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Dong-Man Khu
University of Nebraska - Lincoln

Jim Lorenzen
Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, ID 83844, USA

Christine A. Hackett
Biomathematics and Statistics Scotland, Invergowrie, Dundee, DD2 5DA, UK

Stephen L. Love
Aberdeen R&E Center, University of Idaho, Aberdeen, ID 83210, USA

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Interval Mapping of Quantitative Trait Loci for Corky Ringspot Disease Resistance in a Tetraploid Population of Potato (*Solanum tuberosum* subsp. *tuberosum*)

Dong-Man Khu,[1] Jim Lorenzen,[2] Christine A. Hackett,[3] and Stephen L. Love [4]

[1] Panhandle Research & Extension Center, University of Nebraska–Lincoln, Scottsbluff, NE 69361, USA
[2] Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, ID 83844, USA
[3] Biomathematics and Statistics Scotland, Invergowrie, Dundee, DD2 5DA, UK
[4] Aberdeen R&E Center, University of Idaho, Aberdeen, ID 83210, USA

Corresponding author — Stephen L. Love, email slove@uidaho.edu

Abstract
Corky ringspot disease (CRS) resistance is an ideal target trait for breeding using marker-assisted selection (MAS) due to the high variability in field disease incidence that complicates screening for resistance. To develop molecular marker(s) associated with CRS resistance, a linkage map was constructed for a tetraploid population of 92 genotypes in order to conduct quantitative trait locus (QTL) analysis. The population was tested for CRS resistance in an infested screening field for four years. Broad sense heritability and its standard error of CRS disease resistance were 0.80 (±0.16). A total of 892 AFLP, 95 SSR, and 5 SSCP markers were scored and used for testing marker-trait association. One major QTL that explained 43% of the phenotypic variation in CRS resistance was localized on chromosome IX, with flanking AFLP markers AAC-CGT-0347 and ACG-CTG-0588. A minor QTL that explained 12% of CRS resistance was also detected. This minor QTL was associated with distorted marker GGT-CAC-0259, which remained unlinked. Polygenetic nature of CRS resistance was explained by major and minor QTLs association. Conversion of the three AFLP markers associated with this quantitative trait to simple PCR markers will benefit resistance breeding by enabling MAS.

Resumen
La resistencia a la mancha de anillo corchoso (CRS) es un carácter ideal para el mejoramiento mediante la selección asistida por marcador (MAS), debido a la gran variabilidad de la incidencia de la enfermedad en el campo, la misma que complica el tamizado para resistencia. Para desarrollar marcadores moleculares asociados con resistencia a CRS, se construyó un mapa de ligamiento para una población tetraploide de 92 genotipos, con el fin de realizar un análisis de locus de un carácter cuantitativo (QTL). La población fue probada para resistencia a CRS tamizando por cuatro años en un campo infestado. La heredabilidad en sentido amplio de la resistencia a CRS, y su error estándar, fue de 0.80 (±16). Un total de 892 marcadores AFLP, 95 SSR y 5 SSCP fueron tomados en cuenta y usados para probar la asociación marcador–carácter. Un QTL mayor que explicó el 43% de variación fenotípica en resistencia al CRS fue localizado en el cromosoma IX, con marcadores AFLP flanqueantes, AAC-CGT-0347 y ACG-CTG-0588. Un QTL menor que explicó el 12% de resistencia al CRS fue detectado también. Este QTL menor fue asociado con el marcador distorsionado CGT-CAC-0259, el cual permaneció sin ligamiento. La naturaleza poligenética de la resistencia al CRS fue explicado por la asociación de los QTLs mayor y menor. La conversión de los tres marcadores AFLP asociados con este carácter cuantitativo en marcadores simples PCR, beneficiará el mejoramiento para resistencia haciendo posible la aplicación de MAS.

Keywords: broad sense heritability, tobacco rattle virus, marker-assisted selection, AFLP, SSR, SSCP

Introduction

Corky ringspot (CRS) is an economically important disease of potato and is caused by *Tobacco rattle virus* (TRV; Harrison and Robinson 1989). Once infested, a field requires permanent, expensive management inputs to remain in production. In spite of best efforts, some tuber infection will still occur, resulting in losses in marketable yield due to the occurrence of culled potatoes, reduced returns to the grower, and the potential of rejection by buyers. In addition the grower must expend more money to control the disease in the form of fumigating infested potato fields. CRS disease has seriously impacted the potato industry in a few regions of the USA. Specifically, growers in the Pacific Northwest and Florida have experienced serious risk of severe losses due to CRS (Brown and Syke 1973; Mojtahedi et al. 2000; Weingartner and Shumaker 1990). Although considered serious for commercial growers, the presence of TRV is completely unacceptable to seed potato growers. US seed certification programs have classified TRV as a zero tolerance disease (Brown and Mojtahedi 2005) due to the difficulties of control and eradication of the disease once it is distributed on the field. Detection of any TRV symptoms disqualifies a seed lot from being sold for seed.

TRV is transmitted by stubby root nematode species (*Paratrichodorus* and *Trichodorus* spp.) (Jesen et al. 1974; Mojtahedi and Santo 1999). Both TRV and the nematode vectors are reported to infect a variety of crop and weed species (Allen and Davids 1982), thus allowing the disease to survive and propagate. TRV can be partially controlled through proper field management techniques, e.g. by using systemic nematicides or soil fumigation (Cooper and Thomas 1971; Mass 1975; Weingartner and Shumaker 1990) This is a very expensive process, costing in the range of $620–$1,185 per hectare. Some reduction in disease symptoms has been reported after using an appropriate rotational crop, such as alfalfa (Banttari et al. 1993).

Host plant resistance is the best option for control of the corky ringspot disease. Varieties and genotypes with high level of CRS resistance have been noted from several independent breeding programs in Great Britain, Poland, and the USA (Anonymous 1996; Brown et al. 2000; Dale 1989; Dale and Solomon 1988*a*, *b*; Harrison 1968; Richardson 1970; Shumaker et al. 1984; Weingartner and McSorley 1994). Although CRS-resistant varieties have existed for many years, and are not uncommon in European-type varieties, there are few resistant varieties accepted by the US industry. Breeding for resistance to CRS is a challenge despite the availability of resistant parental materials. This is largely due to the complexity of screening procedures caused by variable and shifting disease distribution which necessitates large trials with numerous replications over multiple seasons to adequately confirm resistance. This seems to be an ideal subject for breeding using molecular markers. However, development of such a breeding method has lagged due to the lack of genetic information for CRS resistance.

This study was initiated to complete basic background research that could be used to develop a marker-assisted selection system for CRS resistance in potato. The objectives of this study are to (1) determine the broad sense heritability of CRS resistance, (2) construct a linkage map for a tetraploid, CRS resistant potato population, and (3) determine the map position of the QTL(s) for CRS resistance.

Materials and Methods

CRS Evaluations

Ninety-two genotypes, derived from the cross PA95A33-1(resistance) × A9446-7(susceptible), were randomly selected and tested for CRS resistance. Seed was maintained clonally by the University of Idaho/ARS potato breeding program, Aberdeen, Idaho. Field plots were established in a production field with a more than 50-year history of CRS incidence near Egin, Idaho. Evaluations were performed in 2001, 2002, 2003, and 2004. Five-hill plots were planted in a randomized complete block (RCB) design with four replications in 2001 and 2002. Three-hill plots were planted in a RCB design with eight replications in 2003 and with ten replications in 2004. The spacing was 25 cm between plants within plots and 91 cm between rows. The plots were maintained using a combination of overhead sprinkler and flood irrigation techniques, while employing other management practices that are typical for the region. The herbicides "Matrix" (1.5 ml/ha) and "Roundup" (2.3 l/ha) were applied for weed control. Tubers were harvested using a two-row flat-bed digger. Every tuber harvested in the plot was immediately cut and evaluated for CRS symptoms in the field. The highest severity score (most affected tuber) found in a given plot was recorded as the CRS score for that plot. The scale of severity scores was measured as follows: (1) no symptoms observed, (2) spot or single faint arc, (3) multiple spots or a single clear arc, (4) multiple clear arcs, (5) more than 30% of the flesh affected by necrosis.

Statistical Analysis

Due to the lack of normality of data, $\log_{10}$ transformation of CRS scores (L_{10}-CRS) for each plot was performed prior to carrying out more detailed statistical analyses. Data were analyzed using the mixed procedure in the Statistical Analysis System (Littel 1996). Broad sense heritability was computed on a mean basis of L_{10}-CRS score and was calculated as

$$H = \sigma^2_G/(\sigma^2_G + \sigma^2_{GY}/y + \sigma^2_e/\mathrm{ry})$$

where σ^2_G = variance of genotype, σ^2_{GY} = variance of genotype × year, σ^2_e = error, and y = number of years (due to the un-balanced replications of each years, the mean of 4-year replication 4, 4, 8, 10 was applied), ry = total number of replications. The approximate standard error of the heritability estimate was calculated as

$$\mathrm{SE}\ (H) = \mathrm{SE}\ (\sigma^2_G)/(\sigma^2_G + \sigma^2_{GY}/y + \sigma^2_e/\mathrm{ry})$$

where SE (σ^2_G) = standard error of genetic variance (Hallauer and Miranda 1981).

DNA Extraction

Genomic DNA was extracted from frozen plant tissue using the DNeasy Plant DNA Extraction kit (QIAgen, cat. No. 69106, Valencia, CA) according to the manufacturer's instructions for plant leaf tissue.

AFLP

AFLP assays were performed using a modified version of the method described by Vos et al. (1995). One hundred and fifty nanograms of genomic DNA were subjected to digestion for 2 h at 37°C using 20 U of

*Eco*RI (New England BioLabs, Cat. No. R0101S, Ipswich, MA) or *Pst*I (New England BioLabs, Cat. No. R0140S) with 12 U of *Mse*I (New England BioLabs, Cat. No. R0525S) in 30 µl reactions with 3 µl 10× restriction buffer NEB buffer no. 2 (supplied with restriction enzyme) and 0.3 µl 100× BSA (supplied with restriction enzyme). Five microliters of ligation mix containing 1 µl of 5× ligation buffer (supplied with enzyme), 5 pmol of *Eco*RI or *Pst*I adaptors, 50 pmol *Mse*I adaptors, and 1 U of T4 DNA ligase (New England BioLabs, Cat. No. M0202S) were added to 25 µl of each digest and incubated overnight at 16°C. Pre-amplification was performed in 25 µl reactions containing 1 µl of adaptor ligation product, 50 ng of E00 or P00 and M00 primers, 0.2 mM dNTPs (New England BioLabs, Cat. No. NO0047L), 1X PCR buffer, and 1 U of *Taq* DNA polymerase (New England BioLabs, Cat. No. MO273L). Preamplification reactions were carried out on a MJ Research (Waltham, MA) PTC 200 thermal cycler as described by Milbourne et al. (1997); pre-amplification products were diluted 1:40 using autoclaved distilled water.

Selective PCR amplification was performed using LI-COR IRDye™ 700 or 800 infrared dye-labeled *Eco*RI (E+ANN) or *Pst*I (P+GNN) primers containing three selective nucleotides. Multiplex selective PCRs were performed in 13 µl reactions containing 3 µl of pre-amplified diluted DNA template, 6 ng of IRD700-labeled *Eco*RI or *Pst*I primer, 7.2 ng of IRD800-labeled *Eco*RI or *Pst*I primer, 60 ng of *Mse*I primer (M+CNN), 0.4 mM dNTPs (New England BioLabs, Cat. No. NO0047L), 1 U *Taq* DNA polymerase, and 1.3 µl 1X PCR buffer. Multiplex selective amplification was performed using the following PCR profile: 13 cycles of 30 s denaturation at 94°C, 45 s annealing at 65°C minus 0.7°C per cycle, and 90 s extension at 72°C, followed by 23 cycles of 30 s denaturation at 94°C, 45 s annealing at 56°C, and 90 s plus 1 s per cycle extension at 72°C, and 2 m extension at 72°C. Electrophoresis of samples was performed using a Li-Cor 4300 DNA analyzer. Electrophoresis procedures were conducted according to the applications manual for the 4300 DNA analyzer, using a 48-well comb. The semi-automated Saga^{MX}-AFLP® Analysis Software was used to score AFLP bands. Manual inspection and correction of band scoring data generated by analysis software was also performed.

One-hundred and twenty E+ANN × M+CNN primer combinations and 144 P+GNN × M+CNN primer combinations were initially screened to determine marker profiles for both parental genotypes, five resistant genotypes and five susceptible genotypes. Primer combinations producing clear bands and potentially valuable simplex markers were selected to produce AFLP marker profiles for mapping. The AFLP marker names designate the three selective bases of the forward primer, three selective bases from the reverse primer, and the molecular size of the marker in base pairs (bp). Duplex markers are indicated by "D" and double simplex markers deriving from both parents are designated "B" (bridging). For example, marker name GCA-CAC-0122D indicates a duplex marker amplified with the primer combination *P*+GCA × *M*+CAC with an estimated size of 122 bp.

SSR

Simple sequence repeat polymorphism analysis was performed using 100 primer sets. Forward primers were labeled using LI-COR IRDye™ 700 or 800 Infrared Dyes. Reaction conditions and amplification were performed as described by Milbourne et al. (1998) or Feingold et al. (2005). Separation and visualization of reactions were performed using a Li-Cor 4300 DNA analyzer using procedures described in the applications manual for the 4300 DNA analyzer. Saga^{MX}-Microsatellite® Analysis Software was used to initially score SSR bands, after which allele calls were manually inspected and corrected if necessary.

SSCP

Single strand conformational polymorphism (SSCP) assay was performed to detect additional co-dominant markers to possibly enhance map resolution. Seven SSCP markers located on potato chromosome IX (Feingold et al. unpublished data) were tested after markers associated with the largest QTL were identified in a linkage group that was a homolog of chromosome IX. Primer sequences and adequate annealing temperatures are listed in Table 1. SSCP analysis was based on the methods of Hongyo et al. (1993). Thermal cycling reactions were performed in 13 µl reactions containing 50 ng of DNA template, 1 µM of forward primer and reverse primer, 0.4 mM dNTPs mixture, 1 U *Taq* DNA polymerase, and 1.3 µl 1X PCR buffer. Amplification was performed using the following PCR profile: 36 cycles of 60 s denaturation at 94°C, 120 s annealing at the appropriate temperature (Table 1), and 90 s extension at 72°C. Amplified PCR products were denatured and single stranded DNAs were separated by MDE gel (Cambrex Bio Science, Cat. No. 50620, Rockland, MI) electrophoresis at room temperature as described in protocols for SSCP supplied with MDE gel solution. Following electrophoresis, DNA bands were visualized by silver staining as described by Switzer et al. (1979).

Construction of Linkage Maps

Linkage analysis was performed as described by Bradshaw et al. (2004*b*), using the software program TetraploidMap (Hackett and Luo 2003). TetraploidMap is designed to develop linkage maps for autotetraploid species. It is suitable for handling markers scored on two parents and their full-sib offspring. TetraploidMap handles

Table 1. Chromosome IX specific SSCP markers used to construct linkage maps.

Marker/primer sequence	No.	Marker class[a]	Annealing temperature
TC57528-9-12[b] *F: catcaccgatgacaacctcaaagc* *R: tggaacaagcttcagagccactc*	12	SD	63
BG095693-9-1R (alternative name: Cyp76B1) BG095693-9-2R/ *F: ttaggacaaataacaacagtgg* *R: agggtcagccaaatccttgg*		SD	63
TC58960/ *F: agttccaccagggccatcac* *R: acgttgatcttcccagcaac*		D	60

[a] *S* simplex marker, *D* duplex marker
[b] Co-segregating markers

both codominant and dominant molecular markers in all possible configurations, and takes into account the presence of null alleles in the analysis. It also includes a routine for QTL analysis which was used for QTL analysis.

Interval Mapping of QTLs

The mean CRS score of each genotype for each year and the mean CRS score of each genotype over all 4 years (total 26 replications) were calculated and used for QTL analysis. A preliminary scan of marker-trait associations was carried out using single-marker analysis. Regression analysis of mean CRS scores for each genotype was performed by regressing mean CRS score on a binary indicator variable (1 vs. 0) dataset that included each marker in binary form. The threshold for declaring a significant association was determined by a permutation test (Churchill and Doerge 1994) to overcome problems of multiple testing. The regression analysis and permutation test were performed using the "Map Manager QTX" software program (Manly et al. 2001). The results of single-marker analysis were used to identify simplex coupling linkage groups with promise for containing QTLs for CRS resistance.

Linkage groups showing significant associations with mean CRS score were analyzed further using interval mapping. A detailed description of the theory for QTL interval mapping in an autotetraploid species is given by Hackett et al. (2001); the protocol for interval mapping was summarized by Bryan et al. (2004). The "TetraploidMap" software program (Hackett and Luo 2003) was used for all analytical procedures for the QTL interval mapping.

Results

Phenotypic data — The histogram of mean L_{10}-CRS scores is shown in Figure 1. Using accumulated multi-year trait data contributed to an improved data set that approached normality. Many genotypes were misclassified as resistant in the first and/or second year. However, combining data each year decreased the number of "resistant" genotypes. The estimates of the variance

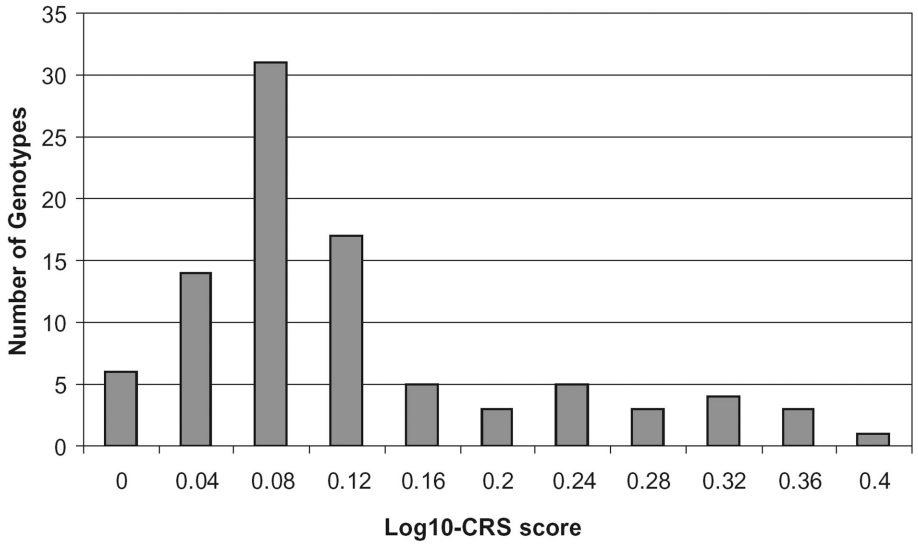


Figure 1. Frequency distribution of mean $\log_{10}$-transformed CRS scores accumulated over years. Lower $\log_{10}$-transformed CRS score represents resistant.

components are shown in Table 2. Broad sense heritability and its standard error of CRS disease resistance was 0.80 (±0.16) in this population.

Molecular marker — Forty-four E+ANN × M-CNN primer combinations and 43 P-GNN x M-CNN primer combinations were selected to generate the AFLP marker profiles used to construct the genetic map. From these 87 primer combinations, 892 bands were scored. A χ^2 goodness-of-fit test with significance level of 5% for all AFLP marker classes was performed to determine if observed segregation ratios were significantly different from expected ratios. Three hundred and thirty-six (336) simplex and 88 duplex markers were scored for female parent PA95A33-1. Two hundred and sixty (260) simplex and 57 duplex markers were scored for male parent A9446-7. One hundred and fifty-one (151) double simplex (bridging) markers were scored for both parental genotypes.

Forty-six SSR primer sets out of 100 SSR primer sets (Feingold et al. 2005; Milbourne et al. 1998) tested produced 95 informative SSR marker bands. Of these, 26 SSR primer sets produced co-dominant markers consisting of two to four simplex and/or duplex and/or double-simplex markers. Three SSCP primers out of seven SSCP primers tested produced five informative SSCP marker bands. SSCP primer TC57528-9 generated co-dominant markers of simplex–duplex combination.

Using software TetraploidMap (Hackett and Luo 2003), mostly complete maps with four homologous linkage groups were developed for chromosomes I, II, III, V, VII, VIII, and IX, using co-dominant SSR markers and duplex AFLP markers (data are not shown). The other chromosomes had maps that consisted of two or three homologous linkage groups. The total map length for parent PA95A33-1 was 2940 cM, which gave an average composite map length for each chromosome of 91.2 cM. The total map length for parent A9446-7 was 1929 cM, with an average composite map length for each chromosome of 79.6 cM (data are not shown).

Table 2. Analysis of variance components for $\log_{10}$ transformed CRS scores (L_{10}-CRS) of 92 genotypes derived from cross PA95A33-1 × A9446-7

Variance components	Estimates	Standard error
Year	0.000377	0.000510
Rep (year)	0.000803[a]	0.000373
Genotypes	0.006656[a]	0.001327
Year × genotypes	0.00246[a]	0.000728
Error	0.0319[a]	0.0011

[a] Significant at 5% level

Single point regression analyses for QTLs — Regression analyses of mean CRS scores for each year against binary data for each marker identified three significant marker-trait associations (ACG-CTG-0588, ATG-CTG-0393, AAC-CGT-0347) that were consistently detected in all four years, as well as for the mean of 4 years (Table 3). Since the mean CRS score for each year was independently generated, consistent detection of marker-trait association is strong evidence for the existence of the QTL on the chromosome that contains these three markers. These three markers were simplex and were tightly linked to each other. This simplex coupling linkage group was identified as a homolog of chromosome IX of PA95A33-1 (female resistant parent; Figure 2). No significant marker-trait associations were detected for this or any other chromosome in A9446-7 (male susceptible parent). Of the three markers that were associated with CRS scores, simplex marker AAC-CGT-0347 explained the highest percentage of the variance in the total mean CRS score, 25%. The regression coefficient of marker AAC-CGT-0347 was negative, indicating that offspring that carried this marker showed a decrease in total mean CRS score and could be said to have greater CRS resistance.

In order to detect smaller QTL effects after accounting for marker AAC-CGT-0347, the residual total mean CRS (R-CRS) score was calculated from regression on the marker AAC-CGT-0347 using the following equation:

$$\text{R–CRS} = \text{total mean CRS} - \beta I_{\text{AAC–CGT–0347}},$$

where β is the regression coefficient (−0.1) and $I_{\text{AAC-CGT-0347}}$ is an indicator variable for the presence/absence of the marker AAC-CGT-0347. This calculation of R-CRS effectively removed the QTL effect of the marker AAC-CGT-0347 from the trait data. Regression of R-CRS scores on the set of AFLP markers identified two simplex markers that were significantly associated with CRS scores according to the permutation test (likelihood ratio statistics >4.1; Table 3). The marker GGT-CAC-0259 accounted for 12.3% of phenotypic variance in R-CRS scores, and the marker GCA-CAC-0122 accounted for 7.2%. These two markers were tightly linked at a distance of 3 cM. However, both of these markers showed distorted segregation (35 present/57 absent for GGT-CAC-0259, 35 present/56 absent for GCA-CAC-0122), indicating a significant lack of fit from a 1:1 ratio using a χ^2 test at $\alpha = 5\%$. As a result these markers had been initially discarded from linkage mapping analysis. Subsequent analysis with these two markers showed no significant linkage with any markers in the previously constructed linkage groups.

Interval mapping — Interval mapping was used to study more precisely the effects of chromosome IX alleles from PA95A33-1 on the total mean CRS scores. The effects of these alleles (markers mapped on chromosome

Table 3. Regression of mean CRS scores for each year on the markers of PA95A33-1

Year	Chromosome	Marker	R^2 (%)	Test against $R^2=0$	Regression coefficient	Allele
2001	IX	ACG-CTG-0588	11[a]	<0.001	−0.06	A2
	IX	ACA-CTT-0426	10[a]	0.002	0.06	A3
	IX	AAC-CGT-0347	10[a]	0.002	−0.06	A2
	IX	STM1021-9-3	10[a]	0.002	−0.09	A2
	IX	ATG-CTG-0393	9[a]	0.002	−0.06	A2
2002	IX	AAC-CGT-0347	10[a]	0.002	−0.07	A2
	IX	ACG-CTG-0588	9	0.004	−0.07	A2
	IX	ATG-CTG-0393	9	0.004	−0.06	A2
	IX	STM1021-9-5	8	0.006	0.06	A2-A3
2003	IX	AAC-CGT-0347	25[a]	<0.000	−0.15	A2
	IX	ACG-CTG-0588	24[a]	<0.000	−0.15	A2
	IX	ATG-CTG-0393	22[a]	<0.000	−0.14	A2
	IX	ACT-CTT-0323	18[a]	<0.000	−0.13	A2
	IX	BG095693-9-2R	8	0.007	0.08	A3
	IX	BG095693-9-1R	7	0.008	0.1	A3-A4
	IX	STM1021-9-3	7	0.009	−0.13	A1
2004	IX	ACG-CTG-0588	16[a]	<0.000	−0.09	A2
	IX	ATG-CTG-0393	14[a]	<0.000	−0.08	A2
	IX	AAC-CGT-0347	14[a]	<0.000	−0.08	A2
	IX	BG095693-9-2R	11	<0.001	0.07	A3
	IX	ACT-CTT-0323	11	<0.001	−0.07	A2
Total	IX	AAC-CGT-0347	25[a]	<0.000	−0.1	A2
	IX	ACG-CTG-0588	25[a]	<0.000	−0.1	A2
	IX	ATG-CTG-0393	24[a]	<0.000	−0.09	A2
	IX	ACT-CTT-0323	17[a]	<0.000	−0.08	A2
	IX	BG095693-9-2R	9	0.003	0.06	A3
	IX	STM1021-9-5	9	0.004	0.06	A2-A3
	IX	GGT-CAA-0247	8	0.005	0.05	A3
	IX	GTA-CTC-0237	7	0.008	0.05	A3
Regression of residual mean CRS scores	Unassigned	GGT-CAC-0259	12.3[a]	<0.001	−0.07	
	Unassigned	GCA-CAC-0122	7.2[a]	0.0092	−0.05	

R^2 shows the percentage of the variance in mean CRS scores accounted for by the regression.
[a] Significant according to permutation test (with likelihood ratio statistics > 5.9 for 2001, > 9.2 for 2002, > 11.7 for 2003, > 12 for 2004, > 11.5 for Total, and > 4.1 for residual mean.

IX) were initially modeled as additive. As no significant association between total mean CRS scores and markers of A9446-7 (male susceptible parent) were detected by regression analysis, markers from the susceptible parent were not included in the model. The QTL location for total mean CRS score was centered near position 44.0 cM of chromosome IX, between AAC-CGT-0347 and ACG-CTG-0588, and explained 43.3% of the phenotypic variance with LOD score 7.21 (Figure 3, Table 4). The percentage of phenotypic variance explained (43.3%) is more than the 25% in the single-marker regression analysis with ACG-CTG-0588 (Table 4). The QTL likelihood profile is shown in Figure 3, and the QTL parameters at the positions with the highest likelihoods are summarized in Table 4. The QTL effect of allele 2 was significantly higher than for alleles 1, 3, and 4 in size and sign, suggesting that the QTL on chromosome IX is a simplex allele on homolog h2 with significantly different effects than those of the other homologs; i.e., the genotype is Qqqq and gives rise to two gametes, Qq and qq. The mean CRS score associated with each QTL genotype for chromosome IX is shown in Figure 4. The presence of allele 2 (Q12, Q23, and Q24) was associated with a decrease in mean CRS scores of offspring of 0.56, convincingly demonstrating an increase in CRS resistance with the presence of this allele.

The QTL location for year 2003 mean CRS score was located at the same position on the chromosome as was the case with the combined data set (position 44.0 cM on chromosome IX), and explained 43.3% of the phenotypic variance with LOD score 7.11 (Table 3). However, the QTL locations for years 2001, 2002, and 2004 were at a somewhat different position. The QTL location for years 2001, 2002, and 2004 mean CRS were 26 cM, 10 cM, and 38 cM respectively. The QTL effect of allele 2 was greater

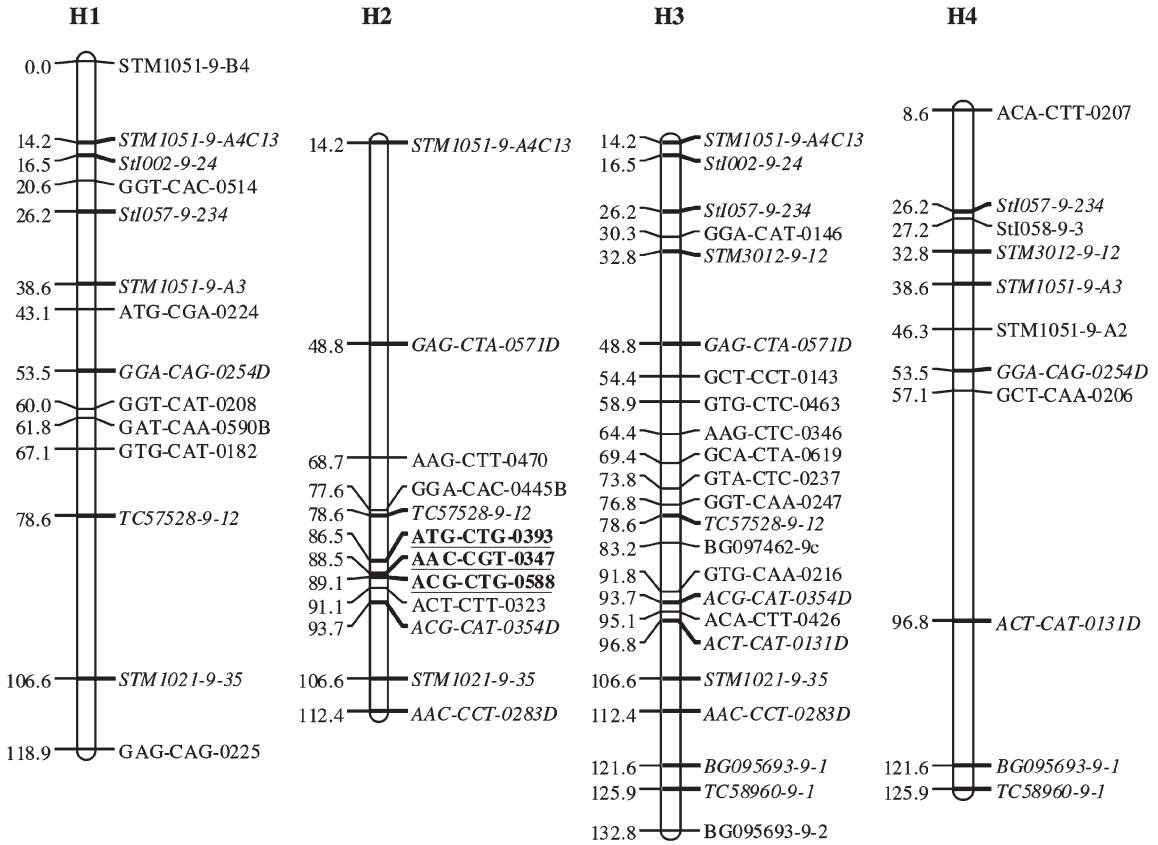


Figure 2. Map of chromosome IX composed of AFLP and SSR markers. Homologous linkage groups are linked by multiple SSR markers (*italic*) which were co-dominantly segregating and duplex markers. SSR marker names begin with the designation StI or STM. Duplex markers are indicated by marker names ending with letter "D". Double simplex markers are indicated by marker names ending with letter "B". SSCP marker names begin with the initials TC or BG.

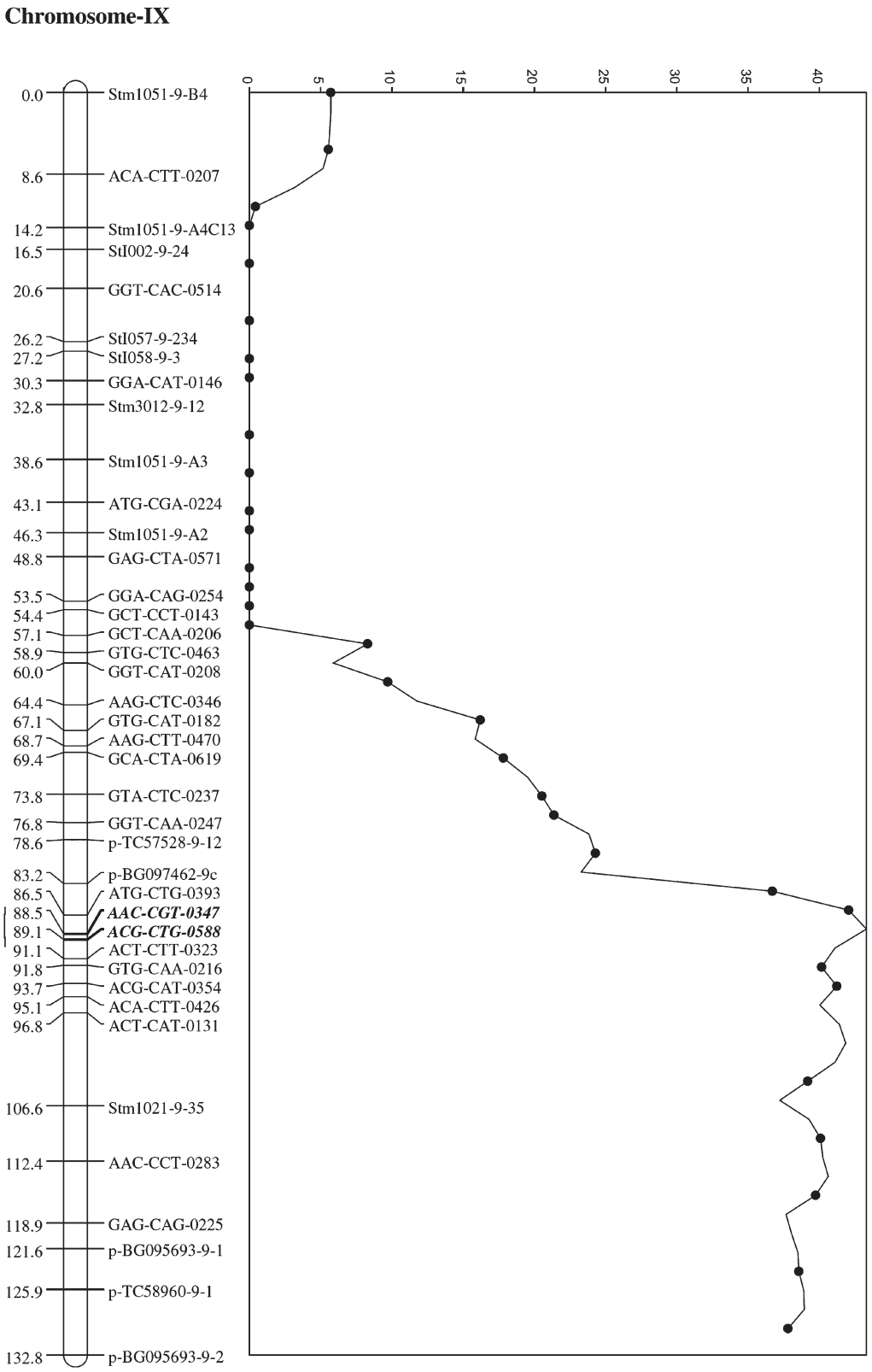
than for the other alleles in years 2001, 2003 and across all 4 years (total mean CRS score), but was not significantly different from allele 4 for years 2002 (Table 3). The predicted QTL location in 2001, 2002, and 2004 was in a portion of the composite chromosome map where only two duplex markers were linked in coupling with the QTL-containing linkage group (Figure 2). The simplex coupling linkage group that contained allele 2 (homolog 2) where the QTL resided, consisted of only four tightly clustered markers located at positions 39 to 46 cM of the integrated map (Figure 2). This limited coverage of homolog 2 of chromosome IX probably caused ambiguity in determining the exact location of the major QTL on homolog 2 of chromosome IX. A more saturated coupling linkage group would be very helpful to determine the location of the QTL.

Effect of minor QTL — The effect of the residual QTL, represented by the presence of marker GGT-CAC-0259 in each offspring, was dissected into the possible gametic genotypes Q12 (e.g. these containing alleles 1 and 2), Q13, Q14, Q23, Q24, and Q34, using the assumed allelic identity indicated by the chromosome IX homolog map (Figure 2). Offspring making up each main QTL genotype were separated into groups with or without marker GGT-CAC-0259. The mean and standard error of each group, separated within each main QTL genotype, is shown in Figure 4. The presence of marker GGT-CAC-0259 significantly decreased the total mean CRS scores in genotypes Q13, Q14, and Q34, i.e. in the absence of the major QTL locus Q2 (Q13, Q14, and Q34; Figure 4), resulting in increased CRS resistance. For genotypes with the Q2 markers, presence of marker GGT-CAC-0259 was significantly associated with lower total mean CRS scores only for the Q24 subset (Figure 4).

Discussion

Acquisition of CRS disease is a function of complex interactions among virus, nematode vectors, and potato plants. Additionally, environmental factors which affect nematode populations affect disease occurrence. All of these factors influencing disease occurrence contributed to the non-uniform disease distribution in the field. The spatial pattern of disease which was indicated by symptom severity of the susceptible check cv. IdaRose, showed that disease "hotspots" were associated with well-irrigated locations. Drier areas with poorer irrigation showed less disease development. However, even the well-irri-

Figure 3. Composite map of chromosome IX and likelihood profile for mean CRS score on Chromosome IX of PA95A33-1.



gated areas showed variable levels of disease distribution. Highly variable occurrence of CRS disease was expected to cause significant G × E interaction. Knowing the high degree of experimental variability common to CRS screening, the large genetic component in phenotypic variance was not expected. The escapes and non-uniform disease distribution would be expected to cause high environmental variance. The strong genetic component is very promising for the mapping research and selection of resistance in breeding population.

Table 4. Estimates of QTL parameters for the QTL location with the highest likelihood on chromosome IX

Year	Location (cM)	R^2 (%) LOD	Constant (SE)	QTL effects, mean, and SE		
				Allele 2	Allele 3	Allele 4
Total mean CRS	44	43.31	0.229	−0.140	−0.040	−0.071
		7.21	(0.028)	(0.022)	(0.021)	(0.021)
2001	26	17.18	0.187	−0.098	−0.042	−0.056
		2.87	(0.037)	(0.026)	(0.027)	(0.027)
2002	10	23.31	0.204	−0.108	−0.048	−0.107
		3.99	(0.037)	(0.028)	(0.027)	(0.027)
2003	44	43.17	0.296	−0.220	−0.069	−0.091
		7.11	(0.043)	(0.034)	(0.033)	(0.033)
2004	38	24.06	0.278	−0.119	−0.087	−0.115
		3.74	(0.040)	(0.031)	(0.031)	(0.030)

The variable constant represents the magnitude and sign for the major QTL.
SE standard error.

Dale and Solomon (1988*a*) and Dale (1989) proposed that the CRS resistance is inherited by a single major gene, possibly subject to modification of expression by minor genes and/or environmental effect. They provided evidence of a polygenic inheritance of CRS resistance combining a major gene effect with minor gene(s) which can modify the major gene effect in their diallel crossing population. Their populations, having a common resistant parent "SCRI10341" were typified by having large portion of genotypes with intermediate resistance. The populations reported in this study showed a similar distribution to those of Dale and Solomon (1988*a*). Distribution of population CRS scores was skewed toward resistance. It is likely that inheritance of this CRS resistance in the population investigated was genetically identical to that reported by Dale and Solomon, inherited in a polygenic manner combining a single major gene with modifying minor gene effects.

TetraploidMap (Hackett and Luo 2003) software was an excellent tool for linkage analysis of tetraploid potato and for the QTL interval mapping. Given an integrated diploid potato map length of about 1,000 cM (Jacobs et al. 1995), the cumulative tetraploid map length based

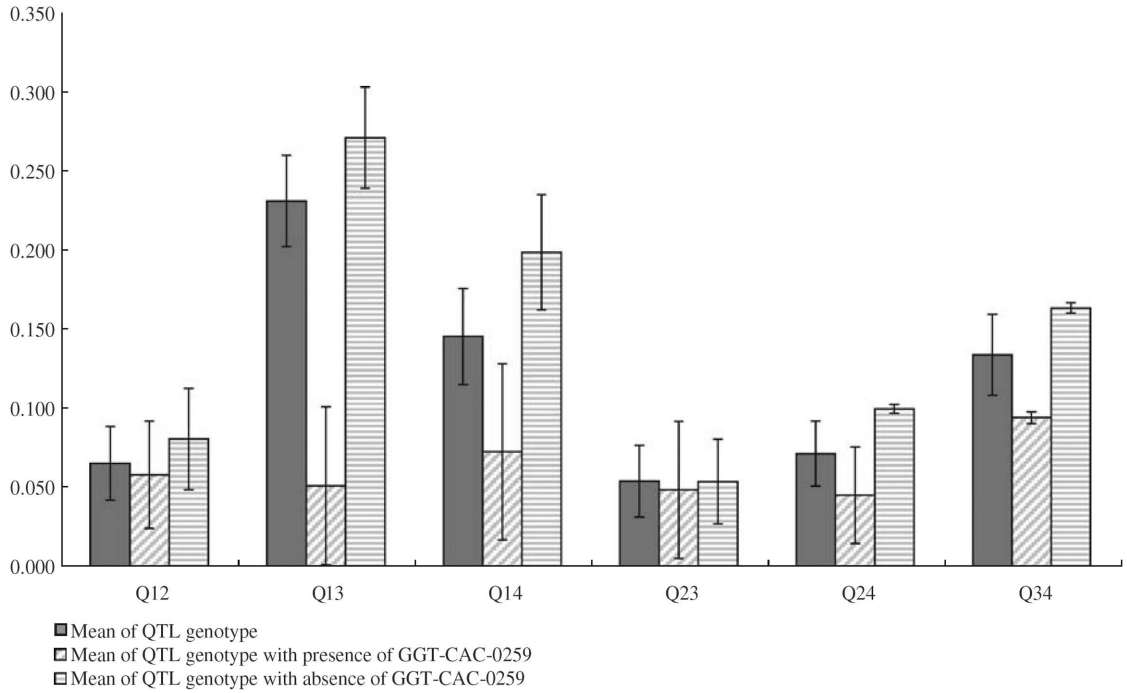


Figure 4. Mean of total CRS scores associated with each QTL genotype for chromosome IX, based on the interval ACG-CTG-0588 to AAC-CGT-0347. Q12 refers to homologs 1 and 2, Q13 refers to homologs 1 and 3, Q14 refers to homologs 1 and 4, Q23 refers to homologs 2 and 3, Q24 refers to homologs 2 and 4, and Q34 refers to homologs 3 and 4.

on coupling-phase simplex markers would be expected to be 4,000 cM. Based on the cumulative length of each homologous coupling linkage, the total map length of PA95A33-1 (2,940 cM) represented approximately 73.5% (2,940 cM/4,000 cM) coverage of the total genome. This genome coverage is larger than tetraploid linkage maps constructed in previous studies that covered 25% of the genome (Bradshaw et al. 1998; Meyer et al. 1998). The derived linkage maps have not yet been resolved into the expected 2×4×12 parental linkage groups, and additional markers will be required to cover the entire genome. The combination of co-dominant SSRs and duplex AFLP markers usually enabled identification of homologous linkage groups. The power of SSR markers to construct genetic maps of tetraploid species was reviewed by Meyer et al. (1998) and Luo et al. (2001). Development of new SSR markers derived from potato expressed sequence tags (ESTs; Feingold et al. 2005) provided additional SSR markers for greater genome coverage. The EST-derived SSR markers showed better resolution on gels than genomic-sequence derived SSR markers in this experiment.

A more precise determination of the interaction of the minor QTL and the major QTL (epistasis, dominant, or additive) was probably hindered by the small size of the population (92 genotypes). The marker GGT-CAC-0259 associated with the minor QTL did not map to an established linkage group. This lack of map coverage for the linkage group containing GGT-CAC-0259 also reduced precision in dissecting the QTL effect and precluded interval mapping analysis. More precise elucidation of the minor QTL effect associated with GGT-CAC-0259 could be achieved with more genome coverage with additional SSR and AFLP markers, and by using a larger population.

Broad sense heritability for CRS scores for this population was 0.80 (±0.16). The percentage of the total phenotypic variation explained by the major QTL on chromosome IX using interval mapping (43%) exceeded the original 27% obtained by simple regression analysis (Tables 2 and 3). A similar increase in the percent of variability explained by an interval mapping approach has been previously observed (Bradshaw et al. 2004*a*, *b*; Bryan et al. 2004). The variation explained by the minor QTL associated with the marker GGT-CAC-0259 was 12.3% in simple regression analysis. Dale and Solomon (1988*a*, *b*) suggested that there is a major dominant gene conferring resistance to CRS disease plus some form of background resistance, possibly of a polygenic nature. Although, the present study could not elucidate the minor gene effect as to whether it is additive, dominant, or interactive, the major gene effect of CRS resistance and presence of a minor gene effect were clearly confirmed using molecular tools.

Several resistance genes have been mapped to chromosome IX of potato, including a minor QTL for late blight resistance (*Pi*; Ghislain et al. 2001), QTL for *Erwinia* species (*Eca*; Zimnoch-Guzowska et al. 2000), *Verticillium* wilt resistance (*StVe*; Simko et al. 2004), minor QTL for cyst nematode resistance (*GPA6*; Rouppe van der Voort et al. 2000), and potato virus X resistance (Nx_{phu}; Tommiska et al. 1998). Relationship between the R-gene complex mapped on chromosome IX and the QTL for CRS resistance can be defined with better resolution of chromosome IX map for this population.

In conclusion, a major QTL was located on potato chromosome IX in the simplex coupling phase. Flanking AFLP markers AAC-CGT-0347 and ACG-CTG-0588 were associated with CRS resistance. More detailed analyses revealed an additional minor QTL associated with an unlinked marker GGT-CAC-0259. Conversion of these three AFLP markers to simple PCR markers should enable future adoption of MAS in breeding for CRS resistance. In addition to MAS, elucidation of relationship between CRS-1 and R-gene cluster located in chromosome IX should be a logical extension of this research.

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