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Identification of molecular markers associated with leptine in reciprocal backcross families of diploid potato

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Abstract *Solanum phureja* clone 1-3 and *S. chacoense* clone 80-1 have a zero and high leptine content in their foliage, respectively. An F_1 hybrid (CP2) was intermediate for the trait, but self-incompatible. Two reciprocal backcross families, PBCp (*phu* 1-3 × CP2) and PBCc (CP2 × *phu* 1-3), and a family of monoploids derived by anther culture of CP2, were characterized for leptine as the aglycon, acetylleptinidine (ALD), content in leaves by gas chromatography. ALD was present in 43 of 87 genotypes in the PBCp backcross, implying simple genetic control by a dominant gene. However, the ALD levels were low compared to CP2. In the PBCc backcross, only 7 of 42 genotypes expressed ALD at a level generally higher than in PBCp. This ratio was significantly different from the 1:1 segregation observed in the reciprocal backcross and suggests a cytoplasmic influence. ALD levels in the CP2 monoploids ranged from 0 to 8,968 $\mu g \cdot g^{-1}$ of dry weight (dw) with 18 individuals expressing ALD and five with 0 ALD content. Ten high (mean ALD = 546 $\mu g \cdot g^{-1}$ of dw) and ten low (mean ALD = 0) individual plants within PBCp and seven high (mean ALD = 3,037 $\mu g \cdot g^{-1}$ of dw) and eight low (mean ALD = 0) individual plants within PBCc were used for bulk segregant analysis (BSA) using 214 RAPD (randomly amplified polymorphic DNA) primers. Three RAPD primers (OPQ-2, OPT-16 and OPT-20) amplified bands exclusively in bulks containing DNA mixes of high ALD producers in both PBCp and PBCc populations. These results suggest that these markers were associated in coupling to ALD content. ANOVAs for ALD content verified association between the markers and the trait. A CAPS (cleaved amplified polymorphic sequence) marker, GP82A, was also significantly associated with ALD production in both the monoploid and the PBCp populations. None of the RAPD markers was associated to ALD in the monoploids but one was associated in repulsion. The monoploid data indicate the likelihood of a recessive gene(s) that controls leptine production, but the backcross data indicate the action of modifying loci.

Keywords Bulk segregant analysis · CAPS · Colorado potato beetle · RAPD · *Solanum chacoense* · *Solanum phureja*

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Introduction

Control of Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say., one of the most destructive pests of the potato crop in North America, has relied heavily on insecticides (Casagrande 1987) and, more recently, on transgenic resistance afforded by transformation of potato with *Bacillus thuringiensis* (Bt) toxin genes (Frutos et al. 1999). CPB has a remarkable ability to develop resistance to insecticides used for its control such that newly developed insecticides exhibit short-lived effectiveness (Bishop and Grafius 1996; Grafius 1997). Even Bt resistance may be short-lived, since selection in the laboratory for resistance to Bt in CPB populations has resulted in more-tolerant insects (Whalon et al. 1993).

Host resistance as part of an integrated pest management program is a possible means of controlling CPB. High levels of leptine glycoalkaloids (Sinden et al. 1986a) or selection for the presence of glandular trichomes on leaves (Plaisted et al. 1992) provide natural forms of resistance. Resistance in wild *Solanum* species, particularly *Solanum chacoense* Bitt., has been associated

with foliar glycoalkaloids not found in *Solanum tuberosum* (Sinden et al. 1986a). Leptines are acetylated forms of solanidine, a glycoalkaloid that occurs in cultivated potato (Deahl et al. 1993). Stürckow and Löw (1961) determined that leptines were the most potent of various *Solanum* glycoalkaloids for CPB resistance. In the mid 20th century, resistant interspecific hybrids were under development in Europe, but this breeding research was discontinued when strong pesticides (such as DDT) became available (Torka 1950; Schwarze 1963). Douches et al. (1998) observed that the combination of high foliar leptines with a *Bt* gene increased effectiveness in controlling the potato tuber moth (*Phthorimaea operculella* Zeller).

Sinden et al. (1986b) reported that only a few genotypes were capable of occurring within rare accessions of *S. chacoense* were capable of synthesizing leptines, suggesting the control of leptine synthesis by a single or few genes. Genes from *S. tuberosum* were dominant for low levels of glycoalkaloids in a cross between *S. chacoense* and *S. tuberosum* (Sanford et al. 1994). In crosses between *S. chacoense* parents with high and low levels of glycoalkaloids, the levels of the F_1 genotypes were closer to the low parent (Sanford et al. 1994, 1996). These observations may suggest dominant suppression of glycoalkaloid expression and that multiple recessive alleles may be required for expression of elevated levels. A limitation of traditional breeding for leptine biosynthesis is the probable additive nature of the trait (Sinden et al. 1984). Hybrids receiving only a single allele from *S. chacoense* express only a fraction of the leptine level found in the *S. chacoense* parent (Veilleux and Miller 1998).

RAPDs (Williams et al. 1990) combined with bulk segregant analysis (BSA; Michelmore et al. 1991) have been used to identify DNA sequences linked to traits of interest in crops, such as scab resistance in apple, *Malus* spp. (Yang et al. 1997), root knot nematode resistance in sweetpotato, *Ipomoea batatas* (Ukoskit et al. 1997), oleic acid concentration in spring turnip rape, *Brassica rapa* ssp. *oleifera* (Tanhuanpää et al. 1996), locus M for sex expression in asparagus, *Asparagus officinalis* L. (Jiang and Sink 1997) and resistance to blackleg (*Leptosphaeria maculans*) in *Brassica napus* L. (Chèvre et al. 1997). Yencho et al. (1998) mapped quantitative trait loci (QTLs) of foliar glycoalkaloid aglycones in progenies of *S. tuberosum* × *S. berthaultii* Hawkes. Several QTLs for the accumulation of solasodine and solanidine (the aglycons of solasonine and solanine/chaconine, respectively) were identified in backcrosses of the hybrid (*S. tuberosum* × *Solanum berthaultii*) to either parent. Two QTLs were identified for solanidine in the backcross to *S. tuberosum*, one of which was also identified in the *S. berthaultii* backcross.

Leptine production is found naturally only in genotypes that are not of commercial importance. One approach to integration of this trait into desirable germplasm is to clone genes involved in their control. These genes can then be introduced into cultivated potato by genetic transformation. However, this requires reasonably simple genetic control. The present research represents a step in this process. This study focuses on phenotypic characterization for leptine glycoalkaloid synthesis, and the use of RAPDs with BSA followed by CAPS (cleaved amplified polymorphic sequences; Konieczny and Ausubel 1993; Jarvis et al. 1994) on individual genotypes to identify molecular markers associated with leptine production.

Materials and methods

Plant material

Two reciprocal backcross populations were established by crossing a self-incompatible hybrid (CP2) between *S. chacoense* clone 8380-1 (*chc* 80-1) and *Solanum phureja* clone BARD 1-3 (*phu* 1-3) to the *phu* parent. *Chc* 80-1 produced high levels of leptine (measured as the aglycon, acetylleptinidine: ALD) glycoalkaloids [7,055–22,261 $\mu g \cdot g^{-1}$ of dry weight (dw) under greenhouse conditions] in its foliage, whereas *phu* 1-3 did not produce leptine glycoalkaloids. The hybrid, CP2, was intermediate for the ALD (2,000–4,000 $\mu g \cdot g^{-1}$ of dw; Rangarajan et al. 2000). The reciprocal backcross populations were designated PBCp for *phu* 1-3 × CP2 (92 genotypes) and PBCc for CP2 × *phu* 1-3 (42 genotypes). The lower-case letters at the end of these family acronyms represent *phu* or *chc* cytoplasm. In addition, a population of 23 monoploids derived by anther culture of CP2 (Chani et al. 2000), and a population of the alternative backcross between CP2 and *chc* 80-1 (CBC; 12 genotypes), were also employed for inheritance studies of leptine glycoalkaloids. The monoploids had been verified by flow cytometry to have half the DNA content of the anther donor and therefore represent true hemizygotes with only a single allele per locus (Chani et al. 2000).

Backcross seedlings were grown in the greenhouse at 25–30 °C day/15–20 °C night. The photoperiod was extended to 16 h using halogen lamps (1,000 W). Leaf samples (2–3 fully expanded young leaves) were taken for preliminary studies of 12 genotypes each of CBC and PBCp during summer 1994, for the entire PBCp population during spring 1997, and for PBCc during spring 1998. The samples were placed in perforated paper envelopes, freeze-dried (Virtis 3l bench-top freeze drier, New York) and frozen at –30 °C. The dry samples were ground with liquid nitrogen, and analyzed by gas chromatography (Lawson et al. 1992). Data for acetylleptinidine (ALD) are presented as $\mu g \cdot g^{-1}$ of dw. Ten selections were taken for both the high and low bulks in PBCp, with nil leptine for the lows and a mean of 546 ± 74 $\mu g \cdot g^{-1}$ of dw for leptine in the highs. In the PBCc population seven and eight genotypes were selected for the high and low bulks, respectively, with nil leptine for the lows and a mean of 3,007 ± 1,305 $\mu g \cdot g^{-1}$ of dw for leptine in the highs.

Preparation of DNA bulks

DNA was extracted from greenhouse-grown leaf samples as described by Doyle and Doyle (1987). Equal volumes of DNA (at concentrations of 10 $ng \cdot \mu l^{-1}$) from each individual plant were pooled to comprise high-leptine and low-leptine bulks for each population.

Screening of primers

A total of 214 RAPD primers from kits A, C, G and Q through Z of Operon Technologies (Alameda, Calif.) was used to screen each pair of bulks. PCR reaction mixtures of 25 µl contained: 20 ng of genomic DNA, 0.6 µM of primer, 200 µM of dATP, dCTP, dGTP, dTTP, 1 × PCR buffer (2.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl, pH 8.3), and 1 U of *Taq* DNA polymerase (Promega,

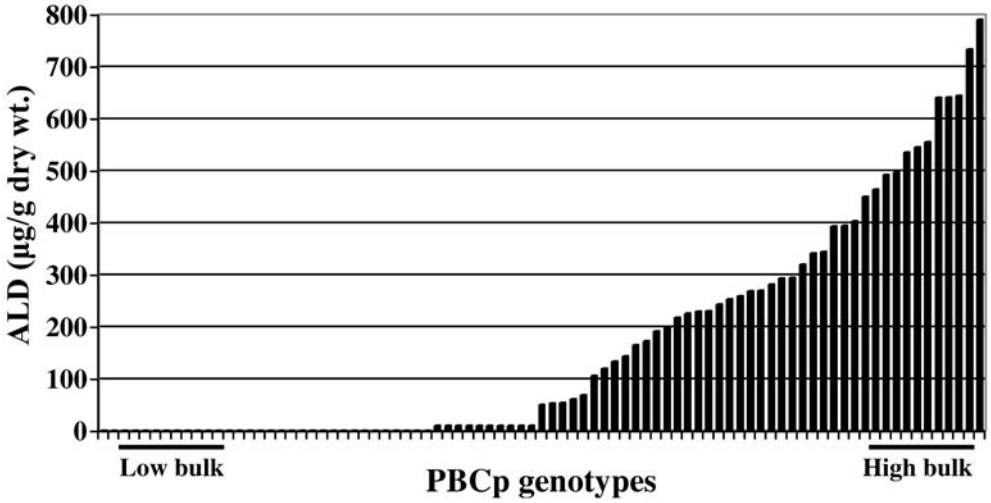

Fig. 1 ALD (leptine) concentration (µg·g^{-1} of dw) in leaf samples of greenhouse-grown plants of 84 individuals from a diploid backcross potato population, PBCp (*phu* 1-3 × CP2); plants selected for the high and low DNA bulks are indicated

Madison, Wis.). Amplifications were conducted in an Amersham Robocycler or in a Perkin-Elmer Cetus Model 480 thermal cycler. In the latter case the reactions were overlaid with a drop of light, sterile mineral oil. The amplification procedure consisted of 45 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 37 °C, 2 min extension at 72 °C, followed by a final extension at 72 °C for 5 min. The amplified samples were separated in 1.4% agarose gels in 1 × TBE buffer (10.8 g trizma base, 5.48 g boric acid, and 0.93 g EDTA/l distilled water) for 3.5 to 4.5 h at 100 V·λ DNA digested with *Eco*RI and *Hin*dIII was used as size marker and a PCR mix with distilled water instead of template DNA as a blank control. After electrophoresis, gels were stained with ethidium bromide (1.5 µg·ml^{-1}) and photographed under UV light. A RAPD primer generating a polymorphic PCR product with the two pairs of bulks was subsequently used in PCR reactions with DNA from the parents and individual plants comprising the DNA bulks, as well as all available individual plants comprising the rest of the families.

CAPS (cleaved amplified polymorphic sequences)

Sequences of 40 mapped RFLP markers cloned from genomic and cDNA libraries were obtained from Gebhardt et al. (1991). These RFLP markers represented most of the 12 chromosomal linkage groups of the potato genome. Each of these marker sequences was transformed to a CAPS by sequence analysis and primer design using CloneWorks, version 1.94 (Anteater Software, Los Angeles, Calif., USA). Amplification reactions (25 µl final volume) were performed with 10 ng of template DNA, 25 mM of TAPS (pH = 9.3 at 25 °C), 50 mM of KCl, 2.5 mM of $MgCl_2$, 1 mM of β-mercaptoethanol, 0.2 mM of each of the four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP and dTTP), 10 ng of each primer and 1 U of thermostable *Taq* DNA polymerase (SuperNova*Taq* polymerase, Madi Ltd, Rishon Le Zion, Israel). CAPS PCR reactions were carried out in an automated thermal cycler (MJ research, Watertown, Mass.). Initial incubation was at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 45–55 °C for 1 min and polymerization at 72 °C for 1 min. A final polymerization at 72 °C was carried out for 7 min after the above cycles were completed. The amplification products were digested with six restriction endonucleases: *Hin*fI, *Dpn*I, *Hae*III, *Rsa*I, *Eco*RI and *Msp*I. Undigested and digested PCR products were visualized by electrophoresis in 1.5% agarose gels stained with ethidium bromide.

Statistical analysis

One-way analyses of variance (ANOVA) were carried out to determine the degree of association between marker bands and ALD concentration. In these analyses the independent variable was a dummy variable in which "0" and "1" represented absence or presence of a polymorphic PCR product, respectively, and the dependent variable was ALD concentration. Prior to the ANOVA, carried out using the SAS proc GLM (SAS 1999), ALD data were transformed to their square root values. Chi-square analysis was done to determine if leptine phenotypes and markers segregated according to a 1:1 expectation. Linkage analyses among markers were conducted using MapManager QTX version b13 (Meer et al. 2001).

Results

Leptine content (expressed as ALD content) was measured in the various populations based on gas chromatographic analysis. From prior research (Veilleux and Miller 1998; Rangarajan et al. 2000) we knew that *chc* 80-1 synthesized high levels of ALD (7,055–22,261 µg·g^{-1} of dw), *phu* 1-3 did not synthesize ALD, and CP2 was intermediate (2,000–4,000 µg·g^{-1} of dw). Our preliminary study of 12 individuals representing the alternate backcross populations between CP2 and either *phu* 1-3 (PBCp) or *chc* 80-1 (CBC) revealed that ALD segregated in the PBCp population (eight genotypes did not express ALD and four individuals ranged from 449 to 2,542 µg·g^{-1} of dw the ALD) but that all genotypes in the CBC population expressed ALD. Although there was considerable variation for ALD expression in the CBC population (1,717–16,316 µg·g^{-1} of dw), we focused on the PBCp population because it segregated for leptine producers and the reciprocal PBCc population was available.

In the expanded PBCp population, 44 siblings produced no leptines and 43 produced low levels ranging from 50 to 790 µg·g^{-1} of dw based on leaf samples of seedlings taken from the greenhouse in the winter (Fig. 1). If we propose production/non-production of leptines to be controlled by a single gene, disregarding absolute quantities of leptines produced per plant, then the ratio does not differ significantly from 1:1 ($\chi^2 = 0.01$, $p = 0.92$). Hence it would be possible that a single gene controlled leptine production in this population. However, because all genotypes expressing leptines were

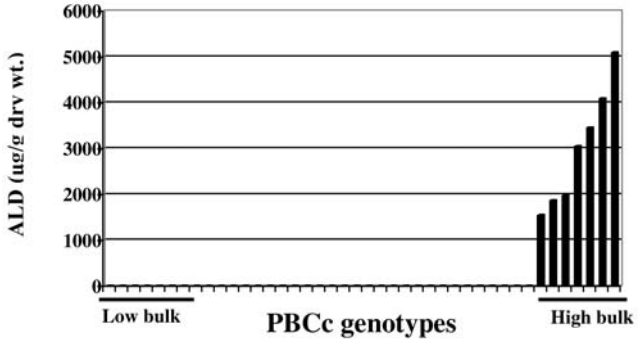

Fig. 2 ALD (leptine) concentration (μg·g^{-1} of dw) in leaf samples of greenhouse-grown plants of 42 individuals from a diploid backcross potato population between, PBCc (CP2 × *S. phu* 1-3); plants selected for the high and low DNA bulks are indicated

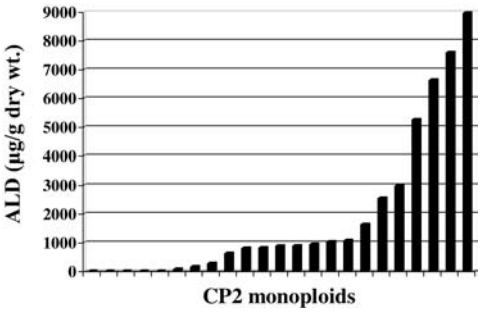

Fig. 3 Variation for ALD (μg·g^{-1} of dw) among 23 monoploid (2n = 1*x* = 12) genotypes derived from interspecific hybrid, CP2 (*chc* 80-1 × *phu* 1-3)

necessarily heterozygous and considerably lower in leptine production than the heterozygous parent, CP2, then additional genes must influence expression. The reciprocal population PBCc, differing from PBCp only by the source of cytoplasm (PBCp has *phu* cytoplasm and PBCc has *chc* cytoplasm), had a different pattern of leptine expression. In this population only 7 of 42 genotypes expressed leptines (Fig. 2), which differed significantly from the 1:1 ratio of leptine producers:non-leptine producers found in PBCp ($\chi^2 = 18.7$, $P < 0.005$). In addition, the amount of leptine (expressed as ALD content) produced by the seven PBCc leptine expressing genotypes ($\bar{x}$ = 3,036 μg·g^{-1} of dw) was generally greater than for those in PBCp ($\bar{x}$ = 546 μg·g^{-1} of dw) and more similar to that of CP2 (2,000 μg·g^{-1} of dw). Hence there would appear to be a cytoplasmic influence on leptine production. Given the 1:1 segregation in PBCp, it would seem likely that leptine genes must be present in many nil producers in PBCc but are not expressed.

A monoploid population consisting of 23 genotypes derived by anther culture of CP2 (Chani et al. 2000) was available for phenotypic analysis and verification of markers associated with leptine production in the backcrosses. Of 23 monoploids examined, five did not produce leptines (expressed as ALD), 14 produced low to intermediate levels (77–2,977 μg·g^{-1} of dw) and four produced high levels (5,258–8,968 μg·g^{-1} of dw; Fig. 3).

BSA revealed five RAPD bands that segregated with ALD concentration. OPQ-2 amplified a fragment (approximately 1,200 bp) present in nine of ten high-bulk selections in PBCp and in five of seven high-bulk selections in PBCc. This fragment was present in one of ten PBCp and in three of eight PBCc low-bulk selections. After 73 individuals in PBCp had been characterized for OPQ2-1200, one-way ANOVA revealed a significant difference for leptine content (ALD) between the 25 genotypes with the band (247 μg·g^{-1} of dw for ALD) and the 48 genotypes without it (126 μg·g^{-1} of dw for ALD). However, in the reciprocal PBCc population, even though genotypes with the marker had more ALD than those without it, ANOVA did not reveal a significant difference (Table 1). MapManager QTX revealed a likelihood ratio statistic (LRS) of 4.8 for OPQ2-1200 in PBCp but only 1.3 for PBCc, explaining 5 and 1% of the variation for ALD, respectively.

OPT-16 amplified a fragment that was associated with leptine (ALD) content (approximately 1,300 bp). This fragment was present in ten of ten PBCp and in six of seven PBCc high-bulk selections. It was present also in six of ten PBCp and four of eight PBCc low-bulk selections. The results of one-way ANOVA were highly significant for PBCp but not significant for PBCc. Mean leptine content (expressed as ALD content) for the 55 genotypes in the PBCp population with the fragment was 210 μg·g^{-1} of dw, and 68 μg·g^{-1} of dw for 30 genotypes without the fragment (Table 1). Segregation for presence:absence of the fragment was significantly different from an expectation of 1:1 ($\chi^2 = 6.78$; $P = 0.009$) in PBCp but not significantly different from 1:1 in PBCc ($\chi^2 = 0.6$; $P = 0.44$). MapManager QTX revealed a LRS of 12.5 for OPT16-1300 in PBCp but only 0.0 for PBCc, explaining 13 and 0% of the variation, respectively.

OPT-20 amplified a fragment that was associated with leptine content in both reciprocal backcross populations. The fragment (approximately 250 bp) was present in all ten PBCp and all seven PBCc high-bulk selections. It was also present in three of ten PBCp and in two of eight PBCc low-bulk selections (Fig. 4). One-way ANOVA revealed that OPT20-250 was the only marker that was a significant source of variation for leptines in both populations; χ^2 did not reveal a segregation pattern different from an expectation of 1:1 for the presence:absence of OPT16-250 in either population ($\chi^2 = 2.71$; $P = 0.1$ for PBCp; $\chi^2 = 0.12$; $P = 0.89$ for PBCc). The mean leptine content for genotypes with and without the fragment in PBCp was 218 and 73 μg·g^{-1} of dw, respectively, and in PBCc, 880 and 157 μg·g^{-1} of dw, respectively (Table 1). MapManager QTX revealed a LRS of 9.1 for OPT20-250 in PBCp and 2.4 for PBCc, explaining 9 and 3% of the variation, respectively.

BSA revealed two additional RAPD primers (OPT-7 and OPW-10) that amplified candidate bands expected to be associated with leptine production in the reciprocal backcrosses. However, when the entire populations were

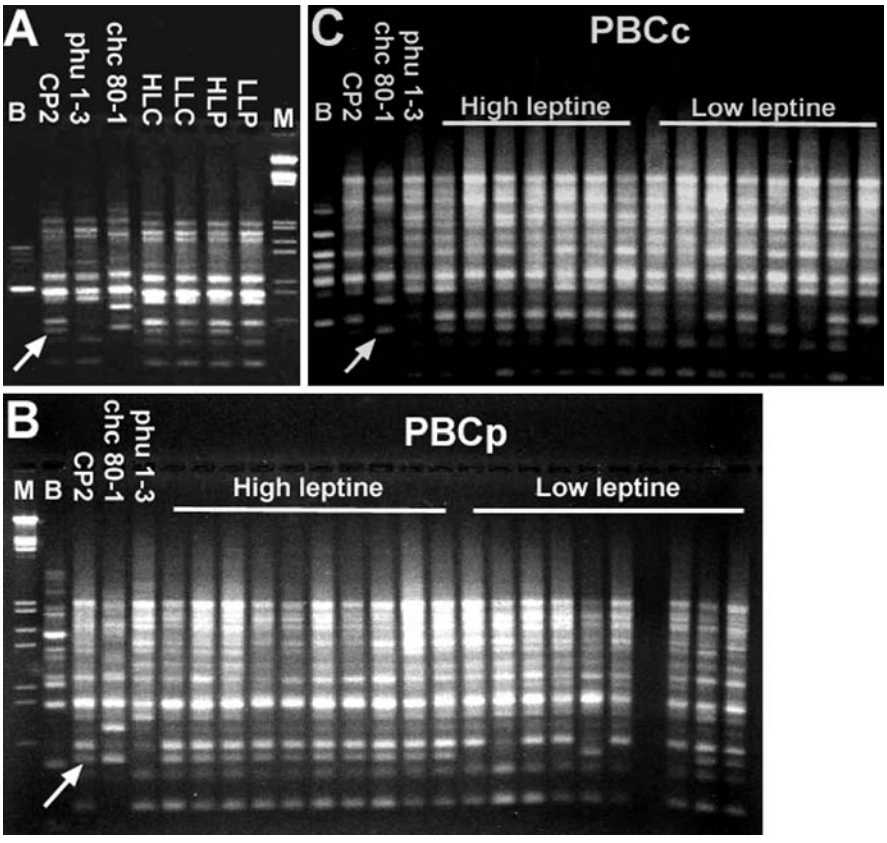

Fig. 4A–C DNA amplification by RAPD primer OPT-20 in potato. **A** Primer screening where a candidate band (indicated by *arrows*) associated with leptines was present in CP2, *chc* 80-1 and the high leptine bulks (HLP for PBCp; HLC for PBCc); the band was absent in *phu* 1-3 and the low leptine bulks (LLP for PBCp; LLC for PBCc). **B** Parents CP2, *chc* 80-1, *phu* 1-3 and individual selections comprising the PBCp bulks; the band was present in all ten high and four low selections. **C** CP2, *chc* 80-1, *phu* 1-3 and individual selections comprising the PBCc bulks; the band was present in six of seven high and three of eight low selections. M=λ DNA digested with *Eco*RI and *Hin*dIII as DNA size markers

Table 1 Mean leptine content (ALD) in $\mu g \cdot g^{-1}$ of dw for genotypes with or without each of six markers (five RAPDs and one CAPS) identified by BSA in reciprocal backcross diploid potato populations derived from an F_1 interspecific (*S. chacoense* × *S. phureja*) hybrid, CP2, and its *S. phureja* parent. χ^2 analysis for expected 1:1 segregation of each marker in both populations is also given

Marker	PBCp population							PBCc population						
	Marker present		Marker absent		χ^{2c}	LRS[d]	%[e]	Marker present		Marker absent		χ^{2c}	LRS[d]	%[e]
	Mean ALD $\mu g \cdot g^{-1}$ of dw	N[a]	Mean ALD[b] $\mu g \cdot g^{-1}$ of dw	N				Mean ALD $\mu g \cdot g^{-1}$ of dw	N[a]	Mean ALD[b] $\mu g \cdot g^{-1}$ of dw	N			
OPQ2-1200	247	(25)	126*[c]	(48)	6.64**	4.8	5	783	(18)	331^{NS}	(21)	0.1^{NS}	1.3	1
OPT7-150	183	(38)	139^{NS}	(47)	0.76^{NS}	0.5	0	824	(16)	381^{NS}	(26)	1.92^{NS}	0.0	0
OPT16-1300	210	(55)	68**	(30)	6.78**	12.5	13	750	(24)	203^{NS}	(18)	0.6^{NS}	0.4	0
OPT20-250	218	(49)	73**	(34)	2.36^{NS}	9.1	9	880	(20)	157**	(22)	0.02^{NS}	2.4	3
OPW10-1400	167	(55)	148^{NS}	(29)	7.44**	0.4	0	717	(25)	256^{NS}	(17)	1.16^{NS}	0.1	0
GP82A	207	(46)	98*	(47)	0.0^{NS}	4.0	4	552	(20)	464^{NS}	(22)	0.02^{NS}	0.1	0

[a] Numbers in parentheses represent the number of genotypes in each class (marker present or absent)

[b] *, **Significant difference ($P < 0.05$ and $P < 0.01$, respectively) for ALD content between marker present and marker absent within a population; NS indicates no significant difference for mean ALD between plants with and without the marker. ANOVA was conducted on the square root of ALD, but means are presented as ALD in $\mu g \cdot g^{-1}$ of dw for ease of interpretation

[c] Test for goodness of fit to 1:1 segregation for individuals with and without each specific band; **significant at $P < 0.01$, ^{NS}not significantly different from 1:1

[d] LRS = likelihood ratio statistic from MapManager QTX for association of the square root ALD to the locus

[e] % = the amount of the total trait variance that would be explained by a QTL at this locus as a percent (MapManager QTX)

screened, ANOVA did not reveal a significant association of either marker with leptine production in either backcross. Despite this lack of significant association by ANOVA, the mean leptine levels for individuals with the markers were higher than for those without the markers (Table 1).

Of the 40 CAPS markers examined, 12 yielded a polymorphic band between the original parental lines, *phu* 1-3 and *chc* 80-1. Nine of these 12 markers, including CP108, yielded a band specific to *phu* 1-3 and three yielded a band specific to *chc* 80-1. These three markers,

Table 2 Mean leptine content (ALD) in μg·g^{-1} of dw for monoploid genotypes with or without each of four markers (three RAPDs and one CAPS) found by BSA and subsequent ANOVAs to be linked to leptine content in the two reciprocal backcross populations

Marker	Marker present		Marker absent[b]		χ^{2c}	LRS[d]	%[e]
	Mean ALD μg·g^{-1} of dw	N[a]	Mean ALD μg·g^{-1} of dw	N[a]			
OPQ2-1200	2,522	(10)	1,841^{NS}	(7)	0.24^{NS}	0.0	0
OPT16-1300	1,604	(12)	3,961^{NS}	(5)	2.12^{NS}	1.9	4
OPT20-250	1,013	(11)	4,742*	(6)	0.94^{NS}	4.1	16
GP82A	2,884	(14)	309**	(9)	0.70^{NS}	10.7	34

[a] Numbers in parenthesis represent the number of genotypes in each class (marker present or absent)
[b] *,**P (F) < 0.05 and P (F) < 0.01, respectively; ^{NS}statistically not significant. ANOVA was conducted on the square root of ALD but means are presented as ALD in μg·g^{-1} of dw for ease of interpretation
[c] Test for goodness of fit to 1:1 segregation for individuals with and without each specific band; ^{NS}not significantly different from 1:1
[d] LRS = likelihood ratio statistic from MapManager QTX for association of the square root of ALD to the locus
[e] % = amount of the total trait variance that would be explained by a QTL at this locus as a percent (MapManager QTX)

GP34, GP82 and GP185, were analyzed in the backcross populations. Of these, only GP82, located on potato chromosome 4, was found to be associated in coupling to ALD concentration in both PBCp and the monoploid populations. In PBCp, the marker was present in 46 individuals with an average ALD concentration of 207 μg·g^{-1} of dw compared to 98 μg·g^{-1} of dw for the 47 genotypes without the marker (Table 1); χ^2 analysis did not reveal a ratio significantly different from the 1:1 presence:absence of the marker in either population. MapManager QTX revealed a likelihood ratio statistic (LRS) of 4.0 for GP82 in PBCp but only 0.1 for PBCc, explaining 4 and 0% of the variation, respectively.

Of the four markers significantly associated in coupling with leptines in the backcross populations, only the CAPS marker (GP82A) revealed a similar association in the monoploid population. The 14 monoploids with the marker had a mean of 2,884 μg·g^{-1} of dw for ALD compared to 309 μg·g^{-1} of dw for the nine genotypes without the marker (Table 2). MapManager QTX revealed a LRS of 10.7 for GP82 in the monoploids, explaining 34% of the variation for ALD. Remarkably, RAPD marker, OPT20-250, that was significantly associated in coupling with leptines in both reciprocal backcrosses, was significantly associated in repulsion to leptine expression in the monoploid population (Table 2). Monoploids with the marker had a mean of 1,013 μg·g^{-1} of dw for ALD and those without it had a mean of 4,742 μg·g^{-1} of dw for ALD. For OPT20-250, the LRS was 4.1 in the monoploids, explaining 16% of the variation. None of the markers exhibited skewed segregation significantly different from 1:1, presence:absence, in the monoploid population, according to χ^2 analysis (Table 2); however, the population size was small and there were more monoploids with the marker present, than absent, for all four markers.

MapManager QTX did not reveal linkage between any pair of markers (OPQ2-1200, OPT16-1300, OPT20-250 and GP82A) in any of the populations. One CAPs marker CP108, known to be tightly linked to the incompatibility locus (Gebhardt et al.1991) and polymorphic between *chc* 80-1 and *phu* 1-3, was examined in the monoploid population, so that a linkage analysis could be conducted between it and OPT20-250. No linkage was found between the two markers, implying that OPT20-250 is not linked to the incompatibility locus.

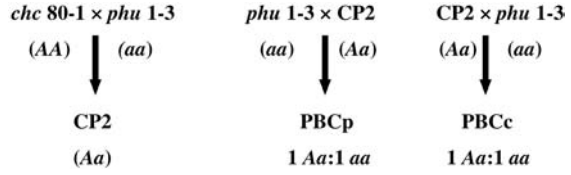

Fig. 5 Expected segregation pattern for RAPD markers linked in coupling to leptine production in reciprocal backcross populations of an interspecific hybrid, CP2 and its *phu* 1-3 parent

Discussion

The source of the leptine trait, *chc* 80-1, represents an unusual clone selected for expression of high levels of leptine glycoalkaloids in leaves. CP2, an F_1 hybrid between *chc* 80-1 and *phu* 1-3 with *chc* cytoplasm, is necessarily heterozygous for genes responsible for leptine production because its *phu* parent produced no leptines. Any leptine-producing progeny from backcrosses of CP2 to *phu* 1-3 would also be heterozygous for genes for leptines. Dominant RAPD markers associated with leptines would be expected to segregate as indicated in Fig. 5. In the PBCp backcross, with *phu* cytoplasm, 43 of 87 genotypes (50%) produced low levels of leptines. However, in the reciprocal backcross, PBCc, genetically similar except for *chc* cytoplasm, only seven of 42 genotypes (17%) produced leptines, but the leptine levels in these selections were much higher than for PBCp leptine selections. Low leptine producers may obscure true genetic ratios as they challenge the limits of detection by gas chromatography. The monoploids, with *chc* cytoplasm, afford the opportunity of viewing segregation for both dominant and recessive genes that control leptines. If recessive genes are responsible for high expression of leptines, as observed in some of the Ronning et al. (1998)

Table 3 Sequences of RAPD and CAPS primers that amplified markers associated with leptine production or incompatibility in potato hybrids

Marker	Associated trait	Type	Sequence
OPQ2-1200	Leptine	RAPD	5′-TCTGTCGGTC-3′
OPT16-1300	Leptine	RAPD	5′-GGTGAACGCT-3′
OPT20-250	Leptine	RAPD	5′-GACCAATGCC-3′
GP82A	Leptine	CAPS	F = GCAGTTGCTCTCTCAAGTTCTGTC R = CCTTCTCTAGGTGATGTTTGCTGG
CP108	Incompatibility	CAPS	F = GTCCATTCGGTTTAGCTGCA R = CTAAAACCCTTCCAAAACACT

chc populations, then these alleles would only have been observed in our monoploid and CBC populations, and not the PBC backcrosses. Indeed, individuals in the monoploid and CBC populations exhibited higher leptine levels than any in the PBC backcrosses. In fact, the distribution of leptine phenotypes in the monoploid population (5 zero: 14 intermediate: 4 high) appeared to fit a two-gene model, where high levels result from a recessive allele at both loci, intermediate levels when one or the other locus has a dominant allele and zero levels when there are dominant alleles at both loci. Dominance or recessiveness is irrelevant in the hemizygous monoploids where either allele is expressed. These high levels in the monoploids were associated with the CAPS marker, GP82A, initially identified in the backcross populations; the three RAPD markers were either irrelevant or associated in repulsion. An absence of linkage between GP82A and OPT20-250 suggests that the two markers are approaching different genes that control leptines. The linkage in repulsion of marker OPT20-250 to leptines in the monoploid population was unexpected. This population represents only a small fraction of the millions of microspores that were placed in anther culture to derive the monoploids. The interspecific hybrid population is also known to suffer from hybrid breakdown (Veilleux and Miller 1998) where some fraction of individuals is inviable. Therefore, it is possible that the process of androgenesis selected for recombinants between OPT20-250 and some linked lethal allele that caused nonrecombinants to perish.

Regarding genes controlling leptines, our study implicates quantitative inheritance involving both dominance at two or more loci that allow low levels of expression, and recessiveness at two or more additional loci that may control high levels of expression. In addition there is evidence of cytoplasmic influence on leptine expression, with the *chc* cytoplasm favoring expression of a dominant allele in the backcrosses. The *chc* populations described by Ronning et al. (1998) segregated according to a single recessive gene model, but significant differences from the expected segregation in some of their populations suggested the presence of modifiers affecting the inheritance and expression of leptines. Our data support this assumption. The behavior of alleles controlling leptine production may differ when observed in different genetic backgrounds. In our hybrids with *phu,* leptine production was at least partially dominant, since the *phu* parent expressed no leptines but low levels were detected in the F_1 and both backcrosses. Sanford et al. (1996) doubled the chromosome number of 2*x chc* 80-1 and crossed the resulting 4*x chc* with *S. tuberosum* (4*x*) clones. The occurrence of leptines in the resulting hybrids led them to propose that the ability to synthesize leptines may be controlled by a few dominant genes and the quantities synthesized may be polygenically controlled, a possibility that is confirmed by our study.

Linkage of a gene controlling leptine production to the self-incompatibility locus on chromosome 1 could help to explain the difference in leptine expression between our reciprocal backcross populations. Ronning et al. (1999) found a SCAR marker (UBC370-1500) that was linked to solanidine production on chromosome 1 of potato, the same chromosomal location of the incompatibility locus. With CP2 as the pollen parent (PBCp population) we expect that all progeny, except recombinants between the incompatibility locus and a marker associated with leptines, will carry the marker, because the *phu* S-allele will not effect pollination. However, with CP2 as the pistillate parent, half of the progeny should have markers derived from *chc* 80-1, regardless of incompatibility. A lack of linkage between OPT20-250 and a CAPS marker (CP108) that is closely linked to the incompatibility locus in potato (Gebhardt et al. 1991) reduces the likelihood of differences between our PBCp and PBCc backcrosses due to incompatibility.

Ronning et al. (1999) reported a molecular marker linked in repulsion to leptine content. This marker was amplified by primer UBC-370 in a segregating population developed from *chc*. Our backcross populations were checked for the presence of this marker, and it was observed in all genotypes except *chc* 80-1 (data not shown). Therefore, just as the UBC-370 marker is present in *S. tuberosum* cultivars that do not produce leptines, it must also be in *phu* 1-3. The genomic region associated with this marker suppressed leptine production; the common presence of this marker in all of PBC backcross genotypes may account for their generally low level of expression.

Our objective was to identify markers associated in coupling to leptine expression in populations that were predominantly *phu*. BSA revealed five RAPD primers (OPQ-2, OPT-7, OPT16, OPT-20 and OPW-10; Table 3) that amplified fragments that appeared to be associated with leptine content in PBCp and PBCc. Even though the trend was similar in PBCp and PBCc (Table 1), the one-way ANOVAs on both populations resulted in significant association for only three markers in PBCp (OPQ2-1200, OPT16-1300 and OPT20-250) and one in PBCc (OPT20-250). The diagnostic band was absent in the low

bulks for PBCp, but it was faint in the low-bulks for PBCc. In PCR for the individual selections in both bulks, the bands that segregated with the trait were present in most or in all of the high-bulk selections, but were also present in several of the low selections. Some of these might be a product of genetic recombination between the marker and the gene controlling the trait. But presence of bands in so many of the low bulk genotypes leads us to question our phenotypic characterization. Leptine analysis was done on leaf samples taken from plants growing under greenhouse conditions where expression of glycoalkaloids may be somewhat suppressed (Maga 1994). Another possibility is that gas chromatography for detection of low levels (less than 100 $\mu g \cdot g^{-1}$ of dw for ALD) of leptines is difficult (Lawson et al. 1992). The smaller population size of PBCc compared with PBCp prevented detection of statistically significant differences in PBCc due to the presence/absence of four of the five candidate markers. However, the trend (i.e., higher leptines when each of the five candidate bands revealed by BSA was present) was similar in both populations. The fragments amplified by OPQ-2, OPT-16, OPT-20 and CAPS GP82A are markers associated with genes controlling the leptine trait, and we believe this is the first report of molecular markers associated in coupling to leptine production in potato.

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