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Mapping of the potato leafroll virus resistance gene, Rlr_{etb}, from *Solanum etuberosum* identifies interchromosomal translocations among its E-genome chromosomes 4 and 9 relative to the A-genome of *Solanum* L. sect. *Petota*

K. B. Kelley · J. L. Whitworth · R. G. Novy

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Abstract Gene Rlr_{etb}, derived from the potato species *Solanum etuberosum*, confers resistance to potato leafroll virus (PLRV). Mapping of this gene would aid in developing marker-assisted selection protocols to facilitate its introgression into cultivated potato. One RFLP marker and 45 cleaved amplified polymorphic markers (CAPs) markers were used to screen an *etuberosum*-derived BC_3 family segregating for PLRV resistance conferred by Rlr_{etb}. Nine markers from linkage group 4 of the tomato map displayed linkage with Rlr_{etb}, however, eight additional markers from linkage group 4 that should have been syntenic with Rlr_{etb} were not. Instead they segregated with 12 markers previously mapped to linkage group 9 of the tomato map, indicative that chromosomes 4 and 9 of *S. etuberosum* have translocated regions relative to the potato and tomato genomes. These chromosomal translocations have placed Rlr_{etb} beyond the end of the published map of linkage group 4 of tomato with the closest marker, C2_At1g42990, mapping 13.6 cM from Rlr_{etb}.

Keywords PLRV · *S. etuberosum* · E-genome · Translocation · Resistance

K. B. Kelley
AgraServ, Inc., 2565 Freedom Lane, American Falls, ID 83211, USA
e-mail: kevin@agraserv.com

J. L. Whitworth · R. G. Novy (✉)
USDA/ARS, Small Grains and Potato Germplasm Research Unit, 1693 S. 2700 W., Aberdeen, ID 83210, USA
e-mail: Rich.Novy@ars.usda.gov

J. L. Whitworth
e-mail: Jonathan.Whitworth@ars.usda.gov

Introduction

Solanum etuberosum Lindl., a wild, non-tuber-bearing relative of cultivated potato (*S. tuberosum* L. subsp. *tuberosum)*, is highly resistant to several potato pathogens, including potato leafroll virus (PLRV), potato virus Y (PVY), potato virus X (PVX), and green peach aphid, *Myzus persicae* (Valkonen et al. 1992a, b; USDA, ARS, National Genetic Resources Program 2003). It is a member of sect. *Etuberosum* which also comprises the non-tuber-bearing species *S. palustre* (formerly classified as *S. brevidens*) and *S. fernandezianum.* These three species are taxonomically and sexually isolated from sect. *Petota* to which cultivated potato belongs (Spooner and Hijmans 2001), and have been characterized as having an E-genome distinct from the A-genome of *S. tuberosum* (Ramanna and Hermsen 1981; Matsubayashi 1991; Perez et al. 1999).

Barriers to hybridization of *S. etuberosum* with cultivated potato were overcome through the use of somatic hybridization (Novy and Helgeson 1994a; Thieme et al. 1999). High levels of resistance to PVY

were reported in somatic hybrids of *S. etuberosum* and their sexual progeny (Novy and Helgeson 1994b; Thieme et al. 1999; Gavrilenko et al. 2003). Resistances to PVY, PLRV, and green peach aphid derived from *S. etuberosum*, also were identified in the BC_1 and BC_2 progeny of somatic hybrids (Novy et al. 2002), and PLRV resistance was shown to be expressed in the BC_3 generation as well (Novy et al. 2007).

The stable transmission of PLRV resistance following three generations of backcrossing to cultivated potato indicate that genomic differences between *S. etuberosum* and cultivated potato have not detrimentally impacted the introgression of this trait. Segregation for PLRV resistance in the two BC_3 populations used in this present study most closely fit a gene model whereby resistance was conferred by a single dominant gene inhibiting the systemic spread of PLRV from infected foliage to tubers (Novy et al. 2007). Localization of the PLRV resistance gene to one genomic region also supports this gene model (Gillen and Novy 2007).

Potato leafroll virus resistance conferred by a single gene demonstrated as heritable following successive backcrossing to cultivated potato would be advantageous to potato breeders. Efforts in developing PLRV-resistant potato varieties have been hampered due to polygenic inheritance of resistance or difficulty in introgressing identified monogenic resistance from donor wild species (Jansky 2000; Solomon-Blackburn and Barker 2001; Taliansky et al. 2003). Of the 13 most widely grown potato cultivars in North America, none of them are resistant to PLRV (Corsini and Brown 2001), even though this virus is considered among the most problematic of the potato viruses on a world-wide scale (Solomon-Blackburn and Barker 2001).

The objective of the present study was to map the location of PLRV resistance in the BC_3 progeny of a *S. etuberosum* somatic hybrid. Currently, selecting for PLRV resistance is done under field conditions and is a time and labor-intensive process. Marker-assisted selection for PLRV resistance would speed this process and would be a great help to potato breeding programs. An earlier study localized the PLRV resistance of *S. etuberosum* to chromosome 4 (Gillen and Novy 2007). This study reports on mapping the location of a PLRV resistance gene derived from *S. etuberosum*, represented by the designation Rlr_{etb}. New information also is presented regarding genomic structural differentiation between the E-genome of *S. etuberosum* and the A-genome of *S. tuberosum* identified during mapping of Rlr_{etb}.

Materials and methods

Plant material

Somatic hybrids were produced by protoplast fusion of the diploid ($2n = 2x = 24$) *S. etuberosum* (PI 245939) (clone designation: 16-1) with a diploid *S. tuberosum* subsp. *tuberosum* haploid-wild species hybrid [US-W730 × *S. berthaultii* (PI 265857)] designated 463-4 (Novy and Helgeson 1994a). BC_1 individuals were produced by crossing somatic hybrid 2-9-3B with the potato cultivar Atlantic, and BC_2 individuals were produced by crossing the BC_1 individual P2-3 with the cultivar Katahdin. The BC_2 individual Etb 6-21-3 was used as the PLRV resistant parent in crosses with the advanced breeding clone A92303-7 to produce a BC_3 family of four individuals designated A00ETB12, and with the potato cultivar GemStar Russet to produce a family of 35 individuals designated as AO1687 (Table 1).

Screening for PLRV resistance

Plant material was previously screened and assessed for response to PLRV infection in the field as reported by Novy et al. (2007), with ELISA testing of harvested daughter tubers conducted to ensure veracity in the classification of response to PLRV infection. The cultivar Liu was used as a PLRV resistant control and the cultivars Russet Burbank and Ranger Russet were used as PLRV susceptible controls.

DNA extraction and marker analysis

Young leaf tissue of potato plants grown in the field or greenhouse was used for DNA extraction as described by Gillen and Novy 2007. The protocol involved either a modification of a CTAB extraction procedure (Doyle and Doyle 1987) or a modification of a nuclei extraction procedure (Bernatsky and Tanksley 1986).

Table 1 Description of breeding clones and cultivars used in the production of two BC_3 progeny families used in mapping PLRV resistance from *S. etuberosum*

Entry	Description	Parentage	PLRV response[a]
16-1	Diploid parent of somatic hybrid	*S. etuberosum* clone (PI 245939)	R
463-4	Diploid parent of somatic hybrid	US-W730 × *S. berthaultii* (PI 265857)	S
2-9-3B	Tetraploid somatic hybrid	463-4 + 16-1	R
Atlantic	Parent of BC_1 clone, P2-3	Wauseon × Lenape	MS
P2-3	BC_1 of somatic hybrid	2-9-3B × Atlantic	R
Katahdin	Parent of BC_2 clone, Etb 6-21-3	USDA 40568 × USDA 24642	MS
Etb 6-21-3	BC_2 of somatic hybrid	P2-3 × Katahdin	R
GemStar Russet	Parent of BC_3	Gem Russet × A8341-5	S
A92303-7	Parent of BC_3	A86332-7 × Ranger Russet	S
A00ETB12	BC_3 family comprised of 4 individuals	Etb 6-21-3 × A92303-7	Segregating for R/S
AO1687	BC_3 family comprised of 35 individuals	Etb 6-21-3 × GemStar Russet	Segregating for R/S

Response to infection by PLRV was obtained from multiple years of field evaluations as reported in Novy et al. (2007)

[a] R = Resistant, S = Susceptible, and MS = Moderately susceptible

RFLP analysis using probe TG443 was carried out following the procedure described by Gillen and Novy (2007). Probe labeling and detection was carried out using Gene Images™ labeling and detection systems (Amersham Biosciences, Piscataway, NJ, USA). A polymorphism specific to *S. etuberosum* and 6.5 kb in size was scored from genomic DNA digested with the restriction enzyme *Eco*RV.

Cleaved amplified polymorphism (CAP) markers were amplified using primers obtained from tomato or potato (Table 2) (Chen et al. 2001; Frary et al. 2005; Wu et al. 2006). Most were COSII markers (second generation conserved ortholog set markers) (Wu et al. 2006), the exceptions being cLEC7B23 (Frary et al. 2005), *ANTL* (a known function gene available at http://sgn.cornell.edu/), and *Dpe-P* (Chen et al. 2001). All markers had been previously placed on the *Solanum lycopersicum* (LA925) × *S. pennellii* (LA716) high-density map (tomato map) (Frary et al. 2005; Fulton et al. 2002; Wu et al. 2006) with the exception of *Dpe-P*, which was mapped in potato (Chen et al. 2001). The tomato map was used as a reference to identify markers mapped to tomato linkage group 4 that likely were syntenic with Rlr_{etb}, and therefore would be useful in establishing genetic linkages to this resistance gene. Due to the length of many PCR fragments (often greater than 1,000 bp), an extended PCR protocol was used as follows: 1 cycle of 94°C for 3 min, an annealing temperature of 55°C for 2 min, 72°C for 1 min 30 sec; followed by 39 cycles of 94°C for 45 sec, an annealing temperature of 55°C for 1 min 30 sec, 72°C for 1 min 30 sec; then a final extension step for 10 min at 72°C. Amplified fragments were screened for both amplicon and restriction site polymorphisms (Table 2). Restriction enzymes were used at a concentration of 0.05 U ml^{-1} with digestion reactions conducted at the enzyme-specific recommended temperatures for 3 h, followed by 20 min of heat inactivation. PCR and restriction digest products were analyzed by gel electrophoresis using 0.7% Seakem® LE Agarose (Cambrex Bio Science Rockland, Inc., Rockland, ME, USA) and 1.15% Synergel™ (Diversified Biotech, Boston, MA, USA). Only markers unique to the *S. etuberosum* parent were analyzed.

S. etuberosum 16-1: S_1 progeny bulk analysis

The original *S. etuberosum* parent, 16-1, which does not produce tubers, and which had previously been maintained in tissue culture was lost in 2004. However, *S. etuberosum* and the other two diploid species within sect. *Etuberosum* are unique among diploid potato species in that they are self fertile and have a high level of genetic homozygosity (Spooner et al. 1992, 1996). Prior to its loss, greenhouse plants of 16-1 had flowered and were selfed to produce S_1 progeny. To obtain DNA samples that closely reconstituted the genetic identity of the original parent, S_1 plants were grown in the greenhouse and leaves bulked together for DNA extraction. Leaf

Table 2 CAPs markers used for mapping the location of *Rlr*$_{etb}$

Marker	Chr.[a]	Forward Primer (5′ to 3′)[b]	Reverse Primer (5′ to 3′)[b]	PCR Product Size (bp)	Enzyme	Polymorphic band size(s) (bp)
C2_At1g19600	4	AAAGTGTAAATATAGGTGCACCAGTTG	ATATGGGCGTTAAGCTCGTTGAG	1500	*Hin*fI	630
C2_At1g42990	4	ATGACCCCGTCGATAAGAAGCG	ACCTCACAGCTGCATCTCTATTCCTC	1100	*Alu*I	400 & 500
C2_At1g74970	4	TCATCATCAACTATCGTGATGCTAAG	ACGCTTGCGAGCCTTCTTGAGAC	1700/1900	Amplicon	1700
C2_At1g76080	4	TAGTATGGAGGAATTGGATGAAGC	TCTTCTCTGCTGTGGAGCTGCAC	1100	*Bst*UI	750 & 500
C2_At1g79600	4	ACCCGAGTACCTTGAGGAGCTCTC	AGATCCAAAAACCTTTAGCCCTTG	1500/1600	Amplicon	1500
C2_At2g45730	4	TCCATTCGGGTCTCTGTTTCAAG	TTTCGCATCTCGTCAATATCTTC	1400	*Mse*I	350 & 550
C2_At3g03990	4	AATCCTGATTTCTTCGATTTCCG	ATGTGGCAAATGTCCCTCAATATTC	1800/Neg[c]	Amplicon	1800
cLEC7B23	4	GGAGAACACGGCTACCTCAG	AGCTGGAAATGAGGTTTTGC	590	*Taq*I	550
ANTL	4/9	TCCACAGGAAATCCATTGAC	TCTTTAGTTCTCTTAGCTAATTCACCA	1650	*Scr*FI	475
C2_At1g10030	4/9	AGCTGTTAGGATGGTGGTTAATGC	ACTCTGTCAAGAAATGACCGAAGGC	2400/2100	Amplicon	2400
C2_At1g35720	4/9	TCTGGACTTTGAGTCCTGCTGAGCG	AAGTGTCTCCAGCAATTGCACGG	–	*Dde*I	890
C2_At1g35720b	4/9	TCTGGACTTTGAGTCCTGCTGAGCG	AAGTGTCTCCAGCAATTGCACGG	1700	Amplicon[d]	850
C2_At1g71810	4/9	TCATGCAGATCCACATCCTGGAAAC	AGTGACAAAATCCTTGGCCAATGC	1070	*Scr*FI	700 & 370
C2_At3g20020	4/9	ATGTTACAACCAACAGACGGCGG	TGAAGTTTTGATGCTGAAAAATTGC	1200/1150	Amplicon	1200
C2_At3g51010	4/9	TCCAAACAATCCCAATGAAGGAAG	ACGCTCTACTCGCTTAATCATTTTC	2300	*Hin*fI	600
C2_At4g09010	4/9	TAAGGGGCTTGATGCTGCTTTG	TAAAGGTCGATTTGACTGCACTTTG	580/610/650	Amplicon	610
C2_At4g39870	4/9	TGATGGAAGTAATTCTAAAGTTTCCG	AACTAGCCCACCAAACACAGCACC	1000/1450	Amplicon	1000
Dpe-P	4/9	CACTACTTTTCAATCTCCTATCCC	GCATAGTCACGAACTTTTTTCC	2400/2700	Amplicon	2400
C2_At1g02910	9	TGAACCCACTCCCACTGCTGAGTC	TGCTGTGTCGAATAGCACAAGAGC	1700/1800	Amplicon	1700
C2_At1g04190	9	TCATTTCTTCGACAGTATGCTGAAGATT	ATTCCATCATTTTGTCCATGCTTCC	1100/1250	Amplicon	1100
C2_At1g05385	9	CCAGAGATCTAATAGCTTTCTCTAGCA	ACGAACAGCTGATGCAGCAAAGG	1220/1100	Amplicon	1220
C2_At2g32600	9	TGAAGGGAATTACTTGGCTCACAC	TGTTTTGTTTCCGGATCAAATTGC	1300	*Hin*dIII	850 & 450
C2_At2g32760	9	TACATCTGCTCTTAATGAAGCTAAGC	TCTCTTTGACAACTTTAGAATGCATTG	800/950	Amplicon	800
C2_At2g37025	9	AACATCACAGGCTCTGGACTGTTTC	ATGCATGTTCGCCAGTTCACTGAC	1350	*Hae*III	1100
C2_At2g37240	9	TCCTTTGTCGCATAAGAGCTGATTATC	ACAAGTGCGACTCCTGCTGCATCC	540	*Hpy*CHIV	300 & 225
C2_At3g09925	9	ATTGAAGCCTCAGATGGAATGGATG	ACATGGGAGTTGTGTAGTAGAAAGGG	900	*Taq*I	310
C2_At3g23400	9	TGGGCTAAACAGAGGTCTTGCTGC	TATAAGTTCAAATTTGTGGGCTAAAG	900	*Msp*I	750
C2_At3g24010	9	AGCATGCAATCAGGATTGCTGATG	AAGTACTGATCGAGCTGCTGAATATG	940	*Dde*I	210
C2_At3g25480	9	ACAAAGTATCCATTCTTTGTTGCTGG	TTATCCCACCTCGGATAGCATAAGC	2100	*Sau*96I	1280
C2_At1g80460	3	TCTTTATAACGCCATTGTTTGGATG	AATCTCAGAATTGCTAATAATCTTTGG	1200	*Msp*I	530 & 730

Table 2 continued

Marker	Chr.[a]	Forward Primer (5′ to 3′)[b]	Reverse Primer (5′ to 3′)[b]	PCR Product Size (bp)	Enzyme	Polymorphic band size(s) (bp)
C2_At2g01770	4 × 3	AGAACCCACAGGCATGGCTCGAC	ATAAGTGCTGTTTCAAAAGCACTCC	950/1200	Amplicon[d]	950
C2_At5g62390	3	TGCTACTAACTGTTGATGCCATTGAG	TTGGGGGTCGATAACATCAAGC	1200	*Hae*III	530 & 630
TG324	3	CACTTGGTTGATGGATAGTG	CTTCTAGTAGTCCAACAGCAACTG	975	*Hae*III	1030
C2_At4g14570	3	AAAGCTTCTTGTAGTTAGAAATCCTGAA	TTTCCAGCTCCCACAGTCCTTATCTG	330/540	*Hin*fI	315
C2_At1g20050	6	ATGATCTAAAATTGCCTGGTTTTG	AATAGCCCTCAAGGACCATGTGG	950	*Scr*FI	850
C2_At1g77470	6	TGCCCTACAATCACGATGTACACG	AAACCACCCTCAGGGACATCAAG	1900	*Scr*FI	650 & 1200
C2_At5g61510	6	AGTTCCTACTGCGCCGCTGCTTC	AGCATGAACAAGTACTGTGTGCCCACG	1510/1700	Amplicon	1510
C2_At5g06370	1	TCTTGGCGTACTGCTTATATTTATGC	TCCAGTTCCTACTTCTTGACCACGTC	1400	*Hpy*CHIV	610 & 550
C2_At5g13030	1	TCCAAGAACAGATACCATTCCAAGAG	AACAGAAGGCAACATTTTCGTATAGC	1600	*Hin*fI	250
C2_At4g09010b	4 × 1	TAAGGGGCTTGATGCTGCTTTG	TAAAGGTCGATTTGACTGCACTTTG	650	Amplicon[d]	650
C2_At1g67700	5	AAGAGGAAATTGTTAGTGGTTGAAGC	ACTGCTGCGAGATTCCTAGCTAGAG	1300	*Dde*I	520
C2_At2g01275	5	TGCCGCTGATTGCTCTTCCCAG	TGCCGTATAACCAGGCCTAAAAG	–	*Msp*I	1000 & 550
C2_At1g14790	5	TGGAGAGAACACTAATATTCTCAAGG	ATGTCTTGATCCCAGCAAACAAAG	1100	*Hin*fI	425
C2_At2g20860	7	ATTGAAGCCACATATACTCATAGAAGC	TCCAGATTTTGCAACTTTCTCTACAC	1180/650	Amplicon	1180
C2_At2g42810	7	TTGCCTTCAAGTGCATGTGTCC	AGAGAGCTTCACGCCATCAACAC	630/900	Amplicon	630
C2_At3g15290	7	TCTGCTATTTTGGCTTCTAATACAAG	ACAATATGTGTCTTCTGATGTATCTGC	1150/900	Amplicon	1150

[a] Chromosome location in the published tomato map. Potential structural differences from the E-genome map are indicated by tomato chromosome/*S. etuberosum* chromosome. Markers not believed to indicate structural differences in that they appear to be secondary amplicons lacking correspondence in size to those published for tomato are indicated by: tomato chromosome × *S. etuberosum* chromosome

[b] COSII markers are described by Wu et al. (2006) and the primers for COSII markers and *ANTL* are published on the SOL Genomics Network (http://www.sgn.cornell.edu/). Primers for *Dpe-P* are published in Chen et al. (2001), and primers for cLECB23 and TG324 are published in Frary et al. (2005)

[c] Neg: Indicates marker did not amplify from susceptible backcross parents and progeny that scored negative for the marker

[d] These amplicon polymorphisms mapped to unexpected locations on chromosomes other that those in published maps

tissue was collected and bulked from 65 S_1 plants; from 131 g of bulked tissue, 15 g was used for a large-scale DNA extraction using a modification of a nuclei extraction procedure (Bernatsky and Tanksley 1986; Gillen and Novy 2007).

To verify that this bulk of S_1 plants provided a DNA sample that approximated the 16-1 parent, two CAPs markers from each of the 12 chromosomes in the tomato map were evaluated for polymorphisms between the progeny bulk and remnant DNA retained of the original 16-1 parent. Each of the markers was evaluated with 12 restriction enzymes (data not shown). The number of enzymes that cut amplified fragments ranged from 5 to 11 out of 12 enzymes with an average of 8.1 enzymes per marker, resulting in a total of 195 restriction sites evaluated. From among these, no polymorphism was detected between the 16-1 parent and the progeny bulk DNA, indicating that the full genome of clone 16-1 was represented in its 65 S_1 progeny. The S_1 bulk DNA was used in the place of the 16-1 parent in this study.

Mapping of RFLP and CAPs markers

Molecular markers were scored in BC_3 individuals as well as parental clones to determine whether polymorphisms were unique to *S. etuberosum*. Only marker fragments unique to *S. etuberosum* were evaluated, as *S. tuberosum* specific markers are present in all progeny of this backcross population. Such *S. etuberosum*-specific markers were expected to be simplex in the BC_2 parent and to segregate for presence/absence in a 1:1 ratio in the BC_3 population. A goodness-of-fit test was performed to determine whether markers fit the expected ratios.

A genetic map was constructed using the statistical program TetraploidMap (Hackett and Luo 2003). Markers were grouped using the cluster analysis function of the program and compared against the tomato or potato map they originated from using a LOD threshold of 3.0. Marker order was determined using a two-point linkage analysis and simulated annealing (Hackett and Luo 2003).

Results

Initial genomic localization studies for PLRV resistance were carried out using a combination of RFLP and SSR markers on six BC_2 and four BC_3 breeding clones of the A00ETB12 family (Gillen and Novy 2007). The marker TG443, mapped to linkage group 4 of tomato and potato, was identified as co-segregating with the PLRV resistance gene, Rlr_{etb} (Gillen and Novy 2007). An additional 35 BC_3 clones from family AO1687 were screened with TG443, and among the 39 BC_3 clones (total across both BC_3 families), TG443 was present in 18 (Fig. 1). The segregation of TG443 with PLRV resistance is outlined in Table 3. Observed segregation for the four classes in Table 3 were tested against a model in which TG443 was unlinked to Rlr_{etb}, with an expected 25% of BC_3 individuals present in each class. A chi-square test using Excel software rejected at the 5% level of significance that TG443 was unlinked to Rlr_{etb}, with a calculated P value of 0.01—corroboration of the linkage of TG443 with Rlr_{etb}. On the basis of the frequency of recombination between TG443 and Rlr_{etb} (represented by the TG443(+)/Susceptible and TG443(−)/Resistant classes in the BC_3), genetic distance between the two is calculated to be 24 cM.

Confirmation of the linkage of TG443 with Rlr_{etb}, allowed the selection of CAPs markers published on the SOL Genomics Network (http://sgn.cornell.edu/) that could be used to saturate chromosome 4 and allow mapping of Rlr_{etb} (Mueller et al. 2005, 2008). Of the 84 CAPs markers that were amplified using PCR in this study, 79 had primers that amplified a fragment near the expected size from *S. etuberosum*. Of these, 62 were tested for amplicon or restriction fragment polymorphisms specific to *S. etuberosum*, and 45 produced polymorphisms that were scored in 39 BC_3 clones. The two markers C2_At1g35720 and C2_At4g09010 produced secondary amplicon polymorphisms that mapped to locations other than the expected synteny groups along with polymorphisms that mapped to expected locations. Of the 5 markers that did not amplify well from *S. etuberosum*, 2 of them were COSII markers (C2_At1g43580 and C2_At3g17210) and the other three used primers designed from tomato sequences (TG223, Brix 9-2-5, and U168526).

When analyzed with the goodness-of-fit test, the majority of markers did not deviate from the expected 1:1 segregation ratio. Exceptions include the following: four from chromosome 3 that formed a linkage

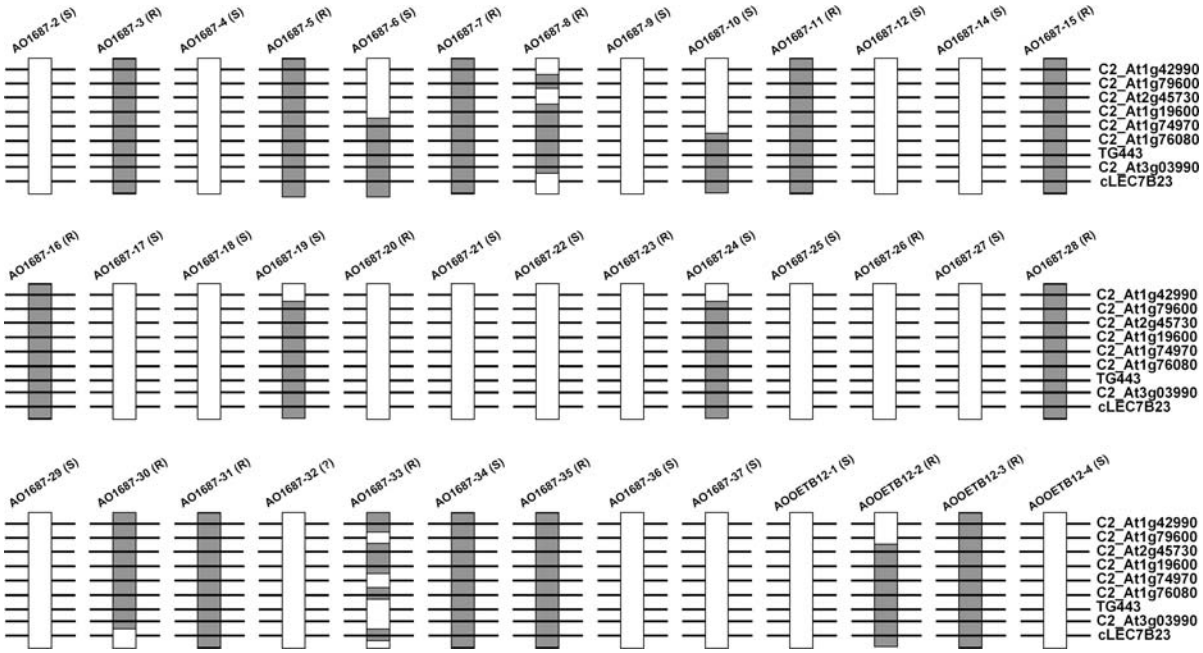

Fig. 1 Ideogram of *S. etuberosum* chromosome 4 in 39 BC_3 progeny of a somatic hybrid between *S. etuberosum* and a *S. tuberosum* haploid × *S. berthaultii* hybrid determined with CAPs and RFLP markers. Each chromosome is marked with nine markers, placed in the order that minimized the number of recombinants. See Fig. 2 to see how this order differs from that of the published tomato map of these markers. Markers present in the respective BC_3 are represented by *black bars* over *gray area*s, and absent markers are represented by *black bars* behind *white areas*. The score for PLRV resistance of each BC_3 clone is indicated by (R) for resistant clones and (S) for susceptible clones following each clonal designation. The exception being clone AO1687-32 for which its response to infection by PLRV remains undetermined

group (C2_At2g01770, C2_At5g62390, TG324, and C2_At4g14570) had a lower than expected number that scored positive for the markers from *S. etuberosum*; and three markers, C2_At2g20860, C2_At2g 42810, & C2_At3g15290, from linkage group 7 of the tomato map that scored positive for these markers from *S. etuberosum* in 38, 38, and 37 out of 39 BC_3 clones, respectively.

Table 3 Segregation of TG443 and Rlr_{etb} in 38 BC_3 individuals

	Rlr_{etb} present (Res.)	Rlr_{etb} absent (Susc.)
TG443 (present)	13 (34%)	**5 (13%)**
TG443 (absent)	**4 (11%)**	16 (42%)

Number of individuals and the percentage they represent of the total number analyzed are given for each of four classes. Non-recombinant classes are in roman and recombinant classes are bold-faced. If TG443 and Rlr_{etb} were unlinked (i.e., independent segregation), nine to ten BC_3 (25%) would be expected in each of the four classes

Localization of Rlr_{etb}

Nine markers unique to *S. etuberosum* from linkage group 4 of the tomato map were identified as being linked with Rlr_{etb} (Fig. 2). The scores for the 39 BC_3 clones with these nine markers are displayed in Fig. 1. The order of the markers in the figure is arranged to minimize the number of recombinants. All nine markers co-segregated with Rlr_{etb} in 26 out of 38 clones, as measured by the presence of all nine in PLRV resistant clones and their absence in susceptible clones. Potential recombinants include three resistant clones that scored negative for all nine markers, one susceptible clone scored positive for all nine markers, and eight clones that scored positive for some markers and negative for others (Fig. 1).

Linkage analyzes of these nine markers indicate that Rlr_{etb} is located outside the limits of this map at a potential distance of 13.6 cM from the marker C2_At1g42990 (Fig. 2). The order that minimized the number of recombinants was not conserved with

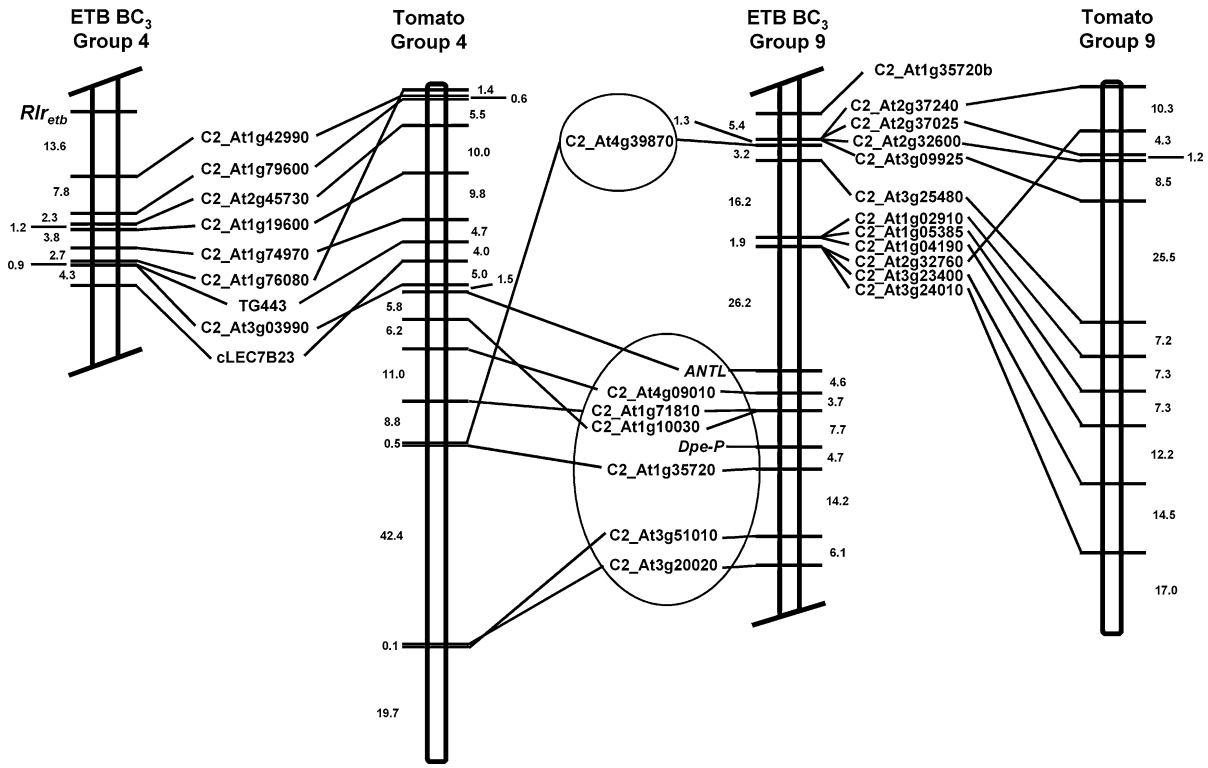

Fig. 2 Linkage maps generated by TetraploidMap of *S. etuberosum* synteny groups 4 and 9 in the BC_3 progeny of a somatic hybrid between *S. etuberosum* and a *S. tuberosum* haploid × *S. berthaultii* hybrid determined with CAPs and RFLP markers. The published tomato map of these markers is included for comparison (Wu et al. 2006) and to show the proposed structural differences within the *S. etuberosum* chromosomes. The tomato map of chromosome 4 is inverted compared to the published map (Wu et al. 2006). The proposed location of the PLRV resistance gene Rlr_{etb} is shown on the map. *Circled* markers were mapped to chromosome 4 in the tomato map, but formed a separate synteny group that aligned with markers from chromosome 9 of tomato

the tomato map (Fig. 2) (Wu et al. 2006). cLEC7B23 and C2_At3g03990 switched places in the marker order of BC_3 progeny compared to the tomato map, while C2_At1g76080, which should have been most tightly linked to Rlr_{etb}, given the tomato map marker order, was instead closer to the other end of the synteny group.

E-genome structure

Markers from across linkage group 4 were analyzed to determine whether one could be located that was more tightly linked to Rlr_{etb}. Instead, 8 markers that mapped beyond C2_At3g03990 across the remainder of linkage group 4 of the tomato map formed a separate synteny group (Fig. 2—markers referenced are circled). ANTL, which mapped 1.5 cM from C2_At3g03990 on group 4 of the tomato map, did not show linkage with this marker in the *etuberosum*-derived BC_3. In all, 17 markers mapped to linkage group 4 of the tomato map were scored. Markers most closely linked to Rlr_{etb} were at the extreme end of the published tomato map (C2_At1g42990 and C2_At1g79600). At the other end of the published tomato map of chromosome 4, C2_At3g51010 is located 19.7 cM from the beginning of the map. The 17 markers are spaced an average of 7.3 cM apart across the tomato map.

Markers from chromosomes other than 4 that had been identified by Gillen and Novy (2007) as present in the resistant parent, Etb 6-21-3, were used to screen the BC_3 population. This effort was undertaken to identify translocated regions of *S. etuberosum* now associated with the section of chromosome 4 linked to Rlr_{etb}. Based on previous segregation analysis (Gillen and Novy 2007), and on the identification of TG10, a marker from linkage group 9, as being linked to

markers from linkage group 4 of tomato and potato in the published map for the E-genome (Perez et al. 1999), chromosome 9 was selected as a potential candidate for this translocated region. Twelve markers unique to *S. etuberosum* distributed across linkage group 9 of the tomato map formed a synteny group with the 8 markers at the end of chromosome 4 of the tomato map that had not previously integrated with markers of linkage group 4 of the BC_3 population (Fig. 2). Linkage analysis with TetraploidMap indicates ANTL from chromosome 4 is 26.2 cM from C2_At3g24010 of chromosome 9 in the BC_3 progeny (Fig. 2).

Additional markers unique to *S. etuberosum* from chromosomal regions other than 9 that were present in the resistant parent, Etb 6-21-3, also were analyzed for possible linkage to Rlr_{etb}. Marker selection focused on chromosomal regions known to contain resistance genes in Solanaceous species (Grube et al. 2000) according to the 'SOLanaceae Function Map for Pathogen Resistance' compiled by Christiane Gebhardt (https://gabi.rzpd.de/) (Gebhardt and Valkonen 2001; Meyer et al. 2005). In addition to chromosomes 4 and 9, this included regions of chromosomes 1, 3, 5, 6, and 7 (Table 2). No additional markers linked to Rlr_{etb} were identified. However, synteny groups were formed for linkage groups 3 and 6 with markers in chromosomal regions that previously were separate linkage groups in the published map of the E-genome (Perez et al. 1999) (Fig. 3). These results indicate that these unconsolidated sections of chromosomes 3 and 6 of the E-genome may in fact be from the same chromosomes as expected based on the tomato and potato maps. Other than those results already mentioned, markers located on the same linkage groups in the tomato map were grouped into predicted synteny groups in the BC_3 population in this study (chromosomes 1, 5, and 7- data not shown) (Table 2) with the exception of C2_At1g14790, which did not segregate with the other two markers from chromosome 5 and was not grouped by TetraploidMap with any other scored markers.

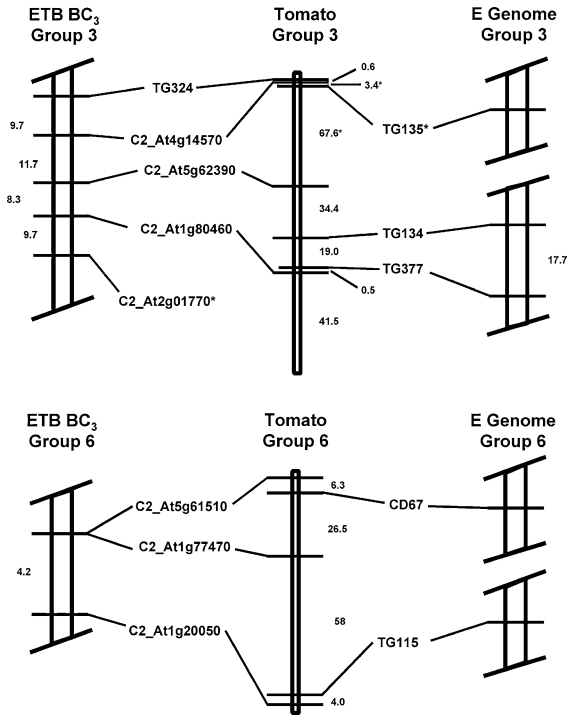

Fig. 3 Linkage maps generated by TetraploidMap of *S. etuberosum* synteny groups 3 and 6 in the BC_3 progeny of a somatic hybrid cross between *S. etuberosum* and a *S. tuberosum* haploid × *S. berthaultii* hybrid (Novy and Helgeson 1994b) determined with CAPs markers. The maps generated by this study are on the *left*, with the published tomato map of these markers (Wu et al. 2006) in the *center*, and the E-genome map (Perez et al. 1999) on the *right*. Comparison between the maps indicates potential consolidation of unlinked sections of chromosomes 3 and 6 in the E-genome (Perez et al. 1999). C2_At2g01770, which is from the tomato map of chromosome 4, produced a secondary amplicon polymorphism that segregated with markers from chromosome 3

Discussion

COSII markers (Wu et al. 2006) were used for the majority of the marker analysis of the current study. A previous study with this material had used mainly RFLP markers and SSR markers developed in tomato (Gillen and Novy 2007). In the current study, PCR-generated markers were emphasized due their advantages over RFLP markers with respect to ease of use and applicability to marker assisted selection, with lesser amounts of DNA required relative to RFLPs—an advantage when extracting DNA from breeding populations having large numbers of individuals. SSR markers were initially evaluated, but many of them did not amplify well from the *S. etuberosum* parent. Unlike the SSRs evaluated in this and the previous study (Gillen and Novy 2007), the primers for COSII markers were designed based on a consensus

sequences across several species (Wu et al. 2006). This design strategy likely enabled the high level of success attained at evaluating *S. etuberosum* specific polymorphisms with COSII markers in the current study. The size of the *S. etuberosum*-derived, BC_3 population used in this study did not appear to have impacted the grouping and ordering of the COSII markers relative to tomato (Figs. 2, 3). With few exceptions, the majority of markers not associated with putative translocations showed good concordance in both grouping and order with the tomato map—such concordance would not have been expected if the population size used for mapping Rlr_{etb} had been inadequate for this purpose.

The loss of *S. etuberosum* specific marker fragments in the BC_3 population indicates that recombination is taking place between *S. etuberosum* and *S tuberosum* chromosomes supporting previous reports of recombination in the BC_2 by Gillen and Novy (2007). This is in agreement with previous studies of *S. etuberosum* and *S. palustre* crosses with tuberous *Solanum* species that indicate recombination takes place in spite of a bias against homeologous pairing (McGrath et al. 1996; Ramanna and Hermsen 1982; Williams et al. 1993). Williams et al. (1993) observed a lack of recombination in chromosomes 4 and 9 of *S. palustre* with *S. tuberosum*. However, despite the observed structural differences between chromosomes 4 and 9 of *S. etuberosum* and *S. tuberosum* in the present study, the ideograms for chromosome 4 of the BC3 in Fig. 1 depict single and multiple cross-over events are occurring between the distinctly different E- and A-genomes of the two species. This suggests that Rlr_{etb} can be integrated into the genome of cultivated potato, and that there is potential for marker assisted selection of Rlr_{etb} in potato breeding programs. However, observed discrepancies from a 1:1 ratio in chromosomes 3 and 7 and may indicate a potential bias toward loss of chromosome 3 and retention of chromosome 7 of *S. etuberosum*.

Translocations between chromosomes 4 and 9 of the E-genome were not reported in the published map of the E-genome (Perez et al. 1999). However, only markers from the center of the tomato map of chromosome 9 were evaluated in that study, which may have been too distant to detect this translocation event. Also, unlike the present study, markers used in chromosomes 3 and 6 of the published E-genome map were not able to be consolidated into single groups (Perez et al. 1999). A reduced level of recombination between chromosomes 3 and 6 of *S. etuberosum* and *S. tuberosum* may have allowed detection of these groups in the present study. Perez et al. (1999) reported greater recombination values in the E-genome compared to the A-genome which would make linkage between distant markers more difficult to detect. Also, the use of COSII markers, not yet identified and mapped at the time when the E-genome map was published, also provided additional saturation of chromosomes 3 and 6 allowing consolidation of previously separate synteny groups.

This study has identified a COSII marker, C2_At1g42990, at a genetic distance of 13.6 cM from Rlr_{etb}—an improvement in linkage relative to RFLP marker TG443 at a distance of 24 cM. Our attempts at identifying markers more closely linked to Rlr_{etb} for use in marker assisted selection led to the identification of translocations among chromosomes 4 and 9 of the E-genome of *S. etuberosum* relative to the A-genome of *S. tuberosum*. Translocations between chromosomes 4 and 9 of the E-genome had not previously been reported and support previous reports of structural differentiation between the two genomes (Perez et al. 1999). Translocations between chromosomes 4 and 9 have confounded our efforts to develop molecular markers more tightly linked to Rlr_{etb}. However, a larger population of 115 BC_4 individuals has been developed and was screened for response to infection by PLRV in 2008. This BC_4 population with its larger number of individuals will be characterized using additional mapped markers from candidate chromosomes thought to be associated with chromosome 4 of *S. etuberosum* via translocations. This approach should allow for the further mapping of the location of Rlr_{etb} and the identification of molecular markers applicable for use in marker assisted selection for this unique source of PLRV resistance.

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