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Genetic architecture of genes from the wild potato plant (*Solanum pinnatisectum)* showing resistance to the Colorado potato beetle

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Abstract

The Mexican wild potato species *Solanum pinnatisectum* is an important gene source for resistance to Colorado potato beetle and late blight. Diploid progenies segregating for resistance genes to Colorado potato beetle were developed by crossing *S. pinnatisectum* with *S. cardiophyllum*. The hybrid lines were resistant to Colorado potato beetle. A resistant hybrid line from this cross was selected and backcrossed as a female to *S. cardiophyllum* to generate a backcross progeny. This backcross progeny was tested for genetic architecture of the Colorado potato beetle resistance gene. Resistance percentage of the test lines over the control was determined for statistical analysis. The Chi square test result showed a significant 1:3 (resistance: susceptible) ratio in this backcrossing population, indicating that resistance in *S. pinnatisectum* to Colorado potato beetle may be controlled by two independently segregating major genes having two respective loci, acting in a complementary epistatic manner. The availability of Colorado potato beetle resistance genes, along with late blight resistance genes in *S. pinnatisectum* may enable breeders and geneticists to develop a single cultivar through the gene pyramiding method.

Keywords: Colorado potato beetle, backcross, potato, resistance genes

Abbreviation: CPB- Colorado potato beetle; (*S. pnt)- Solanum pinnatisectum;(S. cph)- Solanum cardiophyllum*

Introduction:

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is the most destructive insect pest of cultivated potato (*Solanum tuberosum* L.) worldwide. Both adults and larvae feed on potato foliage. They can rapidly reduce tuber yield by 30 to 50% (McLeod and Tolman, 1987). Potato growers rely mostly on synthetic chemicals to control this pest .Thirty-four percent of the total insecticide use on potatoes is for control of CPB, more than that used on any other insect- pest of potato (AGBIOS, 2001). However, beetle populations can rapidly develop resistance to insecticides (Stewart et al., 1997; Wilkerson et al., 2005) as well as the microbial insecticide *Bacillus thuringiensis* subspecies *tenebrionis* (Rahardja and Whalon, 1995).. In addition, use of pesticides is encountering growing public opposition and they are being gradually restricted by regulatory agencies (Casagrande, 1987; Hare, 1990). These unsustainable approaches result in high input costs; pollution to environments and risk to

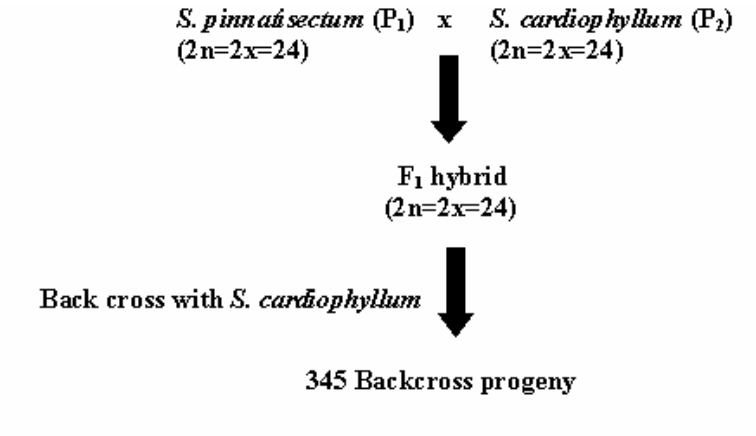


Fig 1. Pedigree of the wild backcross progeny.

health (Kennedy and Barbour, 1992). In addition, organic potato producers have a limited number of options for controlling CPB (Wilkerson et al., 2005). Hence the use of tolerant varieties would be a valuable option in pest management programs. Improvement of varieties is dependent on the availability of genetic variation for many resistance and other quality traits. Many of these traits have been found in related (diploid) wild species of potato. Wild potatoes are important sources of genes for resistance to CPB. Among the 228 wild species growing naturally from the south-western United States to Central Chile (Spooner and Hijmans 2001), the Mexican wild species are considered important donors of genes to potato for pest and disease resistance (Bamberg et al., 1994; Douches et al., 2001; Chen et al., 2003). *Solanum pinnatisectum*, Dunal (*S. pnt*) (2n=2x=24) a Mexican wild diploid species, was identified as a new source of resistance to CPB (Bamberg et al., 1994; Chen et al., 2003). At present, very few genetic studies have been conducted to understand the genetic factors involved in CPB resistance. Quantitatively inherited plant resistance to insects complicates the breeding process because many crosses must be made and large populations have to be evaluated to identify individuals possessing the optimal combination of genes for resistance and adaptation (Yencho et al., 1996). Breeding efforts are further complicated due to plant-insect interaction, bioassay variability, high degrees of genetic variability and mobility of resistance (Kennedy and Barbour, 1992; Yencho et al., 1996). For a long time, genetic analysis and mapping in cultivated potato have been difficult. Genetic analysis of potatoes is also complicated by the severe inbreeding depression that occurs in this crop (Jacob et al., 1995). Finally, the most important bottleneck is the tetraploid nature of cultivated potato, which makes it extremely complicated to understand genetic analyses as well as the detection of CPB resistance genes.

The transfer of useful resistance genes from *S. pinnatisectum* to another potato gene pool has significant value in providing genetic and molecular marker information on resistance traits such as resistance to CPB. It may also provide useful segregating populations for providing insight to interspecific incompatibilities of *S. pinnatisectum* (Chen et al., 2004a). In addition, characterizing the genetic architecture of important traits in wild species can be used to confirm the role of "candidate" genes hypothesized through physiological or biochemical work in contributing to the variation in resistance. Our study on the architecture of resistance genes contributes to the genetics and mechanistic basis of *S. pinnatisectum* mediated resistance to Colorado potato beetle in an interspecific backcross progeny and provides a foundation for future studies to develop insect resistant potatoes. This information is the first step towards predicting possible genetic architecture of CPB resistance gene in *S. pinnatisectum*.

Table 1. Analysis of variance of Colorado potato beetle resistance percentages

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Test lines	346	57.14	0.165	1.40	0.0001
Residual	694	81.65	0.117		

Materials and Methods

Plant Material

The progenies used in this study were previously developed and described by Chen *et al.*, 2004. A brief description is as follows. Diploid progenies segregating for the resistance gene to CPB were developed by crossing *S. pinnatisectum* (2n=2x=24) accession no. PI 275233 (P_1, male) with *S. cardiophyllum* (2n=2x=24) accession no. PI 186548 (*S. cph,* P_2, female). The genotypes were selected on the basis of their reaction to CPB. *S. pinnatisectum* had high levels of resistance to CPB, while *S. cardiophyllum* was found to be susceptible (Bamberg et al., 1994; Chen et al., 2003). The hybrid line F_1 was backcrossed as a female to *S. cardiophyllum* in order to generate backcross progeny (Figure 1). Backcross progeny was maintained vegetatively as *in vitro* tissue culture plantlets and tubers since the first propagation as seedlings. Three hundred and fortry-five lines from the backcross population were selected to analyse the genetic architecture of CPB resistance gene. The 345 backcross progenies were grown in the greenhouse at the Lethbridge Research Station, Alberta, Canada. Tubers of each line were planted in 15 cm diameter × 12 cm deep plastic pots filled with Alaska peat moss soil mix and placed in a greenhouse with a 16-h photoperiod and mean daily temperature of approximately 20-25ºC. Metal halide multivapor growth lights (1500W) supplemented natural lighting..

Insects

A laboratory colony of the Colorado potato beetle was maintained at Lethbridge Research Station, Canada. Colonies were maintained in an insectary at 25 ± 1°C, 70 ± 7% RH and 16:8 (L:D) photoperiod on unsprayed potato foliage. Only newly emerged adult beetles (less than one week old) from a single, summer generation, were used for this experiment.

Bioassays

The backcross family, consisting of 345 lines, as well as *S. pinnatisectum*, *S. cardiophyllum* and the F_1 hybrid, were screened for leaf consumption by adult beetles, using a choice test bioassay (Underwood et al. 2000). A susceptible *S. cardiophyllum* plant was used as the control plant for this choice test. All assays were conducted using leaflets, 3-4 nodes below the apex of the plants at the vegetative stage. Three leaflets of the same size were collected from each target line, as well as the control plant, and placed in three 9 cm × 1.5 cm Petri dishes containing moistened What man No. 1 filter paper. A single laboratory-reared adult beetle was added into each Petri dish and was allowed to feed on the leaflets. The experiments were conducted in a growth cabinet and each beetle was allowed to choose between two leaflets each from the target lines and the control plant (*S. cardiophyllum*) per Petri dish (Zvereva et al., 1998, Yencho et al., 1996). Beetles were maintained for 24 hours without feeding before the experiment. The dishes were transferred to a growth chamber set at 25ºC (day) to 20ºC (night) and illuminated with a 63W growth lamp set to provide a 16h: 8h (L: D) photoperiod. The average light intensity was 275-300 micromoles m^{-2} s^{-1}. There were three replicate dishes per backcross progeny (345 lines), *S. pinnatisectum* and F_1 hybrid, giving a total of 1041 Petri dishes. The proportion of the consumed area in percentage was visually estimated on each leaflet after 8 hours data. This choice test bioassay was tested for all backcross progenies *S. pinnatisectum* (P_1) and F_1 hybrid.

Data analysis

Bioassay for beetle resistance in potato plants was measured as the relative preference of a beetle for the control over the target lines. A calculation of resistance percentage in plant lines was developed using the preference index calculation given by Kogan and Goeden (1970). The resistance percentage

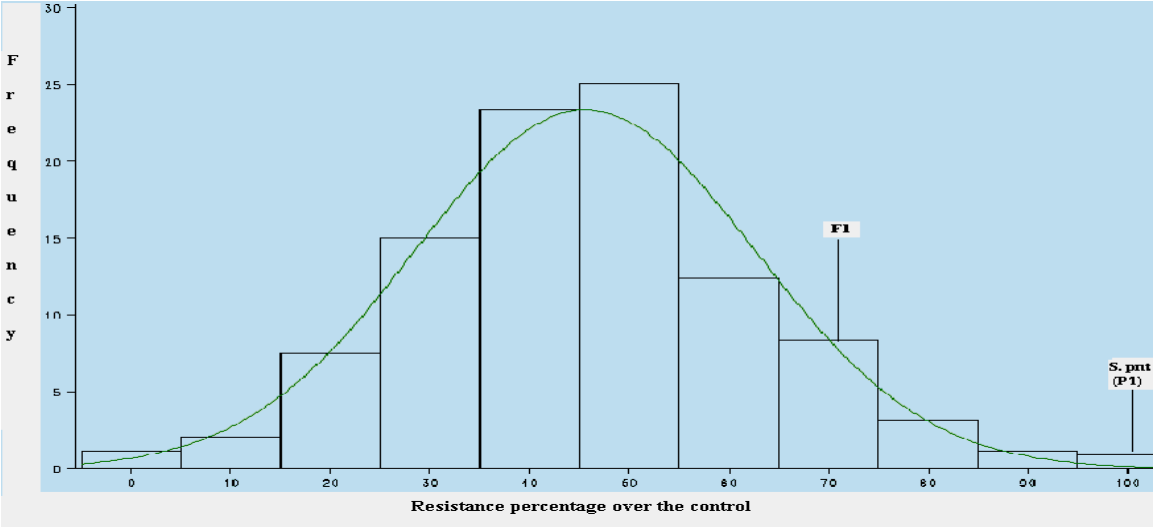


Fig 2. Frequency distribution of resistance percentage values for the backcross progeny. The arrows show the mean resistance percentage for *Solanum pinnatisectum* and F_1

of the test lines over the control was measured using the modified formula R= 100 X [C/(C+L)], where C and L indicated the amount of damage to the control and test line respectively in each Petri dish. Mean of the three replications was used for the calculation of the mean resistance percentage (Underwood et al., 2000). The R-value measures the relative resistance percent over the control on a 0 to100 scale. A value of R= 50±10 % indicates similarity to the control plant (no difference in feeding between the test lines and control plants), whereas R> 60% indicates resistance of the test lines in comparison to control plant and the rest of the values (R≤ 60%) indicate plant susceptibility.

Statistical analyses were performed on the arcsine square root transformed resistance percentage for each treatment to normalize their distribution (Zar, 1999). Normality distribution was tested using the Anderson-Darling method (PROC UNIVARIATE; SAS Institute 2003). Analysis of variance (ANOVA; PROC GLM; SAS Institute 2003) was conducted to determine whether target lines differed significantly in response to CPB damage. Based on a statistical comparison (t-test) (PROCTTEST; SAS Institute 2003), the lines were classified as either resistant or susceptible. A χ^2 test (PROC FREQ; SAS Institute 2003) was used to check the fit of resistance and susceptible classes versus that expected for single (1:1) and two gene (1:3) models using mean data.

Results

The number of genes contributing to the variance of quantitative characters within and between populations are fundamental to the mechanism of heredity. Analysis of variance on the transformed data showed significant difference between the lines (Table 1). The normality test was performed on transformed data using the Anderson-Darling method (Stephens, 1974) and the data fit closely to the normal distribution model (Figure 2) (Sachs, 1992). When the cumulative frequencies are plotted against each other, the resulting straight line suggests that this sample has a normal distribution and allows further parametric statistics.

Genetic study of resistance

The *S. pinnatisectum* parent showed 98± 1.0 % resistance over the control whereas hybrid parent showed 69± 6.4 % resistance over the control. The resistance levels of the parent *S. pinnatisectum* and

Table 2. Chi-square test and possible modes of segregation of back cross progeny

Parental genotypes (F1 x P2)	Phenotype	Hypothesis	Observed ratio	χ^2	P
One gene	Resistant : Susceptible	1:1	81: 264	96.02	<.0001
Two gene	Resistant : Susceptible	1:3	81: 264	0.35	0.5541*

* No significant differences between the expected and observed frequencies; therefore hypothesis is accepted

hybrid parent is also shown in Figure 2. The wide range of distribution pattern for the percent resistance values appeared to be continuous and unimodal. Pair t-test for equal mean showed significant differences of mean between resistant lines and susceptible lines over the control plants. The probability value of paired t test for equal to mean was also found to be significant in *S. pinnatisectum* and F_1 hybrid.

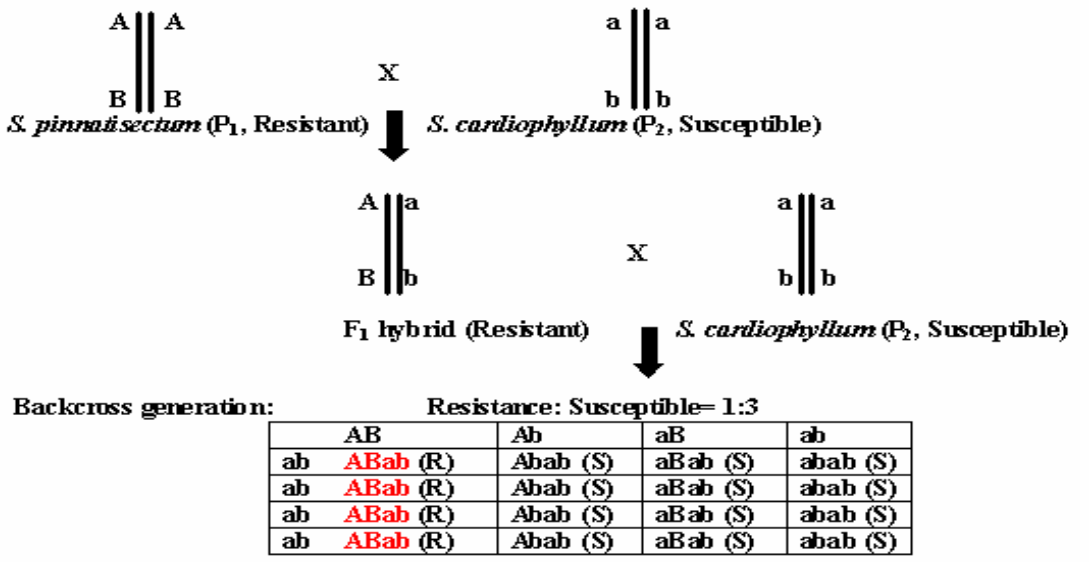
Inheritance of major genes towards resistance to CPB

To test for the possibility of major gene control, the backcross population was segregated into 2 phenotypic classes namely resistance and susceptible based on the resistance percentage. A resistance percentage of >60% indicated resistance over the control plant and <60% indicated susceptibility similar or over the control plant. The ratio observed for the back cross population was 81 resistant: 264 susceptible. Attempts were made to investigate the possibility of polygenic inheritance by observing the frequency distribution of the backcross progeny. The following genetic hypotheses were tested on the back cross population: (1) A 1: 1 resistant to susceptible ratio for one gene controlled trait and (2) a 1:3 resistant to susceptible ratio for two gene controlled trait (Table 2). The resultant division of backcross progeny into 81: 264 (resistant: susceptible) confirmed a 1:3 ratio using χ^2 tests. As the degree of freedom was equal to 1, the calculated value of chi-square was corrected for continuity by Yates correction. A chi-square test of this hypothesis indicated no significant difference between the observed and expected ratios for the backcross population (P>0.05).

Discussion

The availability of 2x hybrids derived from a resistant *S. pinnatisectum* genotype and a susceptible *S.cardiophyllum* genotype and backcross (BC) generation provides unique genetic stock at the diploid level for genetic and molecular study for CPB resistance (Chen et al., 2004a). The observed frequencies were plotted against the predicted normal distribution (Figure 2) and showed high agreement between each other, indicating more than one gene as playing key roles for expressing resistance to CPB in this population. Before trying to identify the molecular markers, it is very important to have a clear idea about the genetic inheritance of CPB resistance genes. Natural plant and breeding populations of crop plants show qualitative and quantitative phenotypic variation for resistance to pests and pathogens. Qualitative resistance is characterized by two distinct phenotype classes, resistant and susceptible, and follows Mendelian inheritance. In contrast, quantitative resistance is characterized by continuous phenotypic variation ranging from high susceptibility to high resistance among the recombinant individuals within a progeny. Such resistance is controlled by more than one gene. In the case of one gene control resistance to CPB, the segregation of a backcross population will be 1:1. If it is two genes, the segregation of a backcross population will be 1:3 (Skiba et al., 2004). The chi square test of this backcross population confirmed that the resistance gene is controlled by two genes.

Let A and B be two genes having two alleles AA and BB at their respective locus for resistance to CPB in *S. pinnatisectum*. Similarly aa and bb alleles are present at their respective loci in susceptible parent *S. cardiophyllum*. Phenotypically, all F_1 hybrids were more resistance than the susceptible parent but less resistance than the *S. pinnatisectum*. From this segregating progeny it was found that if two resistance loci (A and B) are present together in one line, that line will show resistance to CPB. It was clear from the analysis that none of the resistant lines in the backcross population showed similar resistance power to CPB in *S. pinnatisectum* and each resistant



	AB	Ab	aB	ab
ab	ABab (R)	Abab (S)	aBab (S)	abab (S)
ab	ABab (R)	Abab (S)	aBab (S)	abab (S)
ab	ABab (R)	Abab (S)	aBab (S)	abab (S)
ab	ABab (R)	Abab (S)	aBab (S)	abab (S)

Fig 3. Genetic architecture of resistance gene to the Colorado potato beetle for the backcross progeny.

genotype had a slightly different quantitative resistance percentage value. This finding further indicates that resistance in *S. pinnatisectum* to CPB may be controlled by two independently segregating major genes having two respective loci, acting in a complementary epistatic manner (Figure 3). Similarly, two major QTLs were found on chromosomes 1 and 8 for resistance to CPB in a backcross progeny of *S. tuberosum* × *S. berthaultii* (Yencho et al., 1996). In the case of heterozygous parents, loci can have two alleles, which can segregate either as 1:1, 1:3 (Echt et al., 1992). The Chi square test result also showed the significant 1:3 (r: s) ratio in this backcrossing population, which indicates resistance and susceptible genes are present in the F_1 hybrid.

Although the specific protein or amino acid for CPB resistance present in the wild species *S. pinnatisectum* was not determined in the present study, it is believed that leptine glycoalkaloids (Sinden et al., 1980; Tingey 1984; Yencho et al., 1996), glandular trichomes and polyphenol oxidase (Bonierbale et al., 1994) are three natural insect-host resistance mechanisms available in wild potato. Several investigations were done to identify the location of the major genes for leptine (Hutvagner et al., 2001) and trichome type A (Bonierbale et al., 1994) for CPB resistance in chromosome 1 and 6 respectively. QTL mapping of foliar glycoalkaloid aglycones (Yencho et al., 1998) and trichome mediated insect resistance (Bonierbale et al., 1994) was identified in interspecific backcross progenies of *S. tuberosum* and *S. berthaultii*. Consistent QTL for insect resistance in chromosome 1 was observed in two reciprocal backcross potato progenies of *S. tuberosum* × *S. berthaultii* (Yencho et al., 1996). On the other hand, the presence of different levels of polyphenol oxidase also leads to the entrapment and death of small insects (Gregory et al., 1986) and reduces the survival and oviposition rate of CPB (Casagrande 1982; Wreight et al., 1985; Yencho and Tingey 1994). However, only three RFLP markers in the polyphenol oxidase locus were identified from 15 BAC clones of *S. pinnatisectum* (Chen et al., 2004b).

Incorporating multiple resistance genes into a single genotype may be accomplished through the use of a combination of different components of CPB resistance from wild species with other sources found in cultivated potato. Because the introgression of genes from wild species to cultivated potato requires a great effort, use of genotypes that have multiple resistance genes as parents for the crossing would be advantageous. The availability of *S. pinnatisectum*

CPB resistance genes, along with other recently identified resistance genes in some North American potato cultivars and breeding lines, may enable breeders to incorporate resistance to late blight and CPB into a single cultivar through gene pyramiding (Douches et al., 1997). This basic genetic architecture of CPB resistance gene in *S. pinnatisectum* potato plant may be used as a reference framework for achieving enhanced control over CPB.

Acknowledgements

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