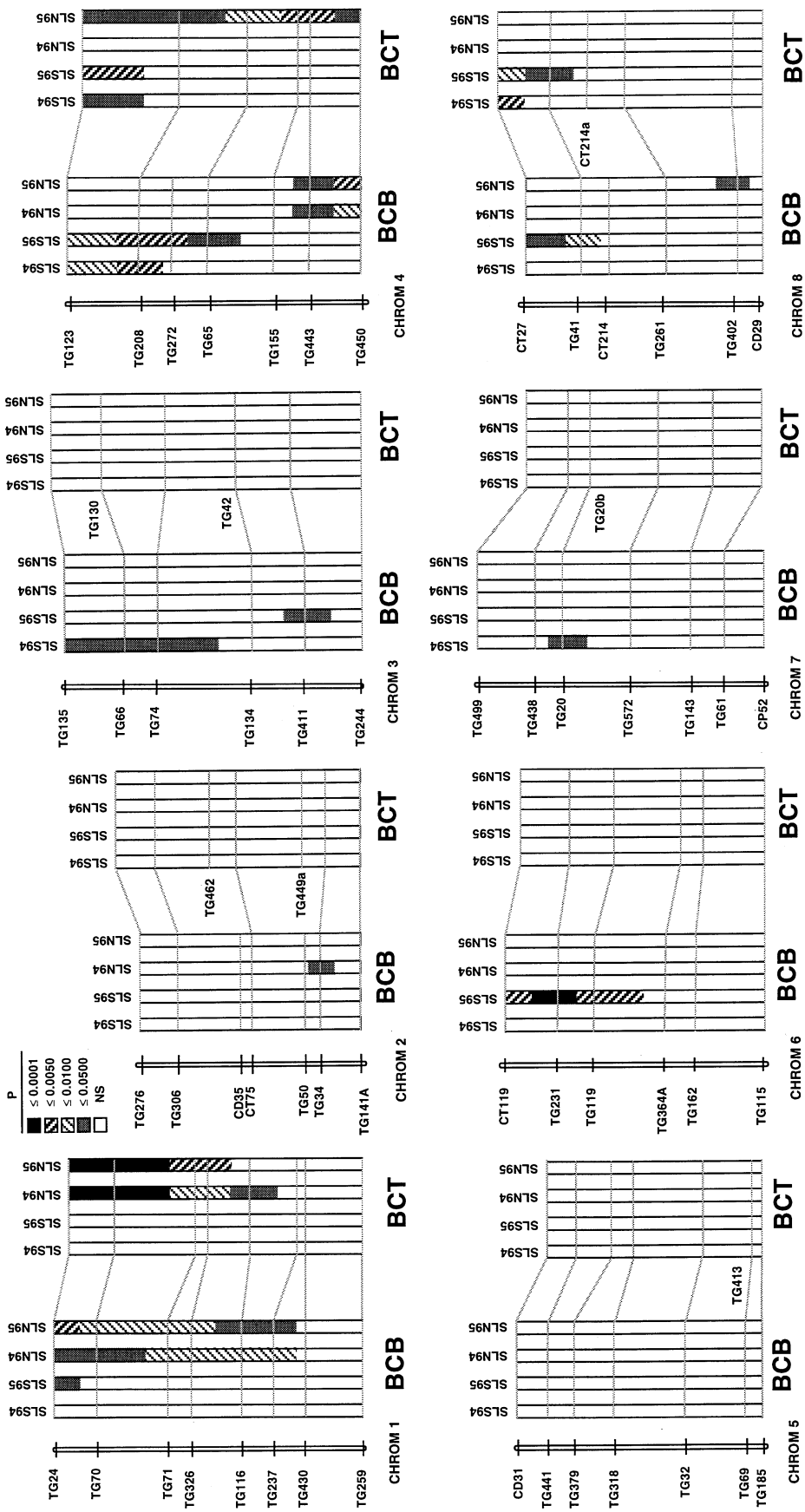
Three QTLs were identified for solasodine accumulation in BCB. These were located on chromosomes 4, 6, and 12 in marker intervals TG123-TG272, CT119-TG231, and TG263a-TG360, respectively (Table 2 and Fig. 4). At the loci on chromosomes 6 and 12, substitution of a *tbr* allele for a *ber* allele resulted in decreased solasodine accumulation (Table 2). Tests for the effect of segregation of loci in the recurrent *ber* parent indicated that the same locus on chromosome 12 near TG263 identified for segregation of the alleles from the hybrid also significantly influenced the expression of solasodine, further indicating the importance of this locus on solasodine accumulation. At the locus on chromosome 4 in the interval TG123-TG272 an allele from *tbr* increased accumulation of solasodine. This result was surprising because *tbr* alleles were not expected to increase accumulation of solasodine.

Averaged over 1994/1995, the QTLs on chromosomes 4 and 12 each explained approximately 8% of the variation in solasodine in BCB, while the QTL on chromosome 6 explained approximately 5%. When the data for these markers were combined and analyzed by multiple regression in 1994 and 1995, all three were significant in the model ($P = 0.05$), there were no epistatic interactions observed among them, and they explained approximately 20% of the variation in the concentration of solasodine in BCB. Tests to identify epistatic interactions between loci other than the major QTLs uncovered indicated that the locus near marker TG263a on chromosome 12 interacted epistatically with a locus near TG44 on chromosome 11 in both 1994 ($P < 0.01$) and 1995 ($P \leqslant 0.003$). The phenotypic effect of marker genotype at these loci on accumulation of solasodine is shown in Table 3. In both years, the effect of allelic substitutions at marker TG263a, resulting in increased solasodine accumulation, was much greater when TG44 was homozygous *ber*. Including the interaction term in the above-mentioned model increased the amount of variation explained in solasodine accumulation in BCB to 22% and 28% in 1994 and 1995, respectively.

QTLs for solanidine in BCB

In addition to the QTLs identified in BCB for solasodine, two additional QTLs were identified for the

Fig. 4. See page 569 for legend



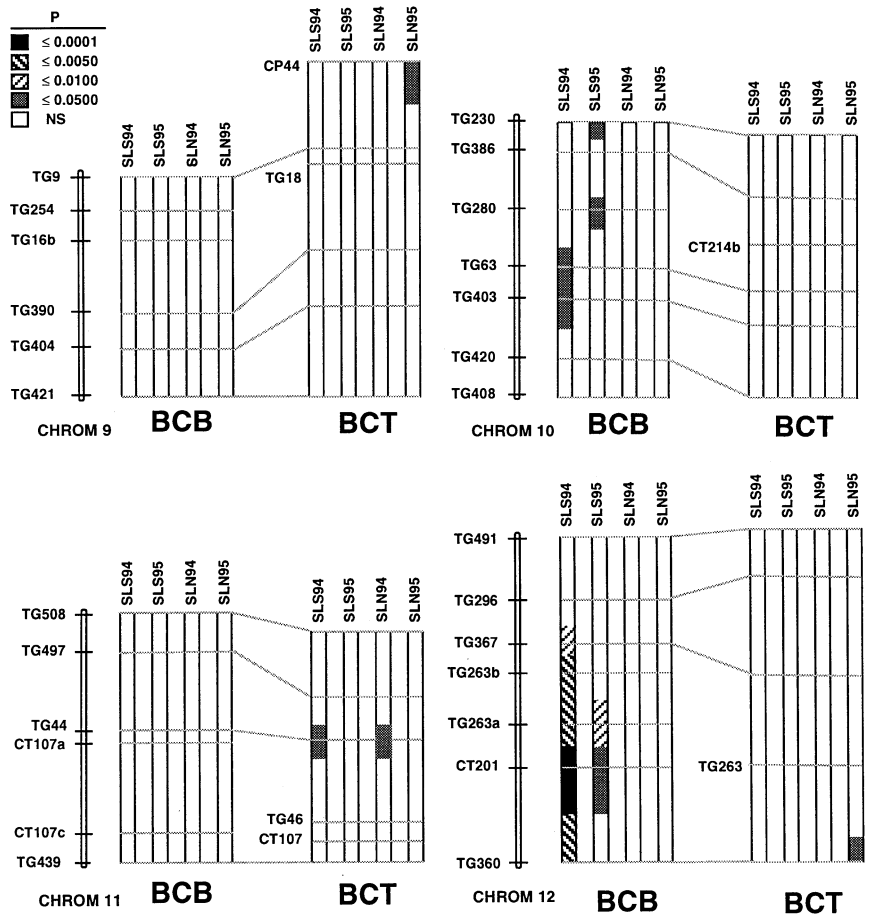


Fig. 4 Location of QTLs for solasodine (*SLS*) and solanidine (*SLN*) accumulation in BCB and BCT. *Vertical bars* represent the chromosomes, and estimated locations of the RFLP markers are indicated along the chromosomes in BCB and BCT. The location of QTLs and single-point ANOVA significance level (*P*) in each cross are indicated by the *shaded boxes*. Markers located to the *left* of the QTL box plots of BCT indicate markers which were not used in BCB. For distances between marker loci and original map data see Bonierbale et al. (1994)

synthesis of the solanidine. These were located on chromosomes 1 and 4 along marker intervals TG70-TG71 and TG443-TG450, respectively. At each locus an allele from *tbr* increased the accumulation of solanidine (Table 2). The QTL along marker interval TG70-TG71 explained approximately 5% of the variation in solanidine accumulation in 1994/1995, and this was also noted in BCT (Table 4), while the QTL located near marker interval TG443-TG450 explained approximately 7%. When the marker data at these loci were simultaneously analyzed using multiple regression both loci were significant ($P = 0.05$), and they explained approximately 10% of the variation in solanidine concentration in BCB. No epistatic interactions were noted for the accumulation of solanidine between these or any other additional loci in BCB. Analysis of alleles segregating in the recurrent *ber* parent indicated that an allele from *ber* near marker TG185 on chromosome 5 also influenced the accumulation of solanidine (Table 2). The effect of this locus on solanidine accumulation was weak, but it was consistently noted in both years and explained approximately 4% of the variation of these compounds.

QTLs for solasodine in BCT

Two QTLs for the accumulation of solasodine were located near marker intervals TG123-TG208 and CT27-TG41 on chromosomes 4 and 8, respectively (Table 4 and Fig. 4). The QTL on chromosome 4 was also present in BCB, and its effects were consistent with that observed in BCB (i.e., in each case an allele from *tbr* increased the accumulation of solasodine). In both years, each QTL accounted for approximately 6% of the variation in solasodine levels. An additional QTL for solasodine accumulation was indicated on chromosome 11 near TG44 in 1994 but not in 1995. At this locus, an allele from *ber* increased the concentration of solasodine in BCT. When all three loci were compared simultaneously using multiple regression for 1994 and 1995, they were significant ($P = 0.05$), acted additively, and explained approximately 10% of the variation in solasodine. Tests for interaction between these and other loci indicated no consistent interactions between loci. Analysis of the effects of segregation of loci in the recurrent *tbr* parent on the accumulation of solasodine indicated that a QTL with a weak, but consistent, effect was located near marker TG130 on chromosome 3 (Table 4).

Table 2 QTLs detected for solasodine and solanidine accumulation in BCB. Parental source of QTL, percent variance explained, and effect of RFLP genotype on solasodine and solanidine glycoalkaloid concentration are shown. The genotype classes BB, BT, and BB′ represent both alleles derived from *S. berthaultii*, an allele from *S. berthaultii* and *S. tuberosum*, and different alleles derived from the recurrent *S. berthaultii* parent, respectively

Trait/marker	Chromosome	Source	*P*	Percent variance explained	Mean ± SE[a] (*n*)	
Segregation from hybrid parent					BB	BT
Solasodine – 1994						
TG208	4	TBR	0.0038	7.7	13.33 ± 0.68 (44)	16.61 ± 0.79 (63)
TG231	6	BER	0.0821	2.8	16.11 ± 0.70 (57)	14.17 ± 0.67 (52)
TG263a	12	BER	0.0006	10.4	17.08 ± 0.83 (55)	13.35 ± 0.65 (55)
Solasodine – 1995						
TG208	4	TBR	0.0015	8.1	13.60 ± 0.93 (48)	17.32 ± 0.70 (71)
TG231	6	BER	0.0010	8.6	17.87 ± 0.79 (60)	14.09 ± 0.79 (63)
TG263a	12	BER	0.0071	5.8	17.66 ± 0.78 (56)	14.56 ± 0.80 (68)
Solanidine – 1994						
TG70	1	TBR	0.0201	5.0	0.16 ± 0.05 (54)	0.99 ± 0.35 (54)
TG443	4	TBR	0.0225	4.8	0.15 ± 0.05 (51)	0.96 ± 0.33 (57)
Solanidine – 1995						
TG70	1	TBR	0.0078	5.7	0.12 ± 0.09 (67)	0.93 ± 0.30 (56)
TG443	4	TBR	0.0220	4.3	0.12 ± 0.11 (57)	0.81 ± 0.26 (66)
Segregation from *S. berthaultii* parent					BB	BB′
Solasodine – 1994						
TG280	10	BER	0.0255	4.6	16.19 ± 0.74 (64)	13.65 ± 0.81 (45)
TG263	12	BER	0.0004	14.8	17.43 ± 1.10 (39)	12.76 ± 0.66 (41)
Solasodine – 1995						
TG280	10	BER	0.0092	5.5	17.18 ± 0.73 (71)	14.16 ± 0.88 (52)
TG263	12	BER	0.0058	8.2	17.61 ± 0.99 (42)	13.92 ± 0.86 (50)
Solanidine – 1994						
TG185	5	BER	0.0318	4.2	0.21 ± 0.11 (58)	0.98 ± 0.35 (51)
Solanidine – 1995						
TG185	5	BER	0.014	4.8	0.14 ± 0.12 (65)	0.87 ± 0.28 (59)

[a] Concentration (mg/g dry weight)

QTLs for solanidine in BCT

A major QTL located on chromosome 1 near marker TG70 was identified in BCT for solanidine in 1994 and 1995. The effective allele at this QTL was derived from *tbr*, and it was highly significant ($P = 0.00001$) accounting for approximately 17% and 22% of the variation in solanidine concentration in 1994 and 1995, respectively (Table 4 and Fig. 4). This same QTL was also detected in BCB where the effect was from *tbr*, and it explained approximately 5% of the variation in solanidine accumulation (Table 2 and Fig. 4). A second putative QTL located near TG443 on chromosome 4 was identified, but its effect was not as strong as the QTL on chromosome 1 and it was not significant in both years of the study (Table 4). Tests for interaction between TG70 and other loci indicated epistasis with loci located near intervals TG155-TG443 on chromosome 4 and CT119-TG231 on chromosome 6 in 1994 and 1995 (Table 5). When all three loci were included in a multiple regression model that included interaction terms for TG155 and TG231 for the 1994 data, all three were significant ($P = 0.05$) and the model explained approximately 31% of the variation in the concentration of solanidine. However, when this same model was

Table 3 Mean concentration (mg/g dry weight) of solasodine in (*n*) individuals in each of eight di-genotypic classes for TG263a and TG44 in BCB. Note that the effect of an allele substitution (BB vs BT) was much greater when TG44 was homozygous for *ber* (BB) than heterozygous (BT)

TG44	1994 TG263a		1995 TG263a	
	BB	BT	BB	BT
BB	18.6 (28)	12.2 (26)	20.0 (28)	13.5 (29)
BT	15.4 (26)	14.4 (29)	15.1 (27)	15.4 (39)

Table 4 QTLs detected for solasodine and solanidine accumulation in BCT. Parental source of QTL, percent variance explained, and effect of RFLP genotype on solasodine and solanidine glycoalkaloid concentration are shown. The genotype classes TT, TB, and TT′ represent both alleles derived from *S. tuberosum*, an allele from *S. berthaultii* and *S. tuberosum*, and different alleles derived from the recurrent *S. tuberosum* parent, respectively

Trait/marker	Chromosome	Source	*P*	Percent variance explained	Mean ± SE[a] (*n*)	
Segregation from hybrid parent					TT	TB
Solasodine – 1994						
TG123	4	TBR	0.0428	3.7	2.49 ± 0.56 (64)	1.11 ± 0.25 (49)
CT27	8	TBR	0.0042	7.3	3.17 ± 0.89 (39)	1.15 ± 0.17 (72)
Solasodine – 1995						
TG123	4	TBR	0.0040	7.0	2.76 ± 0.49 (64)	1.02 ± 0.27 (52)
CT27	8	TBR	0.0055	6.6	3.11 ± 0.76 (42)	1.35 ± 0.18 (73)
Solanidine – 1994						
TG70	1	TBR	0.0000	16.5	1.72 ± 0.39 (48)	0.16 ± 0.04 (65)
TG443	4	TBR	0.0708	3.0	1.04 ± 0.26 (76)	0.31 ± 0.11 (33)
Solanidine – 1995						
TG70	1	TBR	0.0000	21.7	2.72 ± 0.48 (50)	0.31 ± 0.10 (67)
TG443	4	TBR	0.0052	6.8	1.80 ± 0.34 (78)	0.36 ± 0.13 (36)
Segregation from *S. tuberosum* parent					TT	TT′
Solasodine – 1994						
TG130	3	TBR	0.0120	5.6	1.03 ± 0.22 (55)	2.71 ± 0.61 (58)
Solasodine – 1995						
TG130	3	TBR	0.0301	4.0	1.25 ± 0.27 (53)	2.56 ± 0.49 (64)

[a] Concentration (mg/g dry weight)

applied to the 1995 data, the interaction between TG70 and TG231 was not significant. Dropping the interaction term for TG231 from the model did not affect the fit of the model appreciably. The influence of this locus on solanidine accumulation is thus equivocal. Analysis of the effects of segregation of loci in the recurrent *tbr* parent on the accumulation of solanidine revealed no further QTLs.

Discussion

Touzet et al. (1995) noted that very little is known about the biological nature of quantitative or natural variation in terms of the contribution of individual genes and their biochemical functions. To date, most QTL studies of plant species have examined traits expressed as gross morphological phenotype (e.g., height, yield, fruit shape, time to flowering, disease and insect resistance). Although such traits are the result of biochemical and genetic factors which underlie them, these studies provide little insight into the relationship between quantitative variation in phenotype and metabolism/gene expression. Recent investigations of maize (Causse et al. 1995; Touzet et al. 1995; Byrne et al. 1996) have advanced our understanding of this relationship.

Causse et al. (1995), in a study designed to elucidate the impact of carbon-enzyme activities on growth in maize, identified QTLs for early growth traits, concentration of carbohydrates, and the activity of four key enzymes (sucrose phosphate synthase, ADP-glucose pyrophosphorylase, invertases, and sucrose synthase) involved in carbohydrate metabolism. By comparing the locations of QTLs identified for the phenotypic measures of growth with the locations of maize biochemical attributes (i.e., carbohydrate concentration and enzyme activity levels) and previously cloned structural genes (candidate genes) of three of the four enzymes studied, Causse et al. (1995) provided evidence of the importance and potential impact of key carbon-metabolizing enzyme activities upon early maize growth.

Similarly, Byrne et al. (1996) conducted QTL analyses of maysin concentration in maize silks, a trait associated with resistance to corn earworm, and interpreted their results from a regulatory and biosynthetic perspective with respect to previously mapped loci in the phenylpropanoid/flavonoid pathway. They found that while both regulatory and structural loci were essential components in the flavonoid pathway, regulatory loci were key leverage points for determining maysin concentration. However, they also noted that the various branches of the flavonoid pathway interacted considerably to determine the expression of this trait (Byrne et al. 1996) and, by extension, resistance to corn earworm.

Table 5 Mean concentration (mg/g dry weight) of solanidine in (*n*) individuals in each of eight di-genotypic classes for markers TG70 and TG155 (**A**) and TG70 and TG231 (**B**) in BCT. Note that when marker TG70 was homozygous *tbr* (TT), solanidine accumulation was greatest when markers TG155 and TG231 were also homozygous TT

A

TG155	1994 TG70		1995 TG70	
	TT	TB	TT	TB
TT	2.17 (33)	0.17 (43)	3.79 (33)	0.42 (46)
TB	0.73 (15)	0.10 (20)	0.69 (16)	0.10 (21)

B

TG231	1994 TG70		1995 TG70	
	TT	TB	TT	TB
TT	3.58 (10)	0.12 (20)	4.01 (11)	0.27 (21)
TB	1.24 (38)	0.20 (44)	2.36 (39)	0.30 (45)

Unfortunately, the genetics of many biosynthetic pathways have not been thoroughly investigated, particularly where metabolites are inherited in a quantitative fashion, such as the glycoalkaloids in the Solanaceae. By means of QTL analyses of "biochemical phenotypes" (i.e., enzymes, and secondary plant compounds and their intermediates) it is possible to begin to characterize these previously refractory systems. Complex biosynthetic pathways are not readily amenable to classical, quantitative genetic analyses, but the mapping of genes (QTLs) which determine the accumulation of key intermediates or biochemical metabolites is possible. In this study, QTLs affecting the accumulation of the *Solanum* steroid alkaloid aglycones solasodine and solanidine have been mapped. This work provides heretofore inaccessible information on the molecular genetic basis of the inheritance of the steroid alkaloids solasodine and solanidine, and glycoalkaloids in general, and the biochemical pathways leading to their production.

The proposed biosynthetic pathways and intermediate structures leading from cholesterol to solanidine and solasodine are very similar (Fig. 1b) (Osman 1984; Tschesche and Spindler 1978; Ripperger and Schreiber 1981). One might therefore assume that a single pathway with the same series of enzymes and genes is involved from cholesterol to the intermediates teinemine and solaverbascine. The present and previous studies, however, suggest that two separate pathways are involved. This hypothesis is supported by three lines of evidence presented in previous experiments. First, the three-dimensional structures of solanidine and solasodine differ significantly. This is in a large part due to their stereospecific configurations, solanidine being 25S and solasodine being 25R, (Heftmann 1983; Schreiber 1979). When the three-dimensional structures of solanidine and solasodine are superimposed, the difference is striking, particularly in the D, E, and F rings (Stanker et al. 1994). Second, analyses of solasodine biosynthesis in *S. laciniatum* (in which solasodine is the only steroidal alkaloid detected; Tschesche and Brenneche 1980) and solanidine in *Veratrum grandiflorum* (in which solanidine is found at high levels; Kaneko et al. 1976, 1977) have stereochemically distinct intermediate compounds in the respective pathways. This is consistent with separate enzyme systems and genes. A similar case has been observed for solasodine (25R) and tomatine (25S) (Petersen et al. 1993). Third, in the Solanaceae, the majority of species found with solanidine or solasodine contain either one or the other, rarely both, and the identification of an individual plant which does contain both has been reported, to our knowledge, only once (Ripperger and Schreiber 1981; Van Gelder and Scheffer 1991). Chemotaxonomic studies of the genus *Solanum*, with steroidal alkaloids as indicators of divergence traits, lend further support to this observation, strongly suggesting that the occurrence of solanidine or solasodine is species-specific. This is particularly the case in the subsection *Potatoe*, where solasodine is rarely found (Petersen et al. 1993).

The QTL analyses of solasodine and solanidine in BCB and BCT provide additional evidence that distinct genes code for their accumulation. A number of QTLs for solasodine and solanidine accumulation were segregating in the BCT and BCB populations in this study (Fig. 4). These QTLs probably represent structural and/or regulatory genes in the biosynthetic pathways of solasodine and solanidine.

The major QTL detected on chromosome 12, in the region of markers CT201 and TG263a, is a candidate for a recessively inherited gene which leads to the accumulation of solasodine (Fig. 1b). This proposal is supported by: (1) the major effect of this QTL on solasodine levels over 2 years in the BCB population (Table 2); (2) the direction of the gene action, (i.e., alleles from *ber* were associated with increased levels of solasodine); (3) the fact that this locus significantly influenced solasodine accumulation in both the hybrid and recurrent *ber* parent; and (4) the absence of an effect for this chromosomal region in the BCT population.

Similarly, the major QTL detected on chromosome 1, in the region of markers TG70 and TG24, is a candidate for a partially dominant gene which leads to the accumulation of solanidine (Fig. 1b). This proposal is supported by: (1) the major effect of this QTL on solanidine levels over 2 years in the BCT population (Table 4); (2) the direction of the gene action (i.e. alleles from *tbr* are associated with increased levels of solanidine); and (3) the fact that a corresponding effect for this chromosomal region was observed in the BCB

population [i.e., heterozygotes (BT) had higher levels of solanidine than homozygotes (BB) at this locus].

For each of the major QTLs, the mode of inheritance and direction of gene action are consistent with the proposed biosynthetic pathways (Fig. 1b). The remaining QTLs identified in BCB and BCT probably represent additional structural and/or regulatory genes for biosynthesis of solasodine or solanidine. In BCB, two additional QTLs for the accumulation of solasodine were located on chromosomes 4 and 6 near markers TG208 and TG231, respectively. Another two were identified in BCT, near markers TG208 on chromosome 4 and CT27 on chromosome 8. The QTL near TG208 on chromosome 4 was expressed in both BCB and BCT which was similar to the QTL identified for solasodine accumulation on chromosome 12. However, at this locus, and at CT27, alleles from *tbr* increased solasodine accumulation. This effect was unexpected as genes from *tbr* would not, *a priori*, be expected to increase solasodine concentration. Similar cases in which hidden genetic variation is unmasked in wide-species crosses have been noted in progeny of *Lycopersicon esculentum* × *L. pimpinellifolium* crosses (Grandillo and Tanksley 1996). Indeed, it has been suggested that such cases of transgressive segregation may provide new sources of favorable genetic variation for plant breeding (De Vicente and Tanksley 1993). Similarly, Laurila et al. (1996) have noted that in somatic hybrids between *S. brevidens* and *S. tuberosum*, genes can recombine yielding new types of glycoalkaloids with results contrary to expectations.

Compared to solasodine, fewer QTLs were identified for solanidine accumulation in BCB and BCT. Further, the QTLs identified accounted for more of the variation (approx. 30%) in solanidine concentration, and none of these were derived from *ber*. Only three epistatic interactions were detected – one in BCB and two in BCT. In each case, the major QTLs identified in BCB and BCT (TG263a and TG70, respectively) interacted epistatically with loci located on another chromosome. However, since the number of comparisons between loci to be made in each contrast was low, and their effects were not exceptionally strong, it is difficult to draw conclusions about the importance of these interactions. Nevertheless, the three loci which interacted epistatically with the two major QTLs on chromosome 1 and 12, were also QTL for either solanidine or solasodine biosynthesis in either BCB or BCT (Fig. 4). Such an interaction is consistent with their involvement in the biosynthetic pathway of these steroid alkaloids.

This work provides previously unavailable information on the molecular genetic basis of the inheritance and accumulation of solasodine and solanidine, and the production of foliar glycoalkaloids in potato. Of further interest would be comparisons of the foliar and tuber glycoakaloid levels in this same set of clones. Sanford et al. (1996a) have indicated that foliar and tuber glycoakaloid levels are moderately correlated ($r = 0.41$, $P < 0.01$). Further, the synthesis of glycoakaloids has been shown to be organ-specific; that is, glycoakaloids are not translocated from leaves to tubers (Sanford et al. 1992). Thus, it is likely that there is some overlap in the gene(s) controlling glycoalkaloid production in leaves and tubers. Separate QTL analyses of foliar and tuber glycoakaloid levels conducted from the same plants could be used to address this question. To our knowledge, structural or regulatory genes for steroid biosynthesis have not been isolated. Therefore, this work does not permit specific determinations of gene assignments or candidate loci for glycoalkaloid production. However, the gene encoding the enzyme that catalyzes the glycosolation of solanidine (solanidine UDP-glucose glucosyltransferase) in the glycoalkaloid pathway has recently been cloned (Moehs et al. 1997). It would be of considerable interest to use a candidate gene approach to map the location of this gene on the potato genome and determine if it coincides with any of the QTLs identified in this study. This information would further contribute to our understanding of the localization and organization of genes for glycoalkaloid biosynthesis in potato.

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