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Identification of molecular markers associated with leptine production in a population of *Solanum chacoense* Bitter

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Abstract *Solanum chacoense* Bitter, a wild relative of the cultivated potato, produces several glycoalkaloids, including solanine, chaconine, and the leptines. The foliar-specific leptine glycoalkaloids are believed to confer resistance to the Colorado Potato Beetle (CPB). Using two bulked DNA samples composed of high- and low-percent leptine individuals from a segregating F_1 population of *S. chacoense*, we have identified two molecular markers that are closely linked to high percent solanine + chaconine and, conversely, to nil/low percent leptine. One of these, a 1,500-bp RAPD product (UBC370-1500), had a recombination value of 3% in the F_1 progeny, indicating tight linkage. UBC370-1500 mapped to the end of the short arm of potato chromosome 1, in the region of a previously mapped major QTL for solanidine, from a *S. tuberosum* (solanidine) × *S. berthaultii* (solasodine) cross. Taken together, these results suggest that either (1) a major locus determining solanidine accumulation in *Solanum* spp. is on chromosome 1 in the region defined by the RFLP markers TG24, CT197, and CT233, or (2) this region of chromosome 1 may harbor two or more important genes which determine accumulation of steroidal aglycones. These findings are important for the genetics of leptine (as well as other glycoalkaloid) accumulation and for the development of CPB-resistant potato varieties.

Key words Glycoalkaloids · Potato · Metabolic pathways · RAPD · Leptine · Insect resistance · *Solanum*

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Introduction

Steroidal glycoalkaloids, such as α-tomatine in tomato and α-solanine and α-chaconine in potato, are natural toxins present in several species within Solanaceae. In foliage, these compounds have been implicated as resistance factors to plant pathogens (Maga 1994). However, nearly all potato glycoalkaloids accumulate in tubers as well, where they can be potentially hazardous for human consumption (Friedman and McDonald 1997). In contrast, leptine glycoalkaloids are foliar-specific and, to date, have been found in only a few accessions of *Solanum chacoense* Bitter (Kuhn and Löw 1961a; Sinden et al. 1986b). The leptine glycoalkaloids are believed to confer resistance to the Colorado Potato Beetle (*Leptinotarsa decemlineata* Say; CPB) (Kuhn and Löw 1961b; Stürckow and Löw 1961; Sinden et al. 1986a; Sinden et al. 1988) and, as such, are considered to be a possible source of genetic resistance for introgression into the cultivated potato, *Solanum tuberosum* L. Genetic resistance is an attractive alternative to high-cost, environmentally damaging pesticides.

S. chacoense is a tuber-bearing, diploid (2n = 2x = 24), self-incompatible species. This highly variable species is found in geographically diverse regions of southern S. America, including Bolivia, Argentina, Brazil, and Paraguay, where both highland and lowland accessions have been collected (Hawkes 1962). The fact that a few genotypes within *S. chacoense* are highly resistant to CPB makes this species of great interest to potato breeders. *S. chacoense* genotypes have been

identified that produce high concentrations and proportions of leptines relative to other glycoalkaloids (Sinden et al. 1986b). Although diploid *S. chacoense* does not readily cross with tetraploid *S. tuberosum*, when the chromosome number is doubled, tetraploid *S. chacoense* produces fertile F_1 hybrids when crossed to tetraploid *S. tuberosum* (Sanford et al. 1996). Resulting hybrids were found to contain intermediate levels of foliar leptines, with none in the tubers. Such results indicate that it may be possible to introgress the gene(s) for foliar-specific leptine synthesis into cultivated potato.

As a percentage of total foliar glycoalkaloids (TGA) in *S. chacoense*, leptine content has been found to be inversely proportional to that of solanine and chaconine (sol/chac) (Ronning et al. 1998), the predominant glycoalkaloids found in *S. tuberosum*. It has been proposed that, in *S. chacoense*, leptines are synthesized by the hydroxylation of solanidine, a common precursor found in several *Solanum* spp., to leptinidine, which is then acetylated to form acetylleptinidine (Lawson 1993; Lawson et al. 1993). Finally, glycosylation occurs to form leptines. Chemically, the leptines are identical to sol/chac except for the acetylation of carbon 23; leptines I and II are the acetylated analogs of α-chaconine and α-solanine, respectively. Leptine I/chaconine differs from leptine II/solanine by the attached sugar (chacotriose and solatriose, respectively).

Recent developments in molecular biology, particularly the polymerase chain reaction (PCR), have made techniques such as random amplified polymorphic DNA (RAPD) (Welsh and McClelland et al. 1990; Williams et al. 1990) readily available for use in breeding and genetics at the molecular level. Molecular markers such as RAPDs that are linked to a desirable phenotypic trait may increase the selection efficiency in a breeding program by decreasing the generation time required for observation of the trait. Also, by making early selection possible needs for valuable field and/or greenhouse space can be lessened. Identification of molecular markers is simplified by bulking DNA from individuals exhibiting the extremes of the phenotype for the trait in a segregating population (Michelmore et al. 1991). Such an approach has been used successfully to identify molecular markers in several crops, including those linked to fruit skin color and scab resistance in apple (Cheng et al. 1996; Yang et al. 1997), tomato spotted wilt virus resistance in tomato (Chagué et al. 1996), nematode resistance in peanut (Garcia et al. 1996), bean common mosaic virus resistance in bean (Melotto et al. 1996), and beet necrotic yellow vein virus resistance in beet (Scholten et al. 1997). Such markers may be useful in applied marker-aided selection for the particular trait, as has been demonstrated for potato virus Y resistance in potato (Hämäläinen et al. 1997).

The goal of the study presented here was to identify molecular marker(s) linked to leptine production in a segregating family of *S. chacoense* and to use this marker to study the inheritance of leptine production. We report here the identification of two molecular markers associated with leptine production, one of which is a RAPD fragment closely linked to high sol/chac (nil/low leptine) content. We also report that the chromosomal location of this RAPD marker on the potato map coincides with that of a previously mapped major QTL for solanidine synthesis (Yencho et al. 1998). These findings have implications for the genetics of leptine and other glycoalkaloid accumulation in *S. chacoense*, and possibly in other *Solanum* species as well.

Materials and methods

Plant material

The *S. chacoense* parents, 55-1 and 55-3 (high and nil leptine as a percentage of total foliar glycoalkaloids, respectively), are sibling selections from PI 320287. Because *S. chacoense* is a self-incompatible outcrossing species, accessions are not inbred but maintained by the intermating or sib-mating of desired individuals. Reciprocal crosses were made between 55-1 and 55-3 to produce two segregating F_1 families (Ronning et al. 1998). Family 9501 consisted of 94 individuals derived from 55-3 X 55-1. Family 9502, derived from the reciprocal cross (55-1 × 55-3), included 60 individuals. Also included was the *S. tuberosum* cultivar 'Kennebec'.

Glycoalkaloids

Total glycoalkaloids (TGA) were extracted from 8 g FW leaf material in acidified ethanol, as described previously (Bushway et al. 1986; Carman et al. 1986; Sanford et al. 1996). Samples were boiled in a steam bath, evaporated to remove the ethanol, and the glycoalkaloid reagent of Carman et al. (1986) was added. The extract was filtered and purified using a pre-conditioned C_{18} Sep-Pak syringe cartridge (Waters Associates, Milford, Mass). Leptines I and II were analyzed from 20 µl of the Sep-Pak eluates as %TGA by the column and HPLC method of Sinden et al. (1986b). The Waters HPLC equipment consisted of a model 510 pump, 717 plus Autosampler, and Lambda-Max 481 variable wavelength detector (Waters, Milford, Mass). Detection was at 205 nm, with the glycoalkaloid α-solanine (Sigma, St. Louis, Mo.) used as the chromatographic standard.

DNA extraction

Nucleic acids were extracted according to the protocol of Dellaporta et al. (1983), with modifications. Two g FW of leaf material was freeze-dried and stored at −20°C until extraction. This material was finely ground in a mortar and pestle, then incubated 10 min in a 65°C waterbath with 15 ml of extraction buffer (100 m*M* TRIS-HCl, pH 8, 100 m*M* NaCl, 50 m*M* EDTA, 1% (w/v) SDS, and 1% (v/v) 2-mercaptoethanol). Five ml of 5 *M* potassium acetate was added, mixed by inversion, and the solution incubated on ice for 20 min. The samples were centrifuged at 23,500 *g* for 20 min at 4°C, and the supernatant filtered through Miracloth (CalBiochem, San Diego, Calif.). Five hundred microliters of 10 *M* ammonium acetate and 20 ml of ice-cold isopropanol were added to the filtrate, and the mixture was incubated at −20°C for 20 min. The samples were then

centrifuged at 4,500 *g* for 15 min at 4°C, and the pellet washed (70% EtOH; 50 m*M* ammonium acetate) and centrifuged again. After air-drying, the pellet was resuspended in 1.2 ml of TE and treated with Rnase A. The final pellet was dried in a DNA Speed Vac 110 (Savant Instruments, Farmingdale, N.Y.) and resuspended in 100 µl sterile ddH_2O. DNA concentration was determined by electrophoresis with lambda DNA standards.

For bulk segregant analysis, equal amounts of genomic DNA from the highest and lowest percent leptine producers from each of the F_1 families (9501 and 9502) were pooled. The resulting bulks each consisted of DNA from 16 individuals.

PCR analysis

Genomic DNA from the two parents and from the DNA bulks was amplified with random (RAPD) 10-base primers (Operon Technologies, Alameda, Calif., University of British Columbia (UBC), Vancouver, B.C.). The RAPD reactions were modified from the method described by Stommel et al. (1997) and consisted of 1 µl of 10× buffer [0.2 *M* NaCl; 0.5 *M* TRIS-HCl, pH 9; 10% (w/v) Triton X-100; 1% (w/v) BSA], 2 m*M* $MgCl_2$, 200 µm each dNTP, 0.3 U Ampli*Taq* DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn.), 2 pmol primer, and 10 ng gDNA, in a total volume of 10 µl. The reactions were subjected to 45 cycles of 94°C, 15 s, 45°C, 15 s, 72°C, 30 s, followed by a 5-min extension at 72°C.

The bulks were also amplified using double-stringency PCR (DS-PCR) (Matioli and de Brito 1995). DS-PCR reactions were performed with varying combinations of single microsatellite (SSR) primers with RAPD primers. For each microsatellite primer/random primer combination, PCR reactions consisted of 10× -II PCR buffer (Perkin Elmer), 2.5 m*M* $MgCl_2$, 200 µm each dNTP, 0.25 U Ampli*Taq* DNA polymerase (Perkin Elmer), 2 pmol microsatellite primer, 7.5 pmol random primer, and 10 ng genomic DNA, in a total volume of 10 µl. Samples were then subjected to 94°C for 1 min, followed by 15 cycles of 94°C, 20 s, 60 s at an annealing temperature dependent on the microsatellite primer; then 72°C, 20 s. This was followed by 25 cycles of 94°C, 30 s; 35°C, 30 s; 72°C, 10 s, with a 60-s ramp. Finally, a 10-min extension at 72°C was added.

Primers or primer combinations producing fragments that were polymorphic between bulks were then used to screen F_1 individuals to confirm linkage. All PCR reactions were performed on a Perkin Elmer 9600 DNA thermocycler, and PCR products were electrophoresed on 1.5% agarose and stained with ethidium bromide.

Sequencing

Individual fragments from primers with confirmed, tight linkage were purified from low-melting point agarose using Elutip-D minicolumns (Schleicher and Schuell, Keene, N.H.) and cloned into pGEM®-T Easy (Promega, Madison, Wis.). Cloned fragments were sequenced with the Silver Sequence™ DNA Sequencing System (Promega) using pUC/M13 forward and reverse primers. Sequence comparisons were performed with the program CLUSTALW (Thompson et al. 1994).

Data analysis

Distribution of percent leptine in these families is discontinuous, forming two discrete classes consisting of individuals containing nil/low proportions of leptines ($\leq$17% of TGA) or high proportions ($\geq$62% of TGA) (Ronning et al. 1998). Individual F_1 progeny were classified into one of these two groups. Observed segregation of high versus low percent leptine was tested for deviation from expectation by χ^2.

RAPD and DS-PCR bands were scored for presence/absence. Associations between the presence or absence of bands and leptine proportion class (high or low) were visually assessed and compared to the parents, 55-1 and 55-3. Recombination values were calculated as the number of recombinants divided by the total number of scored individuals in the family.

A 1500-bp RAPD product and a 420-bp DS-PCR product that were found to be associated with leptine production were subjected to three-point linkage analysis to determine gene order.

Mapping

The 1,500-bp RAPD product with close linkage to leptine production was cloned as described above and used as an RFLP for mapping to the potato and tomato maps. The mapping population for potato was derived from the cross *Solanum tuberosum* × *S. berthaultii* backcrossed to *S. berthaultii* (BCB) (Tanksley et al. 1992). The tomato map was derived from the interspecific cross *Lycopersicon esculentum* cv 'VF36-*Tm2a*' × *L. pennellii* LA716 (Tanksley et al. 1992). Chromosomal location was determined using MAPMAKER (Lander et al. 1987) with LOD greater than 4 in both maps. Map distances were calculated using the Kosambi function (Kosambi 1944).

Results

The parental genotypes, 55-1 and 55-3, were initially amplified using approximately 350 different random (RAPD) primers. Seventy of these primers produced polymorphic fragments between the parents, and were then used to screen the F_1 bulks. Of these 70 primers, 12 were polymorphic between bulks. RAPD primer UBC370 (5′-tcagc cagcg-3′) produced a 1,500-bp fragment (designated UBC370-1500), which was identified from bulk segregant analysis to be associated with nil/low percent leptine. When genomic DNA from individual plants in the F_1 populations was amplified with this primer, the 1,500-bp band was found to be associated with high sol/chac (nil/low leptine) proportion in all but 3 individuals in family 9501 and all but 2 individuals in family 9502 (Fig. 1, Table 1). A comigrating 1,500-bp fragment also appeared in *S. tuberosum* cv 'Kennebec' (Fig. 1).

The F_1 bulks were also amplified with a total of 436 different combinations of microsatellite primers with UBC random primers using the DS-PCR method. Ten of these pairs were polymorphic between bulks, while the remainder were either monomorphic or did not amplify. Of the 10 polymorphic primer pairs, one association was found between products from DS-PCR with RAPD primer UBC518 (5′-tgctc gtcca-3′) and microsatellite primer MISTFDHD-forward (5′-ctttt catcc tgcct atgtc-3′), using an annealing temperature of 51°C. This 420-bp product, designated Mf/518-420, was linked to nil/low percent leptine with 11.9% recombination in the combined F_1 families (9 recombinant individuals were found in family 9501; 8 recombinants in 9502).

In addition to RAPD and DS-PCR analysis, 33 pairs of microsatellite (SSR) primers were used to amplify the

bulks. None of these detected polymorphism between the bulks.

The relative order of the two markers, UBC370-1500 and Mf/518-420, and the locus for leptine production was determined to be Mf/518-420 – leptine – UBC370-1500, with recombination frequencies of 12.6% between Mf/518-420 and leptine and 3.5% between leptine and UBC370-1500.

A partial sequence of UBC370-1500 was obtained from 55-3 and from 'Kennebec.' Both fragments have close homology at the 5′ and 3′ ends (Fig. 2). The cloned fragment UBC370-1500 from *S. chacoense* 55-3 was located at the end of the short arm of chromosome 1 on both the potato and tomato maps, 5.5 cM from CT197 (potato) and between CT233 and CT2 on the tomato map (6.3 and 2.0 cM distances, respectively; Fig. 3). This location coincides with that of a previously mapped QTL for solanidine biosynthesis (Fig. 3a; Yencho et al. 1998).

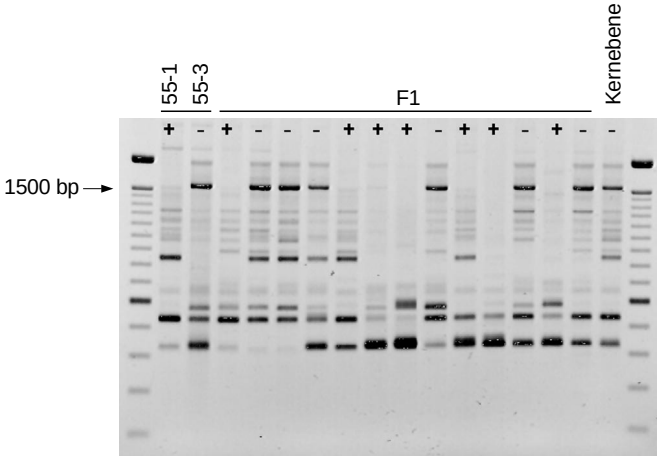

Fig. 1 PCR fragments produced by RAPD primer UBC 370, showing 1,500-bp band asociated with high percent sol/chac (nil/low percent leptine). *S. chacoense* 55-1 and 55-3 are the high- and nil-percent leptine parents, respectively, of the F_1. Also shown is *S. tuberosum* cv 'Kennebec'. + High leptine as a percentage of TGA; − nil/low percent leptine. Gel is 1.5% agarose stained with EtBr; molecular weight markers are a 100-bp ladder (Gibco BRL, Gaithersburg, Md.)

Discussion

Among the random primers utilized, 1 primer produced a fragment closely associated with leptine production and was mapped to chromosome 1 of potato and tomato. This RAPD marker, UBC370-1500, is tightly linked to high percent sol/chac (nil/low percent leptine) in this *S. chacoense* population. One of the DS-PCR primer combinations also produced a fragment that was associated with nil/low percent leptine. This marker (Mf/518-420) was not as tightly linked as that of UBC370-1500. The microsatellite primer for this primer pair was deduced from the GenBank database sequence of *S. tuberosum* mRNA for formate dehydrogenase (accession ♯Z21493) and contains the repeat $(GCT)_5$. To our knowledge, these are the first molecular markers identified that are associated with the production of leptine glycoalkaloids.

To date, no markers have been found linked to high percent leptine. As an obligate outcrossing species, *S. chacoense* should be highly heterozygous. Segregation of leptine proportion in these F_1 populations indicated that a single recessive gene has a large effect on leptine synthesis; however, segregation patterns in inter-F_1 and backcross families derived from the F_1 families 9501/9502 indicated a more complex mode of inheritance for this trait (Ronning et al. 1998). Apparently, at least a second gene or group of linked genes involved in leptine production, which may have complementary effects, is segregating in these families (Ronning et al. 1998) but could not be identified from this set of crosses.

UBC370-1500, the DNA fragment with tight linkage to high percent sol/chac (nil/low percent leptine), was also found in *S. tuberosum* (Figs. 1, 2). The 3′ and 5′ ends of this fragment from the *S. chacoense* genotype 55-3 and those from *S. tuberosum* cv 'Kennebec' are closely

Table 1 Observed segregation of leptine proportion and of presence/absence (scored as 1 or 0) of UBC370-1500 in the F_1 families derived from reciprocal crosses of *S. chacoense* 55-1 and 55-3. Recombination between nil/low percent leptine and presence of band is given

Parentage	Family	Type	Observed segregation			
			Percent leptine (Low : High)[a]	UBC370-1500 (1 : 0)	Recombinants	
					Number	Percentage
F_1 families:						
55-3 × 55-1	9501	L × H	57 : 37*[b]	56 : 38	3	3.2
55-1 × 55-3	9502	H × L	30 : 30 ns	28 : 32	2	3.3

ns and * indicate not significantly different and significantly different at the 5% level, respectively
[a] High leptine is defined as ≥ 62% of TGA as leptines; nil/low is ≤ 17% of TGA (Ronning et al. 1998)
[b] Indicates deviation from expected percent leptine segregation ratios of 1 : 1

Fig. 2a, b Partial DNA sequence of UBC370-1,500-bp cloned fragment from 55-3 and from *S. tuberosum* cv Kennebec. Homology is noted with *asterisks*. The 10-mer random primer sequence is indicated in *boldface*. **a** 5′ end (167 bases sequenced), **b** 3′ end (reverse complement; 183 bases sequenced)

a

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55-3       TCAGCCAGCGATCACTTAAAGCCTTGTTGTCCTAGATTTCATAATAACTTGATCATATTA
Kennebec   TCAGCCAGCGATCACTTAAAGCCTTGTTGTCCTAGATTTCATAATAACTTGATCTAATTA
           ************************************************************  ****

55-3       GTTCATGAGTTCATGTCATCAAGGATAAAGTTTCACTGAACATACAAAAAACAGTAGATA
Kennebec   GTTCATGAGTTCAGCTCATCGAGG-TAAAGTTTCACTGAACATACAAAA---GCTAGATA
           *************  ***** *** ************************     ******

55-3       CTCTGATGCATTAATCACGTAAGCTAATGAGAGAGAGCACATGGCAT
Kennebec   CTCTAGT-CATTA-TCACGTAAG--AAACATATGCATCATTCTACAT
           ****  * ***** *********  **  * *   * **     ***
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b

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55-3       TCAGCCAGCGTATGGAGGGGAAGAGGAGGCTTTCTTGAGAAAAGTTGTTACTCCTATTT-
Kennebec   TCAGCCAGCGTATGGAGGGGAAGAGGAGGCTTTCTTGAGAAAAGTTGTTACTCCTATT--
           ************************************************************

55-3       ATGAAGTGATTGCACGGGTATTGTGTGCATTTTAGTACTATCTGTTTTCTCCAATCTATT
Kennebec   ATGAAGTGATT-CACGG-TATTGAGAGCATTT-AGTACTATCTGTTTTCTC--ATCTATG
           *********** ***** ***** * ****** ******************  ******

55-3       GTATTGAATTTCTATTTTTCTTTGGAACTTGAAGAAAACTTT-CTTACATCTTTT-CTGT
Kennebec   ATG----------ATTTATTCTCAGACTAGAAACTCTACTCT-TCTTCATTCAGA-GGCT
            *           **** *  *  **     **    *** *   * ***         *

55-3       CAT
Kennebec   CAG
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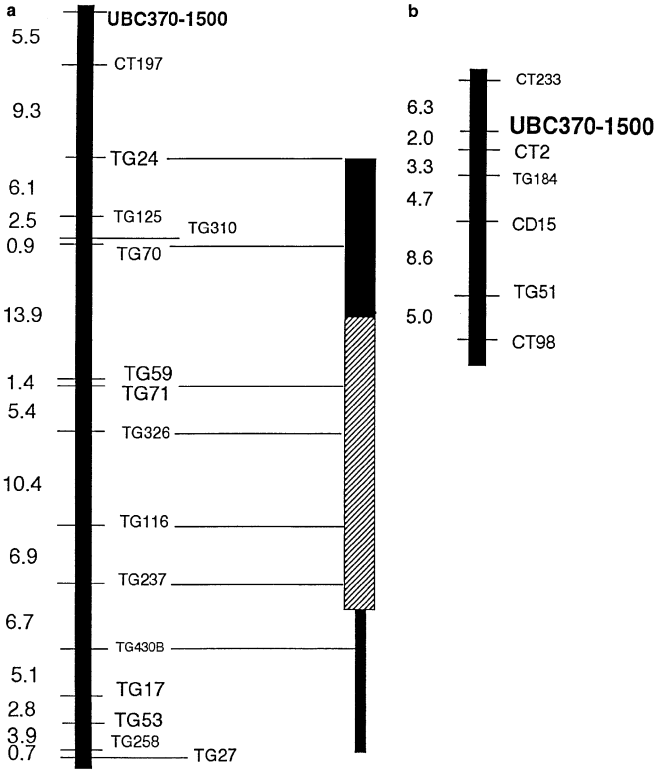
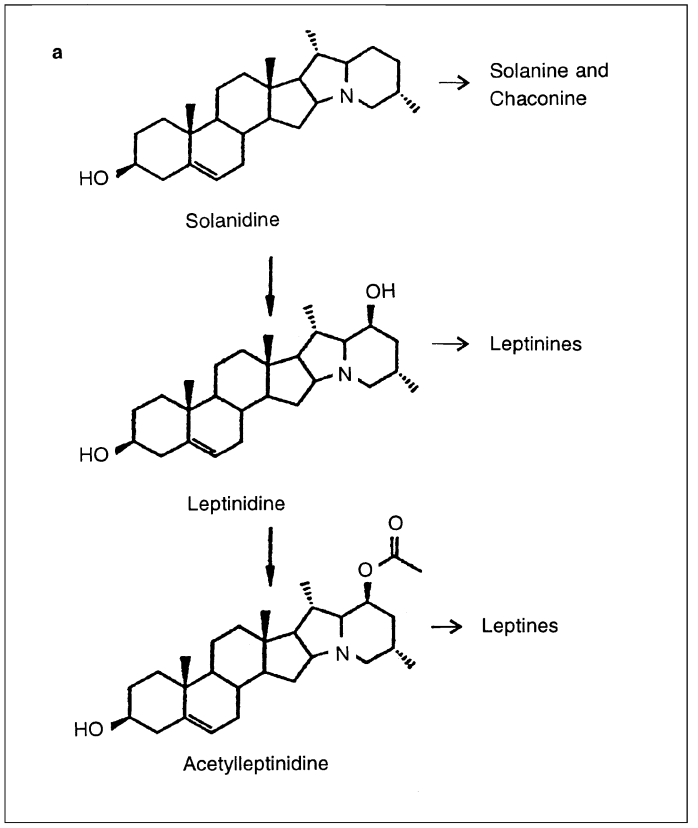
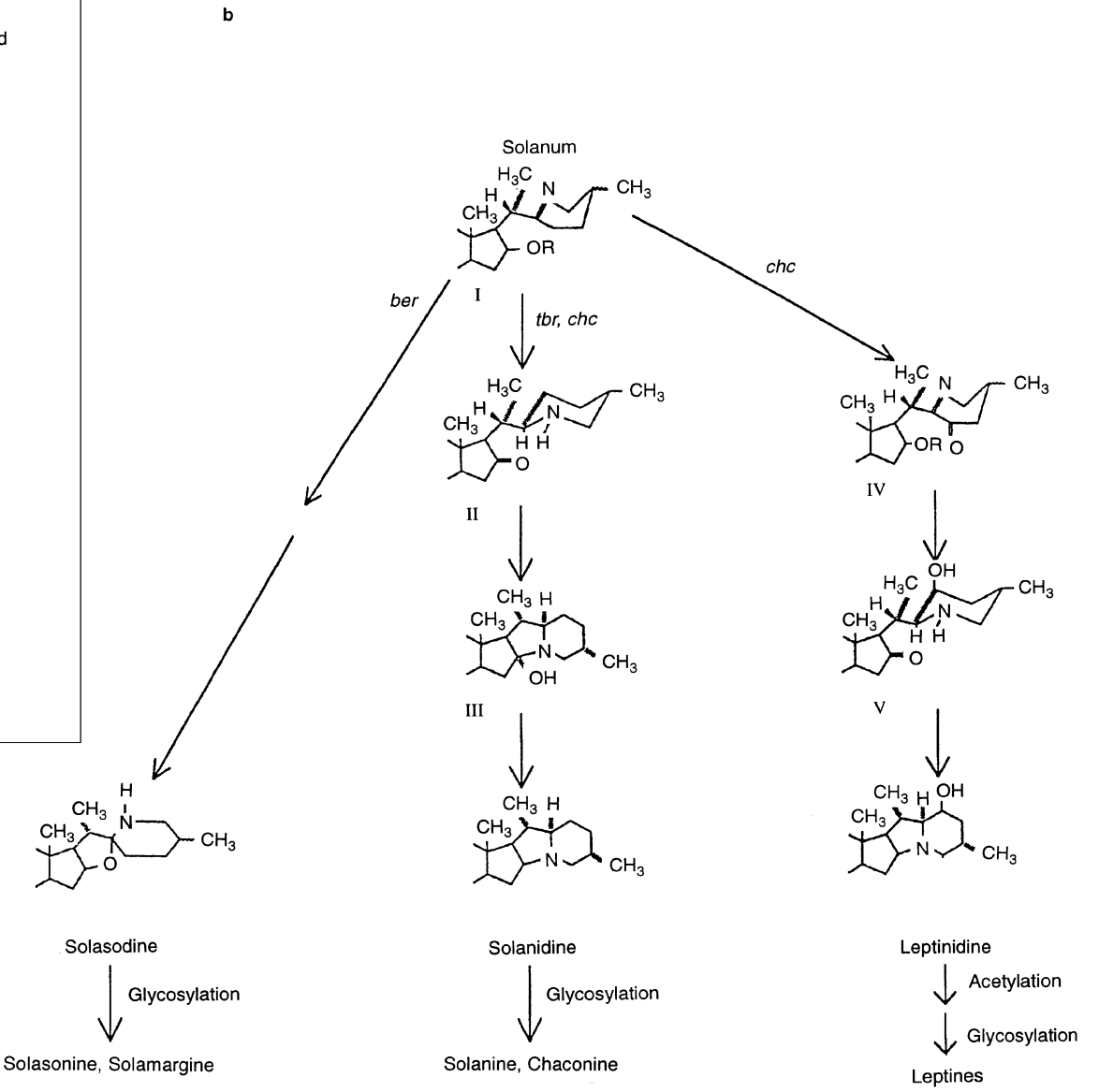


Fig. 3a, b Map location of the fragment UBC370-1500 from *S. chacoense* 55-3 on chromosome 1 of potato (**a**) and tomato (**b**) showing flanking markers. Markers are on the *right*; distances in centiMorgans on the *left*. *Vertical bar* to *right* in **a** shows location of QTL for solanidine production (adapted from Yencho et al. 1998), with estimated locations of RFLP markers. Significance levels are indicated by *shadowing* of the *boxes*: *solid* $P \leq 0.0001$, *cross-hatch* $P \leq 0.0100$

homologous (Fig. 2). If the fragment is associated with a dominant repressor, the same or similar mechanism may be present in *S. tuberosum* preventing leptine synthesis. In a cross between tetraploid 8380-1, a high leptine-producing genotype of *S. chacoense*, and several different *S. tuberosum* cultivars, none of which synthesize leptine, all F_1 hybrids contained leptine in a continuous distribution (ranging from 1 to 62% of TGA), indicating that leptine synthesis involves dominant genes (Sanford et al. 1996). Even though *S. tuberosum* does not synthesize leptine, the amounts and proportions of leptines in these hybrids were significantly affected by the *S. tuberosum* parent. This suggests that different genes may exist in *S. tuberosum* involving leptine synthesis/repression, which, in combination with genes from *S. chacoense* may modify leptine accumulation. In contrast, the *chacoense* × *chacoense* F_1s we report here had a discontinuous distribution, segregating 1 : 1 for high : low percent leptine. This F_1 data, as previously mentioned, indicates control by a single recessive gene, while data from subsequent generations suggests the presence of at least one other as yet unidentified genetic factor. The marker UBC370-1500 may enable the tracking of one of these genes


a
Solanine and
Chaconine
Solanidine
Leptinines
Leptinidine
Leptines
Acetylleptinidine


b
Solanum
ber
tbr, chc
chc
I
II
III
IV
V
Solasodine
Glycosylation
Solasonine, Solamargine
Solanidine
Glycosylation
Solanine, Chaconine
Leptinidine
Acetylation
Glycosylation
Leptines

controlling foliar leptine synthesis and aid in their introgression into cultivated potato.

The relative ease (or difficulty) in introgressing the leptine glycoalkaloids from *S. chacoense* into adapted potato cultivars depends on the number of genes which determine leptine accumulation. Genes that control biosynthetic pathways are probably critical in affecting leptine accumulation. The biosynthetic pathway described by Lawson (1993, partially based on the previous work of Osman et al. 1987) (Fig. 4a) is the most straightforward model, involving the least number of biosynthetic steps: solanidine is hydroxylated on carbon 23 to form leptinidine, which is then acetylated, forming acetylleptinidine, the aglycone of the leptines. This pathway, albeit logical, does not adequately consider the relatively low chemical reactivity of carbon 23 of solanidine. The proposed biosynthetic pathway of Schreiber (1968) (Fig. 4b), although more complex and involving a greater number of steps, may be more plausible, since compound I (an imine with an activated carbon 23 methylene group) has, relative to solanidine, greater chemical reactivity (S. Osman, personal communication; Červinka 1969). This proposed biosynthetic pathway hypothesizes that several genes are involved in leptine biosynthesis. That numerous genes determine leptine production is supported by the observation of Sanford et al. (1996), in that "the ability to synthesize leptines may be controlled by a few dominant genes and the quantities synthesized may be polygenically controlled". To our knowledge, none of the enzymes involved in these biosynthetic reactions have been identified.

Fragment UBC370-1500 from *S. chacoense* 55-3 maps to chromosomes 1 of potato and tomato (Fig. 3). Chromosome 1, along with most of the other chromosomes, are colinear between the two species (Bonierbale et al. 1988). Since a major quantitative trait locus (QTL) for solanidine accumulation has also been mapped to this region of chromosome 1 of potato (Yencho et al. 1998), this is a particularly intriguing finding. This co-localization suggests that a region on chromosome 1 defined by the restriction fragment length polymorphism RFLP markers TG24, CT197, and CT233 may determine solanidine accumulation in *Solanum* spp. If this is the case, then this region may contain genes which affect the accumulation of solanidine or leptinidine in *S. chacoense*, and of solanidine or solasodine in *S. tuberosum* × *S. berthaultii* progeny (see Yencho et al. 1998) (Fig. 4b). It is of interest to note that the direction of gene action in each cross (*S. chacoense* × *S. chacoense*, *S. tuberosum* × *S. berthaultii*) is similar, i.e., the solanidine gene exhibits a dominance effect (Yencho et al. 1998; Ronning et al. 1998).

The identification of UBC370-1500 as a marker linked to a gene involved in leptine production is an important step toward understanding the inheritance of this unique glycoalkaloid and will facilitate a greater understanding of its accumulation and biosynthesis. The marker UBC370-1500 can now be used as a molecular "anchor" to coordinate use of the extensive inventory of molecular tools available in the *Solanum*/*Lycopersicon* inventory in order to fine-map and characterize this very interesting region of chromosome 1. To more clearly understand the apparently complex genetic control of leptine accumulation, we need to identify additional markers within *S. chacoense*. Additionally, by making carefully defined crosses (based on glycoalkaloid phenotypes) between accessions and genotypes of *S. chacoense*, as well as between *Solanum* species, we can generate appropriate recombinant genotypes. Through the integration of molecular techniques, conventional plant breeding and biochemistry, it may be possible to develop a more complete understanding of glycoalkaloid inheritance and biosynthesis within *Solanum*.

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Fig. 4a, b Proposed pathways of glycoalkaloid biosynthesis in some *Solanum* spp. **a** Two-step pathway as proposed by Lawson (1993), showing hydroxylation of carbon 23 on solanidine to form leptinidine, followed by an acetylation step to form acetylleptinidine. **b** Formation of various glycoalkaloids from the common precursor I (adapted from Schreiber 1968, p 122). In this model, genes from *S. berthaultii* direct the precursor to form solasodine, genes from *S. tuberosum* and from most *S. chacoense* genotypes direct formation of solanidine, and genes from leptine-producing *S. chacoense* genotypes form leptinidine. The aglycones are then glycosylated to form either solamargine and solasonine (from solasodine), solanine and chaconine (from solanidine), or the leptines (from leptinidine). *ber S. berthaultii*, *tbr S. tuberosum*; *chc S. chacoense*

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