Hybrid Breakdown in the F₁ between *Solanum chacoense* and *S. phureja* and Gene Transfer for Leptine Biosynthesis

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ABSTRACT. F₁ hybrids between high leptine-producing clones (8380-1, PI 458310 and 55-1) of *Solanum chacoense* Bitt. and anther culture competent or anther-derived clones of *S. phureja* Juz. & Buk. that did not produce leptines were generally weak plants that grew slowly and died before flowering. Exceptional hybrids could be found that were capable of completing a life cycle, especially during the hot summer months in the greenhouse. All F₁ hybrids produced leptines in the leaves but not the tubers, albeit at lower levels than in the *S. chacoense* parent. Anther-derived monoploids from the F₁ hybrids exhibited a range of leptine production from none to levels approaching the *S. chacoense* parent. Backcross populations of an F₁ hybrid to the *S. chacoense* and *S. phureja* parents were examined for leptine production. Backcross hybrids were generally much more vigorous than the F₁ hybrids. All of the *S. chacoense* backcrosses produced leptines ranging from intermediate to high levels; four of the twelve *S. phureja* backcrosses exhibited low leptine levels. A general dominance of leptine synthesis was therefore exhibited, although the nonleptine-producing parent affected the expression of leptines in the hybrids.

Various crossing schemes can be proposed for attempting to transmit the leptine-based resistance to the Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say) from selected clones of *Solanum chacoense* Bitt. (*chc*) into cultivated potato. Leptine glycoalkaloids are specific to particular accessions of *chc* and have been found to reach high levels in leaves of selected clones (Sinden et al., 1986). Sanford et al. (1996) derived hybrids between breeding lines of *S. tuberosum* L. and the chromosome-doubled derivatives of a high leptine diploid clone, 8380-1, of *chc*. All of the resulting tetraploid hybrids produced leptines, indicating a dominant factor in *chc*. There was considerable variation among the hybrids for total leptine as well as total glycoalkaloids in the leaves and tubers, and, as expected, leptines were not found in tubers (Sinden et al., 1986). Chromosome doubling was required because unreduced gametes are absent in 8380-1, making interploid hybridization difficult. A disadvantage of chromosome doubling is that it inevitably involves some inbreeding when breeding at the tetraploid level due to the limited allelic diversity in chromosome doubled material—only two different alleles can exist at a locus instead of the possibility of four for sexually produced tetraploids between heterozygous parents. In addition, because the resulting tetraploid hybrids comprise fully half their genome from unadapted germplasm (*chc*), their agronomic performance may be poor.

Somatic hybrids have also been derived between dihaploid *S. tuberosum* and seedlings of a leptine-producing accession of *chc* (Cheng et al., 1995). Feeding preference assays determined that

Colorado potato beetles consumed three times more of the *S. tuberosum* parent than either the somatic hybrids or the *chc* parent. Somatic hybrids contained lower levels of leptines than the *chc* parent, but verified a dominant characteristic to leptine biosynthesis. Complete somatic hybrids would have comprised the entire genomes of both parents. Hence they would have circumvented the problem of allelic impoverishment induced by chromosome doubling. However, they would still be likely to be limited in agronomic potential because of the inordinate dosage of unadapted *chc* germplasm.

An alternative to doubling the leptine-producing selections of *chc* to derive tetraploid hybrids is to breed at the diploid level using adapted clones of *S. phureja* (*phu*), a primitive cultivated diploid (Haynes, 1972). *S. phureja* has been an abundant source of unreduced gametes (Veilleux and Lauer, 1981). *Solanum phureja* × *S. chacoense* (PC) hybrids have been screened for high 2n pollen production and used to construct highly heterozygous tetraploids after crossing with cultivars of *S. tuberosum* (Bani-Aameur et al., 1991, 1992). In addition, responsiveness to anther culture has been frequently found in *phu*, allowing the possibility to derive monoploids from PC hybrids that could potentially exhibit the high leptine characteristic of *chc* with the better adaptation of *phu*. Such monoploids could be chromosome doubled, followed by a round of breeding at the diploid level to identify better dihaploids (2n pollen producers) with high leptine levels.

The objectives of the present study were to

Table 1. Solanidine content, dry mass basis, of *Solanum phureja* anther donor clone PP5, four of its anther-derived doubled monoploids, and two other *S. phureja* selections.

Clone	Solanidine (mg·g ⁻¹)
PP5	0.43
AD 2-4	0.0
AD 3-4	0.13
AD 4-1	0.82
AD 29-1	2.96
BARD 1-3	3.48
BARD 9-9	1.95

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derive hybrids between anther culture competent clones of *phu* and high leptine producing clones of *chc*, and subsequently examine the leptine levels and response to anther culture of these hybrids and their derivatives.

Materials and Methods

EXPERIMENT 1: TOTAL AND INDIVIDUAL STEROIDAL GLYCOALKALOID CONTENT OF F_1 HYBRIDS BETWEEN *S. chacoense* AND *S. phureja*. Two leptine-producing clones of *chc* (55-1 and 8380-1, PI 458310) were obtained from Steve Sinden, USDA-ARS, Beltsville, Md., and were crossed to two heterozygous clones (PP5 and BARD 1-3) of adapted *phu* (Haynes, 1972) that had been selected for anther culture competence and $2n$ pollen production by the genetic equivalent of first division restitution (Veilleux, 1990). The *chc* clones were also crossed as pollen parents to four homozygous doubled monoploids (AD 2-4, AD 3-4, AD 4-1, and AD 29-1) of *phu*. Monoploids (AM2, AM3, AM4, and AM29) had been obtained by anther culture of PP5 and subsequently doubled spontaneously by leaf disc regeneration (M'Ribou and Veilleux, 1990). Ploidy levels were verified by flow cytometry (Owen et al., 1988). Seedlings of the resulting *phu-chc* (PC) hybrids were planted in the greenhouse in late summer, transplanted to 3.8-L nursery pots, and sampled for foliar glycoalkaloids before flowering by taking two fully expanded young leaves from each of six seedlings per family. The leaf samples were freeze-dried, ground, and assayed for glycoalkaloids by gas chromatography (Lawson et al., 1992). For each sample, the mean of two injections was taken. Family differences were analyzed by SAS ANOVA (SAS Institute, Cary, N.C.) by using the six seedlings per family as replications.

Shortly after samples were taken for alkaloid analysis, when the first flowers began opening, all of the PC hybrids rapidly declined. The plants died within 2 weeks even though no insects or pathogens could be identified after taking samples to the Pest and Disease Clinics, Depts. of Entomology and Plant Pathology, Physiology, and Weed Science, VPI&SU, respectively. New seedlings were started on a monthly basis with the same result, i.e., the plants rapidly declined after a few weeks of slow growth. A few plants from several families survived through the summer months in the greenhouse and eventually flowered and set tubers. A limited number of anther cultures were done on these clones according to Snider and Veilleux (1994) as possible because flowering was sporadic.

EXPERIMENT 2: TOTAL AND INDIVIDUAL STEROIDAL GLYCOALKALOID CONTENT OF ANTER-DERIVED MONOPLIDS. Selected clones (CP2 from 8380-1 x BARD 1-3, RPC4 from AD29-1 x 55-1, and RPC6 from PP5 x 8380-1) were grown in subsequent seasons for anther

culture experiments (Chani, 1997; Veilleux et al., 1993). These selected hybrids were never vigorous during the fall and spring greenhouse seasons but thrived in the summer in the greenhouse when temperatures were too hot for most potatoes. Monoploids obtained from these PC hybrids were identified by flow cytometry (Owen et al., 1988) and sampled for leaf glycoalkaloids as above. Only single plants of each genotype were available for glycoalkaloid analyses, preventing statistical analysis. However, the data exhibit variation within the segregating population.

EXPERIMENT 3. TOTAL AND INDIVIDUAL STEROIDAL GLYCOALKALOID CONTENT OF BACKCROSSES BETWEEN CP2 AND ITS *CHC* AND *PHU* PARENTS. PC clone CP2 was backcrossed to both of its parents (*chc* 8380-1 and *phu* BARD 1-3). The seedlings were grown in the greenhouse in late spring and early summer. Leaf samples were taken for glycoalkaloid analysis as above, again, on single plant basis, to determine variation within the populations.

Results

EXPERIMENT 1: TOTAL AND INDIVIDUAL STEROIDAL GLYCOALKALOID CONTENT OF F_1 HYBRIDS BETWEEN *chc* AND *phu*. Methanolic extracts from parental and selected hybrids, monoploids, doubled monoploids, and backcross progeny did not contain detectable levels of solanidine, leptinidine, or acetylleptinidine. Only upon hydrolysis with HCl were the steroidal alkaloids detected. These results confirmed that solanidine, leptinidine, and acetylleptinidine were present as their respective glycosides, solanine (and/or chaconine), leptinines (I and/or II), and leptines (I and/or II). Throughout the remainder of this paper, glycoalkaloid content of samples is reported as content of the corresponding aglycones, except as noted.

The *phu* clones, *chc* clones and PC hybrids varied in steroidal alkaloid profile and content. The *phu* clones used as parents of PC hybrids contained only solanidine in different amounts (Table 1). Although PP5 had low solanidine content compared to the other heterozygous *phu* clones (BARD 1-3 and BARD 9-9), its anther derived doubled monoploids differed considerably, ranging from no detectable solanidine (AD 2-4) to a much higher content (AD29-1) than exhibited by PP5. As expected, both *chc* clones contained high levels of all three steroidal alkaloids, with the content of acetylleptinidine highest (Table 2). All of the PC hybrids likewise contained all three steroidal alkaloids and the amount differed among the families (Table 2). Among the three PC hybrid families examined that had been derived from crosses with homozygous doubled monoploid *phu* clones, the content of all three steroidal alkaloids reflected the relative amount of solanidine in the doubled monoploid parents, e.g., AD2-4 expressed the lowest level of solanidine and gave rise to PC hybrids in crosses

Table 2. Mean glycoalkaloid content, dry mass basis, in five *Solanum phureja* x *S. chacoense* hybrid families and the *chc* clones used to generate them.

Family	Glycoalkaloid* (mg·g ⁻¹)				
	SD	LD	ALD	Total	% ALD
PP5 x 8380-1	0.69 bc ^y	0.73 cd	1.35 b	2.77 c	54 b
AD 2-4 x 55-1	0.05 c	0.36 d	0.66 b	1.07 c	63 a
AD 3-4 x 55-1	1.73 b	1.02 bc	1.27 b	4.01 bc	33 c
AD 4-1 x 8380-1	3.38 a	1.40 ab	1.54 b	6.31 ab	25 d
BARD 9-9 x 8380-1	3.14 a	1.71 a	2.49 a	7.33 a	33 c
<i>chc</i> 55-1*	2.03	7.50	14.97	24.50	61
<i>chc</i> 8380-1	1.26	7.25	11.32	19.84	57

*SD = solanidine, LD = leptinidine, and ALD = acetylleptinidine; SD, LD, and ALD were products of hydrolysis of solanine + chaconine, leptinines I + II, and leptines I + II, respectively.

^yMean separation within columns by Student-Newman-Keul's test, 5% level.

*The steroidal alkaloid content of *chc* 55-1 and *chc* 8380-1 are shown for comparative purposes only as they were not grown at the same time as the hybrids.

with *chc* 55-1 that had significantly lower solanidine, leptinidine, acetylleptinidine and total glycoalkaloids than the progeny of AD 4-1 x 8380-1 (Table 2), for which the doubled monoploid parent (AD4-1) expressed higher levels of solanidine (Table 1).

EXPERIMENT 2: TOTAL AND INDIVIDUAL STEROIDAL GLYCOALKALOID CONTENT OF ANTHER-DERIVED MONOPOIDS. As stated in the Materials and Methods, all of the PC hybrids that were sampled to compose Table 2 died shortly after the samples were taken. A few plants from a subsequent planting were selected the following summer and subjected to anther culture. These PC hybrids and their anther-derived monoploids were sampled for leaf and tuber steroidal alkaloids. All five of the surviving PC hybrids (CP2, RPC1, RPC2, RPC3, RPC4) contained acetylleptinidine (Table 3), as observed for the weak hybrids (Table 2), with values ranging from 1.14 to 2.0 mg·g⁻¹ dry mass for RPC2 and CP2, respectively. Although these values were much lower than those of the *chc* clones used as parents, the fraction of glycoalkaloids present as acetylleptinidine ranged from 20% to 100%. Two PC hybrids, RPC1 and RPC2, contained only acetylleptinidine (Table 3). The monoploids exhibited the range of total and individual steroidal alkaloids expected from segregation of the interspecific hybrids. Sixty-seven percent (10 of 15) contained no acetylleptinidine or only a trace whereas others, CP2-3 and CP2-103, contained high concentrations approaching that of the *chc* parent (Table 3). Of those not containing acetylleptinidine, many (CP2-7, CP2-314, CP2-341, RPC4-101, RPC4-301, RPC4-331, RPC4-333, RPC4-343A, and RPC2-1) contained leptinidine; only a single monoploid (CP1-1) contained solanidine alone, whereas CP2-314 contained only leptinidine (Table 3). Tuber samples of three sibling PC hybrids (CP1, CP2, and CP3) contained only solanidine at 1.38, 1.51, and 4.55 mg·g⁻¹ dry mass, respectively.

EXPERIMENT 3: TOTAL AND INDIVIDUAL STEROIDAL GLYCOALKALOID CONTENT OF BACKCROSSES BETWEEN CP2 AND ITS *chc* AND *phu* PARENTS. Twelve backcross hybrids from each family, CP2 x 8380-1 and 1-3 x CP2, were grown in the greenhouse and sampled for leaf steroidal alkaloids. Plants in both backcross families were

generally vigorous, although weak plants were occasionally noted. Only vigorous plants were sampled. All of the backcross hybrids to *chc* 8380-1 contained all three steroidal alkaloids, although some contained more acetylleptinidine relative to solanidine and leptinidine (Table 4). The percentage of the steroidal alkaloid present as acetylleptinidine ranged from 14% to 60% for the CP2 x 8380-1 family (Table 4). By contrast, only four of 12 backcross hybrids between *phu* 1-3 and CP2 contained acetylleptinidine and the concentrations were generally much lower than in the *chc* backcrosses (Table 4). The ratio of acetylleptinidine to total alkaloids ranged from 36% to 100%, however. One *phu* backcross (1-3 x CP2-2) contained leptinidine but not acetylleptinidine and seven produced only solanidine at concentrations ranging from 0.20 to 7.14 mg·g⁻¹ dry mass.

Discussion

The simple genetic basis of acetylleptinidine (i.e., leptine) production due to presence of homozygous dominant genes in the selected clones of *chc*, as proposed by Sanford et al. (1996), was supported by the present study. Leptines were synthesized by all of our diploid PC hybrids. However, leptine levels were dependent on the *phu* parent as well, again supporting the data of Sanford et al. (1996), who found that the nonleptine-producing *S. tuberosum* parent affected the synthesis of leptines of their *S. tuberosum* x *S. chacoense* tetraploid hybrids. Our backcross populations were too small to permit sound genetic interpretations; however, when leptines were expressed in the 1-3 x CP2 backcrosses, where heterozygosity would be expected for any genes controlling their expression, the levels were much lower than in the CP2 x 8380-1 backcrosses, in which some plants would have been heterozygous and others homozygous for genes influencing leptine production. In the hemizygous monoploids, leptines could reach high levels, perhaps by elimination of suppressor genes from *phu*. Simple inheritance has also been proposed for other glycoalkaloids present in *chc* (McCollum and Sinden, 1979).

Table 3. Glycoalkaloid levels, dry mass basis, in selected *Solanum phureja* x *S. chacoense* hybrids (CP2, RPC4, RPC6) and their anther-derived monoploids.

Clone	Ploidy	Glycoalkaloid ² (mg·g ⁻¹)				
		SD	LD	ALD	Total	% ALD
CP2	2x	0.94	1.35	2.0	4.29	47
CP2-3	1x	0.69	4.0	15.1	19.8	76
CP2-4	1x	0.23	0.49	0.89	1.6	55
CP2-7	1x	9.79	0.52	TR ^y	10.31	0
CP2-103	1x	0.32	2.42	7.59	10.34	73
CP2-165	1x	1.37	3.86	13.81	19.03	73
CP2-314	1x	TR	0.59	TR	0.59	0
CP2-341	1x	0.69	0.86	TR	1.55	0
RPC1	2x	0	0	1.42	1.42	100
RPC1-1	1x	0.44	0	0	0.44	0
RPC2	2x	0	0	1.14	1.14	100
RPC2-1	1x	0.44	1.84	0	2.22	0
RPC3	2x	0.61	1.73	1.69	4.03	42
RPC3-9	1x	0.08	0.36	0.53	0.97	55
RPC4	2x	3.83	1.97	1.95	7.75	25
RPC4-101	1x	5.97	0.94	TR	6.91	0
RPC4-301	1x	7.39	4.79	0	12.18	0
RPC4-331	1x	9.73	9.30	0	19.03	0
RPC4-333	1x	5.91	0.69	TR	6.6	0
RPC4-343A	1x	1.01	6.09	0	7.1	0

²SD = solanidine, LD = leptinidine, and ALD = acetylleptinidine.

^yTR = trace level <0.1 mg·g⁻¹ dry wt but >0.

Weak PC hybrids have been previously reported. Bani-Aameur et al. (1992) reported that 15 of 48 PC families examined were characterized by weak plants and were discarded from their study. In the present study, with a more limited array of germplasm due to the prerequisite selection for high leptine levels in the *chc* parents, all of the PC hybrids were weak. Those that survived were especially sensitive to environmental conditions; in the fall-winter greenhouse season, none survived whereas in the spring-summer greenhouse season, the better selections thrived. A similar hybrid breakdown phenomenon has been reported in hybrids between *Nicotiana suaveolens* Lehm. and *N. tabacum* L. (Inoue et al., 1994). Seeds could be readily obtained from the cross, but seedlings needed to be maintained at 34 °C to prevent hybrid lethality. Seedlings germinated at lower temperatures became necrotic and died at the cotyledonary stage. The absence of hybrid breakdown in most of our backcross progenies of CP2 x 8380-1 and BARD 1-3 x CP2 may indicate that breakdown is due to an unfavorable genomic ratio in the F₁ hybrids. Birhman et al. (1994) described a mutant in *chc* that resulted in seedling death at the cotyledon stage. In contrast, our weak PC seedlings typically grew slowly for several months before dying.

Some plants contained most or all of their alkaloids as acetylleptinidine (i.e., leptines) with little evidence of the precursors, solanidine and leptinidine, according to the present hypothetical synthetic pathway (Lawson et al., 1993). Knowledge of conversion efficiency would be essential in developing potato with leptine-based resistance because plants with high leaf levels of total glycoalkaloids also tend to have unacceptable levels of tuber glycoalkaloids (Sanford et al., 1996). Hence the most desirable form of leptine-based resistance would be a plant that has low enough total glycoalkaloids to prevent unacceptable levels from accumulating in the tubers but also converts all of its leaf

glycoalkaloids to leptines to confer some measure of resistance to the Colorado potato beetle. Sanford et al. (1994) have observed that introgression of *chc* into *S. tuberosum* can result in elevated glycoalkaloid levels in the leaves even after two backcrosses to *S. tuberosum*. Tuber total glycoalkaloid levels (calculated from total aglycon concentrations) in the present study ranged from 0 to 1.14 mg·g⁻¹ dry mass. Three PC hybrids (CP1, CP2, and CP3) contained >0.30 mg·g⁻¹ dry mass of glycoalkaloids. Backcrosses to *S. tuberosum* effectively reduced the tuber glycoalkaloid levels such that most genotypes occurred below the generally accepted upper limit of 0.20 mg·g⁻¹ dry mass (Sanford et al., 1995).

Leaf concentrations of leptines (calculated from acetylleptinidine concentrations) were sufficient in some PC hybrid families, monoloids and backcross hybrids to confer resistance to feeding by the CPB and reduce larval mass. For example, total leptines (leptine I + leptine II) were approximately 3.78, 3.45, and 3.74 mg·g⁻¹ dry mass in leaves from CP2-3, CP2, and CP2 x 8380-1-3, respectively. Rangarajan et al. (1995) reported that a minimum of 1.00 mg·g⁻¹ dry mass of leptines were required to suppress feeding and 1.98 mg·g⁻¹ dry mass of leptines constituted the ED₅₀. Furthermore, *chc* plants containing 5.70 mg·g⁻¹ dry mass of leptines reduced larval weight by 82% (Sanford et al., 1997). However, leptine concentrations in many plants in the present study may not be high enough to affect survival of CPB larvae. Sanford et al. (1997) found that 2x *chc* containing 8.58 mg·g⁻¹ dry mass significantly reduced larval survival after 5 d of feeding, but genetically comparable 4x *chc* containing 5.70 mg·g⁻¹ dry mass of leptines were intermediate for larval survival after 5 d of feeding and not significantly different from either the 2x *chc* or the Kennebec control. Leptine concentration may be only one component of CPB resistance estimated by larval survival such that selection based solely of leptine level may not be effective.

Table 4. Leaf glycoalkaloids, dry mass basis, of backcross hybrids between *Solanum phureja* x *S. chacoense* hybrids CP2 and both of its parents (*chc* 8380-1 and *phu* 1-3).

Sample	Glycoalkaloid ^a (mg·g ⁻¹)				
	SD	LD	ALD	Total	% ALD
1-3 x CP2-1	0.2	0	0	0.2	0
1-3 x CP2-2	0.86	3.24	0	4.1	0
1-3 x CP2-3	1.71	0	0	1.71	0
1-3 x CP2-4	1.36	0	0	1.36	0
1-3 x CP2-5	1.2	0.96	1.58	3.74	42
1-3 x CP2-6	0.23	0	0	0.23	0
1-3 x CP2-7	5.53	0	0	5.53	0
1-3 x CP2-8	0	0	0.45	0.45	100
1-3 x CP2-9	7.14	0	0	7.14	0
1-3 x CP2-10	0.05	0	0.79	0.84	94
1-3 x CP2-11	2.89	1.56	2.45	6.9	36
1-3 x CP2-12	2.79	0	0	2.79	0
CP2 x 8380-1-3	5.25	11.82	14.98	32.05	47
CP2 x 8380-1-6	1.92	7.92	14.63	24.46	60
CP2 x 8380-1-7	7.62	4.56	7.68	19.85	39
CP2 x 8380-1-11	6.5	11.13	4.6	22.24	21
CP2 x 8380-1-16	1.2	3.41	3.35	7.96	42
CP2 x 8380-1-17	1.2	9.54	1.72	12.45	14
CP2 x 8380-1-19	7.39	12.12	16.32	35.82	46
CP2 x 8380-1-20	5.82	3.0	5.99	14.81	40
CP2 x 8380-1-21	1.8	5.24	7.67	14.71	52
CP2 x 8380-1-22	4.32	9.43	13.29	27.03	49
CP2 x 8380-1-23	5.93	6.35	11.24	23.51	48
CP2 x 8380-1-24	3.63	10.09	16.1	29.82	54

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