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ORIGINAL PAPER

A QTL that confers resistance to Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) in tetraploid potato populations segregating for leptine

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Abstract Genetic resistance to Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) from *Solanum chacoense* has been incorporated in the tetraploid potato selection, ND4382-19, which is highly resistant and contains moderate level of foliar leptines. We recently reported using ND4382-19 progeny, population ND5873 (ND4382-19 × Chipeta), to map two genes that segregated as complementary epistatic genes that allow accumulation of leptinidine (*Lep*) and acetyl-leptinidine (*AL*) on chromosomes 2 and 8, respectively. We describe here the characterization of a second half-sib population NDG116 (ND4382-19 × N142-72). In this population, solasodine from parent N142-72, which has *Solanum berthaultii* in its background, was predominant over solanidine-based alkaloids. Concentrations of solanidine, leptinidine, and acetyl-leptinidine were 15-, 5-, and 14-fold lower than in the ND5873 population. Nevertheless, *Lep* and *AL* mapped to the same locations on chromosomes 2 and 8 of parent ND4382-19, respectively. The two populations were evaluated for resistance to *Leptinotarsa* in field assays, and by detached leaf assay for population NDG116. In both families, QTL analysis identified a major QTL from ND4382-19 on the distal end of chromosome 2, close to the *Lep* locus. The contribution of this QTL to resistance ranged from 11 to 34% for ND5873 at four field sites. Contribution to resistance from the linkage group that contains the gene *AL* for the accumulation of leptine was not detected. In family NDG116, the same chromosome 2 QTL was detected for field and detached leaf assays, explaining 26 and 12% of the variance for defoliation and larval development, respectively. These data may indicate another resistance mechanism besides leptine in the *Leptinotarsa* resistance observed in these populations.

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Introduction

Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) is widely distributed in North America and has invaded Europe and Asia (Jolivet 1991). Its enormous defoliating ability causes significant losses in potato production (Ferro et al. 1985; Jolivet 1991). Its management often requires intensive insecticide application. Unfortunately, *Leptinotarsa* has demonstrated an unusual ability to quickly develop insecticide resistance, including to organophosphates, carbamates, organochlorines, arsenicals, and pyrethroids. In some cases, a new insecticide failed during or immediately

following a single year of use (Forgash 1985; Ragsdale and Radcliffe 2005). Insecticides are also expensive, and associated with concerns about risk to human health and environmental impact.

New varieties with host–plant resistance should have a positive impact on potato cropping systems due to reduced insecticide use for *Leptinotarsa* management, resulting in reduced cost, reduced risks to human health, and less environmental impact (Tingey 1984). One of the most effective sources of host-resistance mechanisms to *Leptinotarsa* is the wild potato species, *Solanum chacoense* (Kuhn and Löw 1961; Sinden et al. 1986b). Resistance to *Leptinotarsa* from this species has been associated with the expression of rare glycoalkaloids known as leptines. Leptines I and II, acetylated variants of solanine and chaconine, respectively, are potent feeding deterrents for *Leptinotarsa* (Sinden et al. 1986b). Experiments demonstrated that 120 mg 100 g^{-1} was required for effective resistance (Sinden et al. 1986a; Sinden et al. 1986b). Plants with high-leptine content were almost immune to *Leptinotarsa*. Leptines are only expressed in aerial tissues and not in tubers, an important consideration with regard to potential consumer toxicity (Friedman 2006). All *S. chacoense* genotypes are able to synthesize solanidine, but few contain leptines. Investigation of segregating families suggested that few genes are required for leptine synthesis (Hutvágner et al. 2001; Medina et al. 2002; Ronning et al. 1998; Sagredo et al. 2006; Sanford et al. 1996; Silhavy et al. 1996; Sinden et al. 1986a).

Solanum chacoense has been used in breeding programs to introgress host–plant resistance, but utilization has been hampered by high levels of tuber glycoalkaloids (Sanford et al. 1996). The tetraploid selection ND2858-1 from an unrecorded *S. chacoense* accession, was highly resistant to *Leptinotarsa* and contained foliar leptine, but its total content of glycoalkaloid (TGA) in tubers was low (Lorenzen and Balbyshev 1997). Characterization of resistant progeny suggested that high leptine content may not be the only causal resistance factor (Lorenzen et al. 2001). Progeny lines ND4382-17 and ND4382-19 were as resistant as high-leptine genotypes of *S. chacoense*, in spite of containing only 16 and 5%, respectively, of the lower effective leptine content described by Sinden et al. (1986a).

Two loci with complementary epistasis for leptine accumulation were recently mapped using a segregating population from resistant parent ND4382-19 (Sagredo et al. 2006). Locus *Lep* enabling accumulation of hydroxylated (C-23) solanidine (leptinidine) was located on chromosome 2; locus *AL* enabling accumulation of acetylated leptinidine (acetyl-leptinidine) was on chromosome 8. Leptinidine and acetyl-leptinidine are the non-glycosylated steroidal alkaloids for leptinines and leptines, respectively.

Another type of resistance in potato is provided by glandular trichomes from *S. berthaultii* (Bonierbale et al. 1994; Tingey 1991). Two half-sib tetraploid populations were used in this study, ND5873 and NDG116. These populations were developed from crosses ND4382-19 × Chipeta and ND4382-19 × N142-72, respectively. The resistant leptine-donor, ND4382-19, was selected for high resistance to *Leptinotarsa*, reasonably low tuber glycoalkaloids, and good agronomic performance (Lorenzen et al. 2001). Parent N142-72 was an advanced genotype selected for beetle resistance at Cornell University that expresses Type A glandular trichomes from *S. berthaultii* (R. Plaisted, unpublished). The latter population was developed in an attempt to combine these resistance sources and determine their compatibility/synergism.

In this report, we demonstrate by QTL analysis of two segregating populations derived from ND4382-19 that leptine content was not the main factor responsible for the resistance to *Leptinotarsa*. Instead, the resistance was explained by another factor on chromosome 2 that was linked to the *Lep* gene involved in the leptinidine synthesis.

Materials and methods

Plant material

The two tetraploid populations in this study, ND5873 and NDG116, were developed at North Dakota State University. Depending on the experiment, plants were grown either from field-produced tubers, or from tissue-cultured plantlets transferred to a greenhouse or environmental growth chamber. Plant tissues for DNA extraction, glycoalkaloid analysis, and glandular trichome analysis were obtained from greenhouse-grown plants. Plants were acclimated and planted in 25-cm diameter × 19-cm deep plastic pots filled with peat-vermiculite medium (Sunshine Mix 1, Fisons Hort., Bellevue, WA) and placed in a greenhouse with a 16-h photoperiod and mean daily temperature of 22°C. Natural lighting of the greenhouse was supplemented by 1,000-W, metal halide lamps. Plants used for detached leaf experiments were maintained in an environmental growth chamber (Percival Model PT-80 chambers with a mix of fluorescent and incandescent bulbs) with a 16-h photoperiod, 25°C temperature, and 80% relative humidity. All plants were fertilized weekly with 15N-7P-14K (Peters, Forgelsville, PA) at 1 g l^{-1}.

Resistance to Colorado potato beetle

The level of resistance to Colorado potato beetle was assessed by field experiment under natural infestations. Field trials were planted as 5- or 10-hill plots in a

randomized complete block design (RCBD) with four replications. Natural infestations of *Leptinotarsa* were promoted and spread by spreader–feeder rows of susceptible cultivars every 4–6 rows in insecticide-free plots. Resistance was estimated as the percentage of defoliation. Beetle defoliation was evaluated at three stages of the pest: (1) emerged overwintered adults, (2) small larvae, and (3) large larvae and newly emerged adults. A single defoliation index was calculated using respective weights of 10, 40, and 50% of these respective defoliation scores. Defoliation by *Leptinotarsa* was evaluated at different locations during 1997 and 1998 (Fargo, ND, 1997; Grand Forks, ND, 1997; Rosemount, MN, 1997; and Crookston, MN, 1998). The NDG116 population was characterized for resistance to *Leptinotarsa* by detached leaf assay (Boiteau and LeBlanc 1992; Sanford and Cantelo 1989) and one field experiment with natural pressure of insects, at McLeod, ND, in 1998. For detached leaf assays, potato leaves from plants grown in a controlled environmental chamber, were placed in 8.5-cm diameter Petri plates with the petioles inserted into tubes of water to maintain turgidity. Five neonate larvae from a colony raised on a susceptible potato cv. were placed on each leaf. The plates were placed in a growth chamber maintained at 25°C with a 16-h photoperiod. After 4 days, the developmental stage of each larva was determinated, and a larval development index score was assigned for each sample (Lorenzen et al. 2001).

Steroidal aglycone analysis

Plants from the ND5873 and NDG116 populations were grown in a greenhouse. Leaves were collected and immediately placed in liquid nitrogen, and then lyophilized. Alkaloid extraction and analysis was performed as described in Sagredo et al. (2006).

AFLP mapping and QTL analysis

DNA from each genotype was extracted from fresh-frozen leaves according to Fulton et al. (1995) and AFLP reactions were performed according to Vos et al. (1995), as described in Sagredo et al. (2006). Linkage analysis and development of tetraploid linkage maps was done according to Hackett et al. (1998) and Sagredo et al. (2006) by assuming random pairing and chromosome assortment and analyzing simplex–simplex coupling; duplex–duplex coupling; and duplex–simplex linkages in coupling or repulsion. A preliminary analysis of resistance to *Leptinotarsa* was performed by regressing the resistance scores vs. simplex markers, using the software MapManager QTX (version 0.14; Manly et al. 2001). Associations between loci and traits were established according to the values of likelihood ratio statistic (LRS) and *P*-value (Haley and Knott 1992). Later, the linked coupling-linked groups that showed the more significant association with resistance to *Leptinotarsa* were analyzed further using the QTL interval mapping method of software TetraploidMap (version 1.0.6; Hackett and Luo 2003; Luo et al. 2001).

Results

Glycoalkaloid content

The parents of family ND5873 (ND4382-19 × Chipeta) are highly resistant and very susceptible to *Leptinotarsa*, respectively. Family NDG116 (ND4382-19 × N142-72) was developed from two resistant parents with different mechanisms of insect resistance derived from *S. chacoense* and *S. berthaultii*, respectively. Analysis of glycoalkaloid aglycones by GC-MS of the two families of ND4382-19 is summarized in Table 1. A detailed description of the foliar content of solanidine (Sol), leptinidine (Lep), and acetylleptinidine (AL) in the parents and 93 progeny genotypes of the family ND5873 was given in Sagredo et al. (2006). In progeny NDG116, a total of 99 individuals were analyzed by GC-MS. This population also contained Sol, Lep, and AL, but almost all genotypes (except two) also contained an additional aglycone, solasodine (Sda) that was present in parent N142-72 (Table 1). Solasodine content ranged from 0.01 mg g^{-1} to 8.80 mg g^{-1} of dry weight, with an average of 2.22 mg g^{-1} (SD = 1.49). The content of this glycoalkaloid was much greater than the other steroidal alkaloids (Table 1). Solanidine content of NDG116 ranged from a trace to 1.16 mg g^{-1} dry weight, with an average of 0.11 mg g^{-1} (SD = 0.19). Leptinidine was present only in 40 genotypes and its content ranged from a trace to 0.21 mg g^{-1}, with a mean of 0.02 mg g^{-1} (SD = 0.04). AL was present in only 18 genotypes, ranging from a trace to 0.06 mg g^{-1}, with an average of 0.01 mg g^{-1} (SD = 0.02) for those plants. Family ND5873 contained much higher levels of Sol, Lep, and AL than NDG116 (15, 5, and 14 times higher, respectively).

Concentration differences aside, segregation patterns for Lep and AL followed a similar pattern for ND5873 and NDG116. Three phenotypic classes were observed for these two aglycones. Class A included genotypes with Sol, Lep, and AL (Sol^+, Lep^+, AL^+); class B contained Sol and Lep, but not AL (Sol^+, Lep^+, AL^-); and class C included individuals that contained only Sol (Sol^+, Lep^-, AL^-). As for ND5873, the segregation analysis of these classes fit a two gene model (M1 and M2) for the synthesis of Lep from Sol and AL from Lep, respectively, both of which segregated as simplex characters with a 1:1 ratio. Linkage between M1 and M2 was not detected.

Table 1 Inheritance of alkaloids in populations NDG116 and ND5873

	Alkaloid (mg g^{-1} DW)			
	Solasodine	Solanidine	Leptinidine	Acetyl-lept
Parents				
ND4382-19	nd*	0.64	1.40	0.69
N142-72	3.93	nd*	nd*	nd*
Chipeta	nd*	0.32	nd*	nd*
NDG116 (ND4382-19 × N142-72)				
Number of plants	$n = 99$	$n = 97$	$n = 40$	$n = 18$
Average	2.22	0.11	0.02	0.01
STDEV	1.49	0.19	0.04	0.02
ND5873 (ND4382-19 × Chipeta)				
Number of plants	nd*	$n = 93$	$n = 48$	$n = 15$
Average	nd*	1.63	0.11	0.16
STDEV	nd*	0.51	0.08	0.13
Ratio ND5873:NDG116		15.0	5.2	14.1

Mean concentrations of solasodine, solanidine, leptinidine, and acetyl-leptinidine are given for each alkaloid. Genotypes not containing specific aglycones were excluded from the respective group

* nd = Not detected

Mapping of *Lep* and *AL* in population NDG116

Using a similar strategy as for ND5873, the putative loci for the synthesis of Lep and AL were determined in a tetraploid AFLP map (Sagredo et al. 2006). The AFLP map was initially developed using a population of 186 individuals. The available phenotypic data of Lep and AL from 99 individuals were used to place these loci in their respective AFLP coupling linkage groups. Seventeen different AFLP primer combinations and 186 progeny genotypes were used to map the NDG116 population. A total of 1,034 segregating AFLP fragments were obtained. Of this total, 821 were polymorphic between the parents; the rest (213) were shared between the parents, but segregated 3:1. The number of polymorphic AFLP fragments from the ND4382-19 and NY142-72 parents was 439 and 382, respectively. Markers were classified by segregation ratio as simplex, duplex, triplex, or distorted. Simplex markers, including those with slight deviation ($P \leq 0.10$) from simplex ratios, were used to map both parents using MapManager QTX. From the leptine donor (ND4382-19) of 278 segregating simplex markers, 266 markers were included in 37 simplex-coupling linkage groups, and 12 markers were unlinked. The 37 linkage groups covered a total map length of 1395.7 cM for ND4382-19. From parent N142-72, of a total of 201 markers, 196 were linked, and 5 markers were unlinked. Forty simplex-coupling linkage groups were obtained, covering a total map length of 1141.6 cM for the N142-72 parent.

Phenotypic markers *Lep* and *AL* were classified as binary markers and mapped in the above simplex-coupling maps. Phenotypic marker *Lep* was linked to Group L20, with closest AFLP marker E-ACG/M-CAAL9 at a distance of 24.53 cM (LOD = 5.0). Analysis of comigrant AFLP markers indicated that this group, L20, from population NDG116 corresponded to the *Lep*-containing chromosome 2 linkage group from population ND5873 (Sagredo et al. 2006) (Fig. 1). The requirement of *Lep* to synthesize AL indicated that only Lep^+ genotypes were informative to study the segregation of *AL*. A sub-set of 40 Lep^+ genotypes was used to analyze the linkage of *AL*. Only 18 of these had detectable levels of AL. Although no linkage was detected by MapManager QTX, simple correlation analysis within this sub-set between *AL* and AFLP markers identified three AFLP markers on group L6 with highly significant correlation ($P < 0.01$) with *AL*. This linkage group shared six common AFLP markers with the same chromosome 8 linkage group in ND5873 that contained *AL* (Sagredo et al. 2006) (Fig. 1).

Resistance to Colorado potato beetle

In progeny ND5873, resistance to *Leptinotarsa* showed a continuous distribution (Fig. 2). Defoliation of 100 progeny genotypes in replicated trials was measured under natural infestation of *Leptinotarsa* during the years 1997 and 1998. Percentage defoliation by *Leptinotarsa* ranged from 4 to 65% at Fargo, ND, in 1997 (F97); from 2 to 43% at Grand Forks, ND, in 1997 (GF97); from 5 to 68% at Rosemount, MN, in 1997 (MN97); and from 8 to 57% at Crookston, MN, in 1998 (MN98). Parent ND4382-19 consistently showed high levels of resistance to *Leptinotarsa* defoliation with 6, 5, 7, and 7% defoliation at F97, GF97, MN97, and MN98, respectively.

In family NDG116 resistance to *Leptinotarsa* was evaluated by detached leaf assay, obtaining larval mortality and development indexes across 184 NDG116 progeny

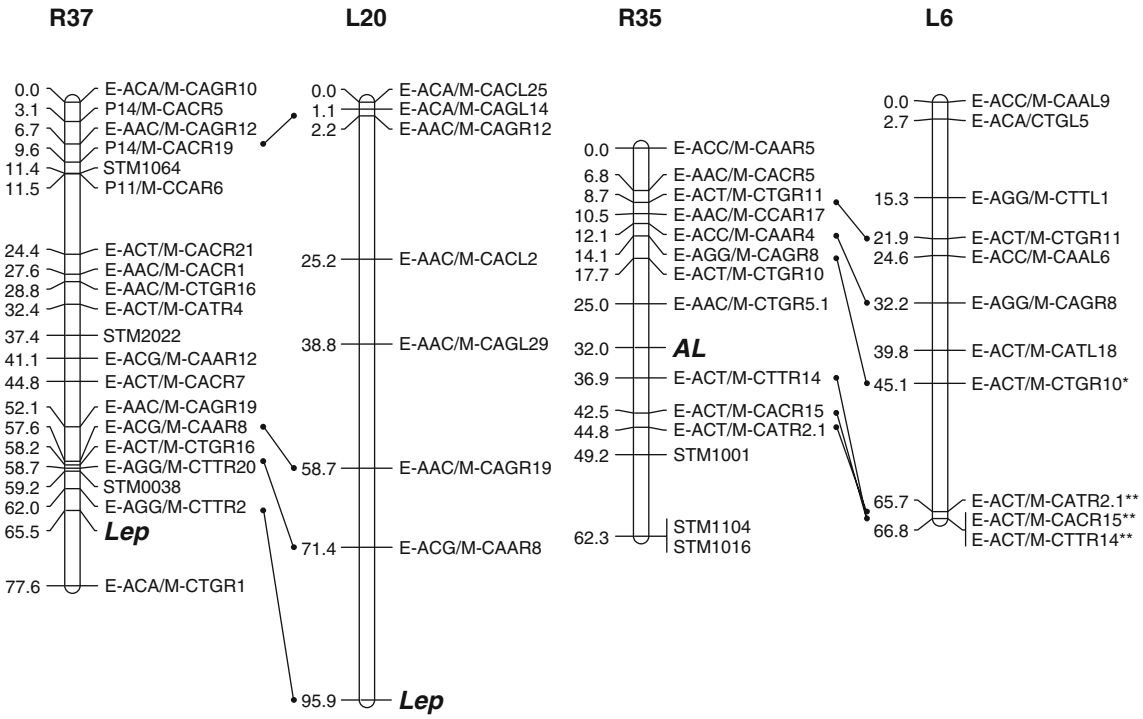

Fig. 1 Linkage groups carry genes *Lep* and *AL* from populations NDG116 and ND5873, from parent ND4382-19. Linkage Groups L20 and L6 share common AFLP markers with R37 and R35, from populations NDG116 and ND5873, respectively. *Lep* and *AL* genes involved in the synthesis of leptinidine and acetyl-leptinidine, respectively. R37 and R35 are homologs of chromosomes II and VIII, respectively (Sagredo et al. 2006). Shared markers are indicated with *lines*. Markers that were significantly associated with acetyl-leptinidine in L6 are indicated by "*" or "**" with $P \leq 0.05$ or $P \leq 0.01$, respectively

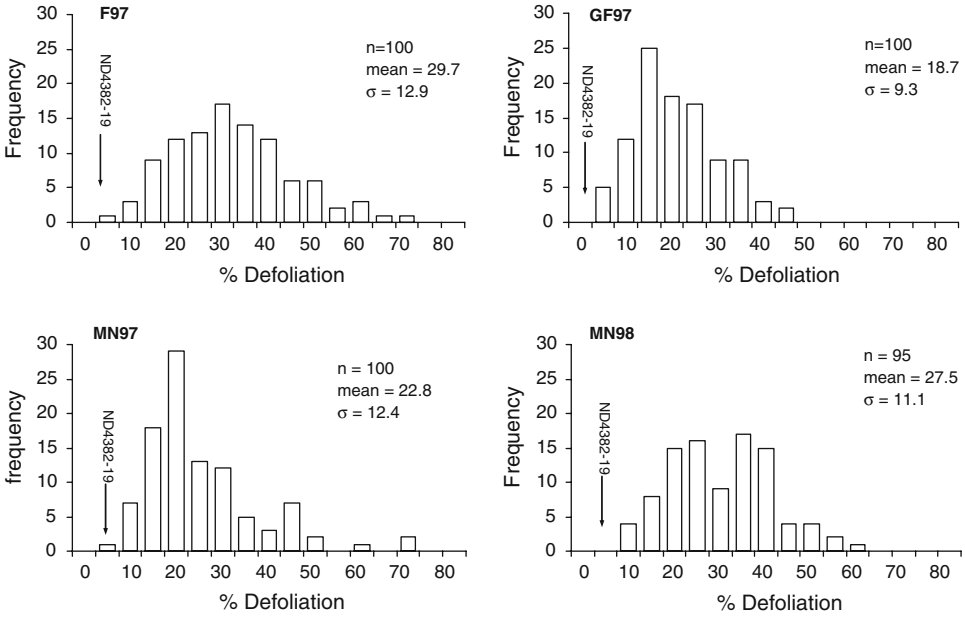

Fig. 2 Defoliation by CPB in four field trials in population ND5873. F97, GF97, MN97, and MN98 represent experiments performed at Fargo, ND 1997, Grand Forks, ND 1997, Rosemount, MN 1997, and Crookston, MN 1998, respectively

genotypes. Parents ND4382-19 and N142-72 were scored as resistant and moderately susceptible with regard to larval mortality index and larval developmental index (Fig. 3). Resistance to *Leptinotarsa* was also evaluated under field conditions for a subset of population NDG116. Sixty-four individuals from the NDG116 progeny were evaluated under natural *Leptinotarsa* pressure at McLeod, ND (1998). Percentage defoliation ranged from 22 to 100% (Fig. 3). N142-72 again showed intermediate resistance (65%) while the leptine donor, ND4382-19, was highly resistant (17% defoliation). Three other field assays were lost due to flooding (1997 and 1998) and hail (1999). Considering data

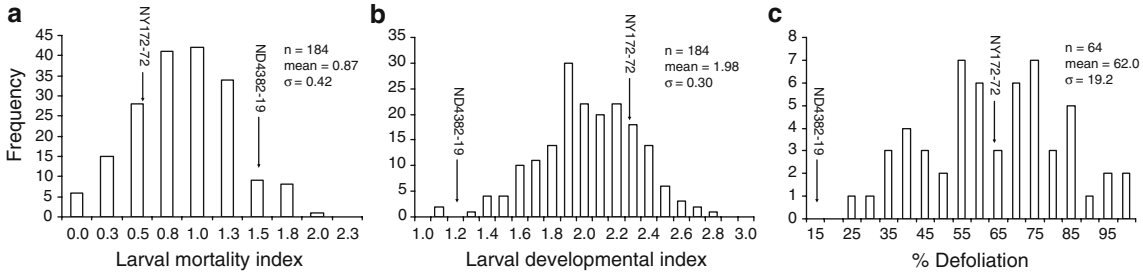

Fig. 3 Segregation of resistance to CPB in family NDG116. The graphs "**a**" and "**b**" illustrate the frequencies of larva mortality and the larval development for 184 NDG116 progeny genotypes, determined by detached leaf assay. The graph "**c**" corresponds to a field defoliation experiment (McLeod, ND, in 1998) with 64 NDG116 progeny genotypes. *Arrows* indicate the rate of defoliation of parents ND4382-19 and N142-72

of 57 common genotypes, the field defoliation experiment showed a highly significant correlation with larval development index ($r = 0.405$, $p < 0.01$) of the detached leaf assay, but not with the larval mortality index.

Relationship between steroidal alkaloids and resistance to *Leptinotarsa*

The possible relationship between resistance to *Leptinotarsa* and the glycoalkaloids, Sol, Lep, and AL, was evaluated in both populations using simple and multiple linear regressions. The aglycone group Sol showed no effect on resistance to *Leptinotarsa* in ND5873 or NDG116. However, Lep showed a significant contribution in population ND5873 ($n = 87$) at three localities, contributing 10% ($P = 0.0025$), 14% ($P = 0.0004$), and 5% ($P = 0.0326$) to the variance for Fargo (1997); Grand Forks (1997); and Crookston (1998), respectively, with no significant effect for Rosemount (1997). However, no significant relationship was observed between Lep and resistance to *Leptinotarsa* in family NDG116. There was a positive small contribution of AL ($n = 87$) at each of the four environments for ND5873, with 7% ($P = 0.013$), 9% ($P = 0.004$), 3% ($P = 0.037$), and 5% ($P = 0.049$) of the variance at Fargo, Grand Forks, Rosemount, and Crookston, respectively. There was no detectable concentration effect among the AL + ($n = 15$) individuals. Interestingly, for family NDG116, AL showed association with larval development when considering all individuals (AL− and AL+, $n = 74$) or only AL+ ($n = 13$), explaining 7 and 36% of the phenotypic variance, respectively. There was a quantitative effect of Sda in family NDG116 that contributed 11% ($P = 0.0037$) and 10% ($P = 0.0346$) to the variance of larvae development ($n = 73$) and field defoliation by *Leptinotarsa* ($n = 47$), respectively.

Association of molecular markers to *Leptinotarsa* resistance

In family ND5873, resistance data from 97 genotypes were used to perform single linear regression analysis against each simplex marker from both parents ND4382-19 and Chipeta, including AFLP simplex markers of 1:1 or 13:15 ratios (Hackett et al. 1998), those markers with slight deviation from a 1:1 ratio (distorted), and SSR markers. The only strong association between markers and *Leptinotarsa* resistance was from a single coupling linkage group from ND4382-19, R37. The closely-linked locus E-ACT/M-CTGR16 explained 27 and 19% ($P < 0.00001$) of the variance in *Leptinotarsa* resistance for 1997 and 1998, respectively. This group also contains the locus enabling Lep accumulation, but it was 7.3 cM from the more informative AFLP marker (Fig. 1), explaining 25% ($P < 0.00001$) and 17% ($P = 0.00002$) of the variance in *Leptinotarsa* resistance in 1997 and 1998, respectively. No marker from the linkage group associated with the synthesis of AL was significantly associated with resistance, even using a more permissive threshold of $P = 0.05$. Loci E-AGG/M-CTTR17 (Group 24) and E-AAC/M-CACR14 (Group 18) from the resistant parent also contributed to the *Leptinotarsa* resistance to a minor degree (8 and 5%, respectively). One locus from the susceptible parent, Chipeta, contributed to the resistance: E-AAC/ MCCAS12 (Group 25). This locus explained 8 and 7%, and 7 and 4% of the phenotypic variance in 1997 and 1998, respectively.

For NDG116, simple linear regression analysis between AFLP markers from both parents and larval mortality only detected a contribution from the leptine donor parent ND4382-19 ($P \leq 0.01$). Of nine associated markers, seven were located in the L15 simplex-coupling linkage group, and two were in Group L13. Markers from L15 group enhanced larval mortality (more resistant) while those in Group L13 had a negative association with larval death (more susceptible), albeit with a small contribution to the variance. The single linear regression analysis for larval development detected a contribution from both parent genomes. From ND4382-19, there were five individual markers, one linked to Group L8, one to Group L18, and three to Group L20. All loci from ND4382-19, except the one from the L18 group, had a negative effect on larval development (increased resistance to *Leptinotarsa*). The

L20 simplex-coupling linkage group contains the phenotypic marker for leptinidine, suggesting the same mechanism that was active in the family ND5873. On parent N142-72, seven AFLP markers were detected by linear regression ($P \leq 0.01$), which were associated on four homologous linkage groups. Three of the four groups contributed to susceptibility, but all had only a small effect on the phenotypic variance (3–5%).

One field assay for Colorado potato beetle was performed at McLeod, ND (1998), with 64 NDG116 progeny genotypes. Single linear regression analysis between the AFLP markers and *Leptinotarsa* defoliation detected significant associations ($P \leq 0.05$) deriving from both parents, ND4382-19 and N142-72. From the leptine donor, there were 10 markers, linked to Groups L14, L20, L21, L24, L28, and L30, and one unlinked marker. The markers associated with simplex-coupling Groups L20 and L28 were associated with resistance. The same markers from Group L20, which were detected in the detached leaf assay, were also associated with resistance in this field experiment. The strongest effect was associated with marker E-ACG/M-CAAL9, with 12% of the variance explained. Markers in Groups L14, L21, L24, and L30 were negatively associated with resistance. From the parent N142-72, nine AFLP marker-loci were detected; all of which contributed negatively to the resistance. One marker from the T25 group presented the major contribution to the variance (11%).

Interval mapping analysis of QTL associated to *Leptinotarsa* resistance on chromosome 2

The analyses above showed that the most significant contribution to resistance to *Leptinotarsa* came from ND4382-19 chromosome 2, represented by homologous groups R37 and L20 for ND5873 and NDG116, respectively. Interval mapping was used to examine the effect of the QTL on this chromosome 2 according to Hackett and Luo (2003) using the software TetraploidMap. Figures 4 and 5 illustrate the four homologous chromosomes for chromosome 2 from ND4382-19 that segregated within the progenies ND5873 and NDG116, respectively. The QTL analysis for resistance to *Leptinotarsa* in ND5873, measured as field defoliation at four different environments, showed a major QTL on the distal extreme of the chromosome 2 (Fig. 4). This region contains gene *Lep*, postulated to be involved in the synthesis of leptinidine from solanidine (Sagredo et al. 2006). Table 2 indicates the main features for this major QTL detected at three different locations in 1997 and one location in 1998. This QTL explained from 11 to 34% of the variance of these trials (Table 2). The same QTL was detected in the family NDG116 by both percentage defoliation in the field and larval development in detached assay, explaining 26 and 12% of the variance, respectively (Fig. 5, Table 2). This locus was not associated with larval death in the detached leaf assay. QTLs from other chromosomes were associated with larval death (data not shown). A second minor QTL for resistance was observed on chromosome 2 in population ND5873 in three of the four field trials (Fig. 4), suggesting that environment affect its expression.

Interestingly, population NDG116 contained a major QTL from parent ND4382-19 on chromosome 2, position 56.00 cM that affected the concentration of Sda (Fig. 5). This QTL explained 41% of the phenotypic variance in Sda, and is best explained by a simple dominant model.

Discussion

The incorporation of genetic resistance in potato varieties should facilitate the management of Colorado potato beetle. Deployment of beetle-resistant cultivars would allow reduced use of insecticides, and may be compatible with other biological control strategies. Several natural enemies of Colorado potato beetle that significantly reduce *Leptinotarsa* populations have been described (Hilbeck and Kennedy 1996; Hough-Goldstein et al. 1993). Maximal depredation of beetles is expected to occur in the absence of insecticides. However, considering the large investment of money for a potato crop and the risk of loss in commercial production, pesticide-free cultivation is not practical at present. Genetic resistance should reduce the risk of loss in the absence of insecticides, which may enable use of biological control agents on a larger scale.

One of the most interesting sources of genetic resistance to *Leptinotarsa* is the wild species *S. chacoense*, which has been associated with leptines. However, the development of leptine-expressing commercial potato varieties that are resistant to *Leptinotarsa* has been a difficult task. Although leptines are only expressed in the foliage, effective deterrent levels of leptine in leaves has been associated with high TGA levels in the tubers, a concern for human and animal consumption (Sanford et al. 1996; Yencho et al. 2000). The observation that some progenies derived from an unrecorded *S. chacoense* were highly resistant to *Leptinotarsa* with only moderate to low-leptine content suggested the possibility of an alternative resistance factor (Lorenzen et al. 2001). In order to study the genetic nature of this resistance, we characterized two segregating tetraploid families, ND5873 and NDG116, from common parent ND4382-19. This highly resistant parent contains a relatively low level of foliar leptine.

A recent report (Sagredo et al. 2006) supported the hypothesis that Lep and AL are synthesized from Sol (Lawson et al. 1993; Osman et al. 1987). Using segregating

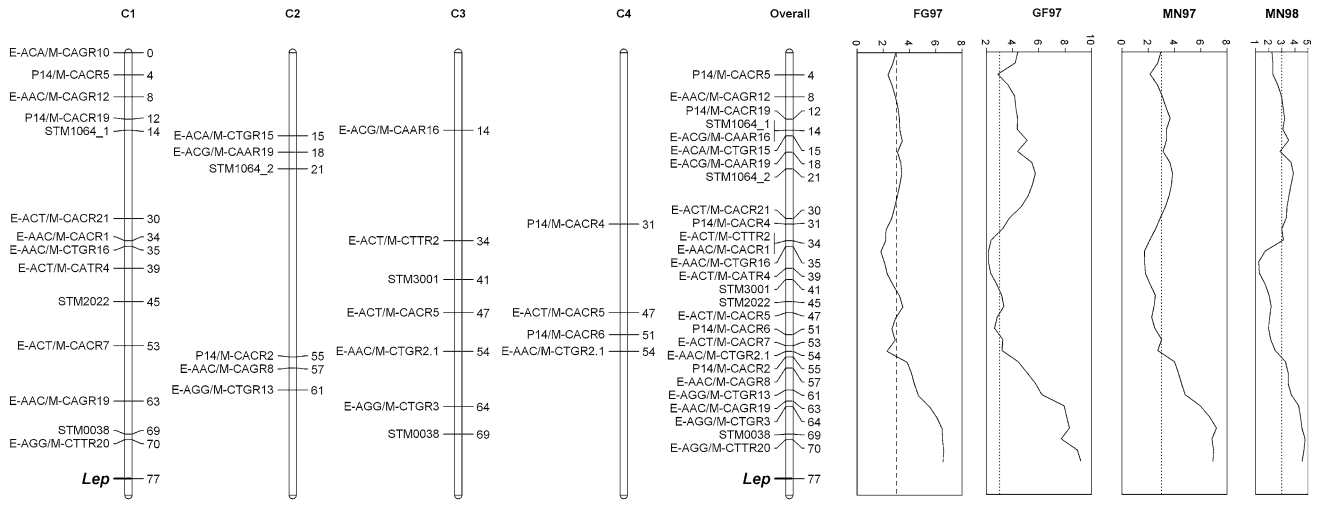

Fig. 4 QTL mapping of CPB resistance using family ND5873. The figure shows the QTL analysis of CPB resistance for the chromosome II using the Tetraploidmap software. F97, GF97, MN97, and MN98 represent field trials at Fargo, ND, in 1997; Grand Forks, ND, in 1997; Rosemount, MN, in 1997; and Crookston, MN, in 1998, respectively. C1, C2, C3, and C4 represent homologs of chromosome 2. The overall group of chr 2 was generated by Tetraploidmap software. The numbers to the right of each linkage group are distances (cM). The graphs to the right of the map are the output of the QTL analyses, where the *X*-axis for each panel represents LOD score. The segmented lines (LOD > 3.0) represent QTL significance threshold

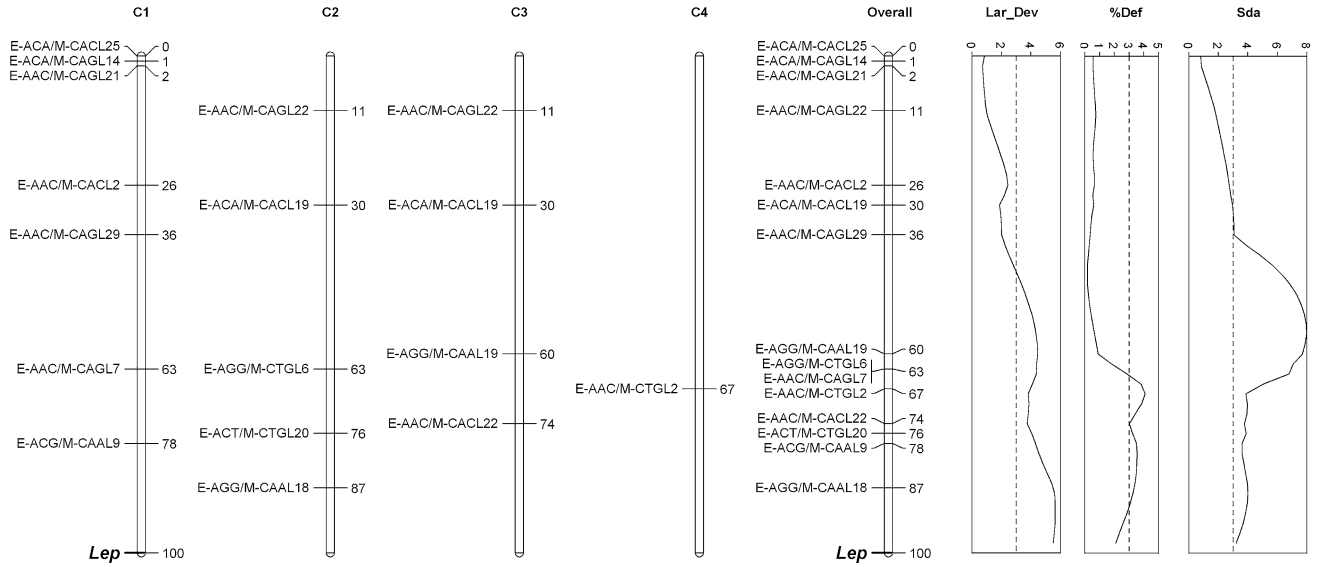

Fig. 5 Major QTLs associated with CPB resistance and content of solasodine in progeny NDG116. The major QTL associated with larval development (Lar_Dev), CPB defoliation in the field (% def) and content of solasodine (Sda) are shown for chromosome II. See Fig. 4 for details

family ND5873 (ND4382-19 × Chipeta), we demonstrated that genes enabling accumulation of Lep and AL have complementary epistasis and map to chromosomes 2 and 8, respectively. Mapping these steroidal alkaloids in a second related population, NDG116, reinforces that previous report. The aglycone groups Sol, Lep, and AL were present at concentrations that were 15-, 5-, and 14-fold lower than in family ND5873, respectively, and another aglycone group Sda was present in all the individuals at much higher concentration (Table 1). Sda was apparently inherited from the parent NY142-72, which has a complicated pedigree derived from *S. berthaultii*, which produces this spirosolane class of alkaloids (Yencho et al. 1998). It may be that the Sda biosynthetic pathway takes precedence over the Sol pathway in potato leaves with genes for Sda synthesis. Mean content of Sda was 20-fold higher than Sol in NDG116 (Table 1). A similar situation was seen in the BCT population, where Sda was the dominant aglycone in a backcross population that was 75% *Solanum tuberosum* (Yencho et al. 1998). That Sda still predominated in N142-72 after 14 generations of

Table 2 QTL interval mapping analysis for resistance to *Leptinotarsa* on chromosome 2 of genotype ND4382-19 from two segregating progenies

Traits	Position (cM)	LOD	% Variance	EMS	QTL Effects on genotypes					
					Q1.2	Q1.3	Q1.4	Q2.3	Q2.4	Q3.4
ND5873 (% def)										
Fargo 1997 (FG97)	72.00	6.61	24.28	119.40	19.93(2.38)	29.51(3.42)	23.39(2.61)	37.83(3.42)	34.42(2.43)	34.70(2.76)
Grand Fork 1997 (GF97)	74.00	9.20	34.14	54.68	11.99(1.64)	14.16(2.32)	14.00(1.78)	28.70(2.25)	21.29(1.62)	22.55(1.86)
Rosemount 1997 (MN97)	68.00	3.48	11.37	113.02	22.14(2.20)	24.51(3.35)	23.78(2.54)	30.85(3.54)	34.24(2.44)	27.52(2.66)
Crookston 1998 (MN98)	70.00	4.77	16.34	106.32	23.28(2.18)	23.95(3.26)	23.27(2.46)	36.19(3.36)	31.26(2.33)	34.05(2.55)
NDG116										
Larva development (Lar_Dev)	92.00	5.65	11.94	0.08	1.856(0.043)	1.89(0.07)	1.82(0.06)	2.032(0.048)	2.07(0.05)	2.13(0.05)
McLeod 1998 (% def)	68.00	4.10	25.95	320.18	33.99(4.961)	17.74(10.97)	24.744(6.34)	33.914(5.798)	60.78(6.96)	47.88(4.31)

The traits columns indicate progeny and locality where the resistance to *Leptinotarsa* was measured in field assays (% def) or detached assay (Lar_Dev). Position, maximal LOD score (LOD), contribution to the phenotypic variance and the error means square (EMS) are given for the respective QTL. The QTL effects are presented as means for the six QTL genotypes, coded Q1.2, Q1.3, Q1.4, Q2.3, Q2.4, and Q3.4 according to the chromosomes inherited from the parent ND4382-19, and their associated standard errors between parentheses. The C1 group corresponded in the two populations but lack of marker coincidence among C2, C3, and C4 groups prevented similar identification for these groups

backcrossing and intercrossing, and in its NDG116 progeny, was surprising. The genetic and regulatory control of this partitioning between alkaloids remains to be elucidated. Sol was detectable in almost all genotypes of the NDG116 population, suggesting that the observed predominance of Sda over Sol is because its synthesis was favored over Sol. The same inhibitory effect was observed over the synthesis of Lep and AL, reinforcing that they are derived from the same biosynthetic pathway as Sol (Lawson et al. 1993; Osman et al. 1987; Sagredo et al. 2006).

Aside from their lower concentration in NDG116, the segregation of Sol, Lep, and AL followed similar segregation patterns as in ND5873. The *Lep* locus mapped to a similar position on chromosome 2 (Fig. 1). The *AL* locus did not map cleanly because of complementary epistasis and the relatively few informative Lep+ genotypes available in this study. However, its relative position as indicated by correlation to neighboring AFLP markers suggested that the *AL* locus is at an equivalent chromosome 8 location in NDG116 (Fig. 1; Sagredo et al. 2006).

Both half-sib families segregated for resistance to *Leptinotarsa*, as measured by field defoliation experiments for ND5873, and by field and detached leaf assays for NDG116 (Figs. 2, 3). A large QTL for resistance to *Leptinotarsa* on the distal extreme of chromosome 2 contributed from 11 to 34% of the variance in defoliation in population ND5873 at four environments. The same resistance QTL in family NDG116 explained 26 and 12% of the variance in field defoliation and larval development, respectively. Other regions of the ND4382-19 genome detected in family NDG116 had minor effects on larval death. The largest QTL for *Leptinotarsa* resistance coincided with the *Lep* locus, which explains the significant association between resistance and presence of aglycone groups Lep and/or AL, observed in families ND5873 and NDG116. The large differences in Lep/AL content between populations ND5873 and NDG116 (Table 1) suggests that Lep concentration may not be the primary determinant of resistance in these families. Therefore, another N4382-19 factor may be responsible for *Leptinotarsa* resistance.

The male parent of population NDG116, NY142-72, was selected for resistance to *Leptinotarsa* and has *S. berthaultii* in its background. One of the objectives in creating NDG116 was to combine mechanisms of resistance to *Leptinotarsa* from *S. chacoense* and *S. berthaultii*, respectively. Combination of genetic resistance mechanisms such as leptines and glandular trichomes could enhance their effectiveness and durability against *Leptinotarsa*. Multiple mechanisms of resistance would be expected to produce more durable resistance. Conventional potato breeding is tedious and time consuming, with incorporation of resistance and development of new varieties taking more than 10 years (Plaisted et al. 1994).

Unfortunately, parent N142-72 showed relatively little resistance to *Leptinotarsa* from North Dakota in both the field experiment and detached leaf assay (Fig. 3), as was observed in other field tests (Lorenzen and Balbyshev, unpublished). Most N142-72 markers detected by QTL analysis were associated with susceptibility. Although the greenhouse conditions may have affected expression of glandular trichomes, and the number of individual genotypes used in the field experiment was relatively low, glandular trichome donor NY142-72 did not appear to contribute substantially to resistance. More experimental data using plants grown under field conditions for detached leaf data and larger population would help to address this issue with more precision. Unfortunately, field experiments were lost to flooding and hail during the experimental seasons.

Chromosome 2 from ND4382-19 also contained a major QTL that affected the content of Sda in NDG116 (Fig. 5). Yencho et al. (1998), using two segregating populations from a hybrid (*S. tuberosum* × *S. berthaultii*) backcrossed with *S. berthaultii* (BCB) and *S. tuberosum* (BCT), found that Sda accumulation was controlled by three QTLs present in chromosomes 4, 6, and 12 in BCB; similarly three QTLs were found in chromosomes 4, 8, and 11 in BCT. In this study, the identified QTL on chromosome 2 that controlled Sda accumulation in NDG116 accounted for 41% of the variability in Sda and was not related with those QTLs detected by Yencho et al. (2000). Therefore, much remains to be elucidated about control of glycoalkaloid content in potato with differing genetic backgrounds. Solasodine content was inversely related to *Leptinotarsa* resistance as assessed by larval development ($R^2 = 0.1297$, $P = 0.002$). It is unlikely that low levels of Sda would cause resistance. It is more probable that this effect of Sda was due to linkage with the resistance factor just described for these populations.

In conclusion, presence of Sda was associated with suppressed accumulation of Sol, Lep, and AL in population NDG116. Nonetheless, qualitative presence of Lep and AL was controlled by loci at the same chromosome 2 and 8 locations as in half-sib population ND5873 (Sagredo et al. 2006). A large QTL resulting in resistance to *Leptinotarsa* was located very near the chromosome 2 locus, *Lep*, in both populations. The lack of a QTL associated with *AL*, and identification of highly resistant individuals that lack leptine or have very low leptine content, suggests that this resistance from *S. chacoense* is qualitatively different from the leptine-dependent resistance reported by previous authors.

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