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S.K. Naess · J.M. Bradeen · S.M. Wielgus
G.T. Haberlach · J.M. McGrath · J.P. Helgeson

Resistance to late blight in *Solanum bulbocastanum* is mapped to chromosome 8

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Abstract Somatic hybrids between potato and *Solanum bulbocastanum*, a wild diploid (2n=2x=24) Mexican species, are highly resistant to late blight, caused by *Phytophthora infestans*. Both randomly amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers that are closely linked to the resistance have been noted by analysis of three different backcross-2 populations derived from two different somatic hybrids. With reference to previously published potato and tomato maps, resistance appears to be on the long arm of chromosome 8 and is flanked by RFLP markers CP53 and CT64. In a population of BC_2 plants derived from a cross between the BC_1 line J10lK6 [(*S. tuberosum* PI 203900+*S. bulbocastanum* PI 243510) ×Katahdin)]×Atlantic, late blight resistance cosegregated with RFLP marker CT88 and RAPD marker OPG02–625.

Key words *Solanum bulbocastanum* · Late blight · *Phytophthora infestans* · Somatic hybrid

Introduction

Late blight resistance is once again demanding the attention of potato breeders worldwide following recent migrations of aggressive metalaxyl-resistant isolates of *Phytophthora infestans* into potato production areas. Crop failures, reduced yields, storage losses, and increased costs of crop protection, both to the grower and to the environment, have resulted from these migrations (Fry and Goodwin 1997). Because no widely grown U.S. potato cultivar has adequate resistance to late blight, the development of high-quality, late blight-resistant potato cultivars is clearly needed.

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S.K. Naess · J.M. Bradeen · S.M. Wielgus · G.T. Haberlach
J.M. McGrath · J.P. Helgeson (✉)
USDA/ARS Plant Disease Resistance Research Unit,
Department of Plant Pathology, University of Wisconsin,
Madison, WI 53706, USA
e-mail: JPH@plantpath.wisc.edu
Fax: +1-608-262-1541

There are several sources of late blight resistance available to the potato breeder, particularly among Mexican *Solanum* species which co-evolved with *Phytophthora infestans* (van Soest et al. 1984). *Solanum demissum*, a Mexican hexaploid species, has been used extensively in potato breeding programs. Much of the resistance to late blight in this species is due to a series of race-specific resistance (R) genes. Unfortunately, the pathogen is able to overcome this resistance when presented with the few R genes introgressed into the genome of present-day potato cultivars (Wastie 1991).

A potentially more durable race-non-specific resistance is present in many of the diploid Mexican species, notably *S. bulbocastanum* (Neiderhauser and Mills 1953). These species are largely sexually incompatible with potato due to differences in endosperm balance numbers (EBN, Johnston et al. 1980). Ploidy manipulations and a series of bridge crosses have been successfully used to effect hybridization between potato and *S. bulbocastanum* (Hermsen and Ramanna 1969; 1973; Ramanna and Hermsen 1971; Hermsen and De Boer 1971). However, reconstituting the recurrent potato parent from the resulting quadruple species hybrids has been difficult and late blight-resistant cultivars have not yet been obtained.

More recently, somatic hybridization has been used to overcome sexual incompatibility between potato and *S. bulbocastanum* (Austin et al. 1993; Thieme et al. 1997). Late blight resistance has been recovered in hexaploid somatic hybrids and has been passed on undiminished to progeny of the first and second backcrosses to potato (Helgeson et al. 1998; Douches et al. 1997). In this paper we describe the mapping of late blight resistance from *S. bulbocastanum* to chromosome *8*.

Materials and methods

Plant material

Plants examined in these experiments were BC_1 and BC_2 progenies descendant from somatic fusion products between *Solanum bulbocastanum* PI 243510 (2n=2x=24) and *S. tuberosum*, PI

23900 (2n=4x=48) (Helgeson et al. 1998). For convenience, the clones of *S. bulbocastanum* and *S. tuberosum* used in this study are designated PT29 and R4, respectively. The BC_1 progenies were obtained from crosses between three different somatic hybrids (J101, J103, and J138) and the *S. tuberosum* cultivars Katahdin (KAT), or Atlantic (ATL). Subsequently, BC_2 progenies were obtained from crosses between selected late blight-resistant BC_1 individuals J101K6 and J101K27 and the potato cultivars ATL and Norland (NOR), respectively. A third progeny group (J103K7 ×A89804-7) was provided by Dr. Joe Pavek, Aberdeen, Idaho. These three BC_2 populations are designated 1K6, 1K27 and LB1, respectively.

Resistance screening

Plants were screened for late blight resistance in greenhouse facilities at the University of Wisconsin Biotron. Five replicates of clonally propagated, 20- to 30-cm plants were arranged randomly on carts for whole plant assays. Plants were sprayed to run-off with a fine mist of *Phytophthora infestans* sporangial suspension prepared from US-8, type A2, Cornell standard ME 93-A2 (WEF#US930287) cultures maintained on rye A medium. The suspension contained approximately 30000 sporangia/ml and was pre-chilled 4 h at 10°C before use. Relative humidity in the greenhouse was maintained at or above 90%. The temperature was maintained at 23°C during daylight hours (15 h) and dropped to 15°C at night. Foliage blight scores were recorded at 4–5, 7, 10–11, and 14–15 days. A blight scale, with 0 indicating a dead plant and 9 no visible infection, was used to visually rate disease severity. The ratings and the ranges of percentage infections associated with the rating value are as follows: 9, no visible infection; 8, <10%; 7, 11–25%; 6, 26–40%; 5, 41–60%; 4, 61–70%; 3, 71–80%; 2, 81–90% 1, >90%; 0, 100% (dead).

In preliminary field trials, late blight resistance segregated 1:1 in BC_1 progeny of *S. bulbocastanum*+*S. tuberosum* somatic hybrids, indicating that late blight resistance in these plants may be due to a single dominant gene or a tightly linked cluster of genes. The late blight data were therefore recoded from quantitative to qualitative for mapping purposes (Table 1). BC_2 clones with average resistance scores of 8.0 or above (10% or less leaf infection) were scored as resistant and those with a resistance score of 6.9 or below (>25% infection) as susceptible. Clones with an average resistance score of 7.0–7.8 were omitted from the initial analysis (5/50, 3/54 and 2/69 individuals in the 1K27, 1K6 and LB1 populations, respectively. The recoded resistance data were included in the MAPMAKER random amplified polymorphic DNA (RAPD) mapping data sets.

DNA extraction

For RAPD analysis, DNA was extracted from single leaves of in vitro-grown material by the miniprep method described in McGrath et al. (1994). A microprep DNA extraction protocol was used when larger quantities of DNA were required for restriction fragment length polymorphism (RFLP) analysis (Fulton et al. 1995).

RAPD analyses

Amplification reactions were carried out in 25-µl reaction mixtures containing 10 m*M* TRIS-HCl, pH 8.3, 50 m*M* KCl, 2 m*M* $MgCl_2$, 0.01% gelatin, 100 µ*M* of each dNTP, 200 n*M* primer (OPERON, Alameda, Calif.), approximately 15 ng template DNA and 1 Unit Amplitaq DNA Polymerase (Perkin Elmer, Foster City, Calif.) using a Perkin-Elmer model 480 thermocycler. The cycling program consisted of an initial 2 min denaturation step at 94°C, followed by 3 cycles of 94°C (1 min), 35°C (1 min), and 72°C (2 min) then by 32 cycles of 94°C (30 s), 35°C (30 s), 72°C (1 min), and a final 5-min extension step at 72°C (McGrath et al. 1996). Amplification products were size-separated on a 1% Synergel (Diversified Biotech, Boston, Mass.) 0.6% agarose gel, stained with ethidium bromide and visualized on a UV transilluminator. RAPD markers were grouped into synteny groups and the synteny groups assigned to chromosomes with chromosome-specific RFLP probes as described in McGrath et al. 1996.

RFLP analyses

DNA samples (10 µg) were digested with *Eco*Rl, *Hin*dlll, *Eco*RV, or *Dra*l at a DNA/enzyme ratio of 7 units per microgram. The fragments were size-separated on 0.8% agarose gels and blotted onto Hybond-N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway, N.J.). Non-radioactive hybridization and detection methods were used following protocols provided by the manufacturer using either the ECL, Gene Images or AlkPhos systems (Amersham Pharmacia Biotech), depending on the probe used. Probes were chromosome-specific tomato genomic and cDNA probes (Tanksley et al. 1992; Bonierbale et al. 1988) as well as potato genomic probes (Gebhardt et al. 1989, 1991). Enzyme/probe combinations which gave useful polymorphisms between the potato and *S. bulbocastanum* genomes are as follows:

chromosome 1: TG301/*Hin*dIII, TG17/*Eco*RI, TG27/*Eco*RI;
chromosome 2: TG31/*Eco*RV, CT75/*Eco*RV, TG34/*Hin*dIII, GP504/*Eco*RI, TG449/*Eco*RI;
chromosome 3: TG56/*Eco*RI, TG130/*Hin*dIII, TG134/*Eco*RI, CT243/*Hin*dIII;
chromosome 4: TG123/*Eco*RI, TG65/*Eco*RI, TG450/*Eco*RI;
chromosome 5: CD64/*Hin*dIII, TG23/*Hin*dIII, TG185/*Hin*dIII;
chromosome 6: TG231/*Eco*RI, CT83/*Hin*dIII, TG25/*Eco*RI, TG275/*Eco*RI, TG115/*Eco*RI;
chromosome 7: TG128/*Eco*RI, TG61/*Eco*RI, TG272/*Eco*RV;
chromosome 8: TG176/EcoRI, GP245(GP170)/*Hin*dIII, TG45/*Dra*I, TG41/*Eco*RV, GP171/*Dra*I, CT245/*Hin*dIII, CP53/*Eco*RV, PPO/*Hin*dIII, TG495/*Eco*RV, CT88/*Eco*RI, TG478/*Hin*dIII, CT64/*Hin*dIII, TG261/*Eco*RI, GP301/*Eco*RI, CT148/*Hin*dIII, CT265/*Eco*RI, CT252/*Eco*RI, CT68/*Eco*RI;
chromosome 9: TG18/*Eco*RV, TG35/*Hin*dIII, TG404/*Eco*RV, CT198/*Eco*RI;
chromosome 10: TG122/*Hin*dIII, TG52/*Eco*RV, CT20/*Hin*dIII, CT124/*Eco*RI, TG63/*Hin*dIII;
chromosome 11: GP125/*Eco*RI, CT168/*Eco*RV, CT55/*Eco*RV, TG57/ *Hin*dIII, TG26/*Eco*RI;
chromosome 12: TG68/*Hin*dIII, CT211/*Eco*RV, TG28/*Eco*RV, CD2/*Eco*RI.

In addition, two RAPD fragments, G02-575 and P09-550, of *S. bulbocastanum* origin were cloned for use as RFLP probes (see below).

Sequence characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) markers

The Original TA Cloning Kit (INVITROGEN, Carlsbad, Calif.) was used to clone RAPD fragments of interest. RAPD fragments were first reamplified using a band-stab technique (Bjourson and Cooper 1992), and 1-µl aliquotes of the reamplification reactions were ligated into the pCR2.1 vector following instructions provided by the manufacturer.

An ABI automatic sequencer (Perkin Elmer) was used to sequence plasmid inserts using fluorescent dye terminators. New primers were designed from sequences internal to the original Operon primer annealing site. Primer sequences designed from the P09–550 fragment were AGG TGG TGG TGG GGT GGG ATA GTG and GTG GTC CGC ACC CAT ATT TCA CCA.

For SCAR markers based on RFLP probes, primers were first designed based on the published probe sequences (Solgenes database:http://probe.nalusda.gov:8300/cgi-bin/browse/solgenes). These primers, 905/906 (GTT GGG CAG AAG AGC TAG/ TTG CCT TAG TCC CCA GAG) and CT64 forward/reverse (GAG GAG

AGA TTC TTG GAC/ TGA GGT TGA TAG TGG GTG), were used to generate fragments from both fusion parents using low stringency polymerase chain reaction (PCR) conditions (see below). The resulting monomorphic fragments were cloned as described. Sequence differences between the CT88 plasmid inserts derived from the two fusion parents (PT29 and R4) were used to design new primers specific to the resistant parent, PT29 (CT88 forward and reverse: TGA GGC TCT TGG CTT TCG/AAT CAA TTG AGC ATC TTG AGA).

For amplification using locus-specific primers 905/906 and CT88 forward/reverse the $MgCl_2$ and dNTP concentrations were increased to 4 m*M* and 300 µ*M*, respectively. The annealing temperature used with primers 905/906 and CT64 forward/reverse was 55°C and for CT88 forward/reverse it was 60°C. The cycling program consisted of an initial 2-min. denaturation step at 94°C followed by 30 cycles of 94°C for 1 min., an annealing step (55°C or 60°C) for 30 s., and an elongation step at 72°C for 1.5 min. These cycles were followed by a final 5-min extension cycle at 72°C.

For amplification of SCAR marker P09-478 a step-down PCR cycling program was used. The annealing temperature in the above cycling program was varied. In the initial 3 cycles it was 72°C, in the following 3 cycles, 70°C and in the final 25 cycles, 68°C.

For CAPS markers, fragments generated following amplification with CT88 primers 905/906 were cleaved in the same reaction tube with 1 U *Taq*I for 1 h at 65°C. The products were size-separated and visualized as previously described.

Mapping

Markers were mapped using the haploid function of MAPMAKER (Lander et al. 1987). Only markers specific to *S. bulbocastanum* were scored and included in the mapping data set. In using the haploid function for mapping in the BC_2 we are assuming that there is a single copy only of each *S. bulbocastanum* chromosome in the BC_1 parent due to preferential pairing of the *S. bulbocastanum* chromosomes in the somatic hybrid. RAPD markers were grouped at an LOD of 3. Easily scored but unassigned markers were recoded (presence of marker recorded as absence and *vice versa*) to test whether any of these markers were segregating in repulsion to other synteny groups. Only markers that give readily distinguished polymorphisms with potato were included in the maps.

Results

In preliminary field trials, late blight resistance segregated 1:1 in BC_1 progeny of *S. bulbocastanum+S. tuberosum* somatic hybrids, indicating that late blight resistance in these plants may be due to a single dominant gene or a tightly linked cluster of genes. Resistance could be passed on, apparently undiminished, to BC_2 (Helgeson et al. 1998) and BC_3 (Fig. 1) populations. Inspection of the preliminary RAPD data indicated that resistance was associated with one synteny group, later determined to be chromosome 8.

Assignment of resistance to chromosome 8 and linkage to G02-625

Late blight resistance was assessed in three different BC_2 populations. Representative results for one of these populations are given in Table 1. The recoded resistance data were included in the MAPMAKER RAPD mapping data sets. Segregation ratios for resistance did not deviate significantly from 1:1 in the 1K27 or 1K6 populations; respectively 58% and 59% of the individuals in these populations were resistant. Both of these populations were derived from somatic hybrid J101. In the LB1 population, derived from somatic hybrid J103, only 37% of the individuals were resistant, a significant deviation from a 1:1 segregation ratio ($0.01<P\ 1{:}1<0.05$).

Fig. 1 Field resistance to late blight derived from *Solanum bulbocastanum* under intense disease pressure in Toluca, Mexico. BC_3 individual A9509-34 (J101K6A22 X A84118-3) is contrasted with the standard susceptible variety Alpha

Late blight resistance maps to chromosome 8 and is linked to RAPD marker G02-625 in all three BC_2 populations (Fig. 2). In the 1K6 population, resistance cosegregates with G02-625 (Fig. 3, Table 1), and in the 1K27 population a single individual (1/50) is recombinant between resistance and G02-625. There is 9% recombination between resistance and G02-625 in the LB1 population; 5/69 susceptible seedlings have the marker and 1/69 resistant seedling lacks the marker.

In all three populations, resistance maps to the end of the RAPD marker synteny group. However, in the LB1 population markers G02-575 and P09-550 segregate in repulsion to the rest of the RAPD synteny group. These markers, when recoded, flank resistance. The increased recombination rate between G02-625 and resistance in the LB1 population may be due to the presence of portions of both PT29 chromosome 8 homologues in the BC_1 parent of this population.

Chromosome 8 in the BC1

RAPD markers G02-575 and P09-550 are heterozygous in PT29 and segregate in the combined BC_1 populations. This is also true for a number of other chromosome 8 RAPD markers which segregate in the BC_1 but are present in all 3 resistant individuals chosen as parents for the BC_2 populations. Late blight resistance also segregates in the BC_1 and it is therefore possible to map resistance in these populations in relation to other segregating

Table 1 Association of RAPD marker G02-625 with late blight resistance in the 1K6× Atlantic BC_2 population. Resistance scores: 9, no visible infection; 8, <10%; 7, 11–25%; 6, 26–40%; 5, 41–60%; 4, 61–70%; 3, 71–80%; 2, 81–90%; 1>90% defoliation; 0, dead. Late blight scores were recoded to R (<10% defoliation) 0, (11–25% defoliation) or S (>25% defoliation) for qualitative mapping purposes. RAPD marker G02-625 is scored as present (Y) or absent (N)

Clone	N	G02	Code	Score	Clone	N	G02	Code	Score
J101K6A1	5	Y	R	9.0±0.0	J101K6A41	5	Y	0	7.8±0.8
J101K6A4	5	Y	R	9.0±0.0	J101K6A61	5	Y	0	7.4±3.0
J101K6A5	3	Y	R	9.0±0.0	J101K6A29	3	N	0	7.0±1.7
J101K6A6	5	Y	R	9.0±0.0	J101K6A11	3	N	S	5.7±1.5
J101K6A9	5	Y	R	9.0±0.0	J101K6A7	3	N	S	5.3±1.5
J101K6A12	3	Y	R	9.0±0.0	J101K6A18	3	N	S	5.3±2.1
J101K6A13	5	Y	R	9.0±0.0	J101K6A30	3	N	S	3.7±2.5
J101K6A15	4	Y	R	9.0±0.0	J101K6A59	3	N	S	3.7±4.6
J101K6A21	5	Y	R	9.0±0.0	J101K6A34	5	N	S	3.6±3.3
J101K6A28	5	Y	R	9.0±0.0	J101K6A60	5	N	S	3.4±1.8
J101K6A32	5	Y	R	9.0±0.0	J101K6A23	5	N	S	3.2±2.2
J101K6A39	5	Y	R	9.0±0.0	J101K6A33	5	N	S	2.2±2.6
J101K6A44	5	Y	R	9.0±0.0	J101K6A3	5	N	S	2.0±3.9
J101K6A45	5	Y	R	9.0±0.0	J101K6A26	5	N	S	2.0±3.9
J101K6A48	3	Y	R	9.0±0.0	J101K6A10	5	N	S	1.4±1.9
J101K6A49	5	Y	R	9.0±0.0	J101K6A38	5	N	S	1.4±1.7
J101K6A51	5	Y	R	9.0±0.0	J101K6A25	2	N	S	1.0±0.0
J101K6A57	5	Y	R	9.0±0.0	J101K6A54	5	N	S	1.0±1.7
J101K6A70	5	Y	R	9.0±0.0	J101K6A58	5	N	S	1.0±2.2
J101K6A2	5	Y	R	8.8±0.4	J101K6A14	3	N	S	0.0±0.0
J101K6A16	5	Y	R	8.8±0.4	J101K6A24	5	N	S	0.0±0.0
J101K6A19	5	Y	R	8.8±0.4	J101K6A37	5	N	S	0.0±0.0
J101K6A22	5	Y	R	8.8±0.4	J101K6A46	5	N	S	0.0±0.0
J101K6A31	5	Y	R	8.8±0.4	J101K6A50	5	N	S	0.0±0.0
J101K6A17	5	Y	R	8.4±0.5			Parental materials		
J101K6A27	5	Y	R	8.4±1.3					
J101K6A42A	5	Y	R	8.4±0.5	R4 no. 2	5	N	S	0.6±0.9
J101K6A42B	5	Y	R	8.4±1.3	PT-29	5	Y	R	9.0±0.0
J101K6A40	5	Y	R	8.2±1.8	J101	5	Y	R	8.4±0.9
J101K6A47	5	Y	R	8.2±1.1	Katahdin	5	N	S	3.4±1.8
					J101K6	5	Y	R	9.0±0.0
					Atlantic	5	N	S	1.8±1.8

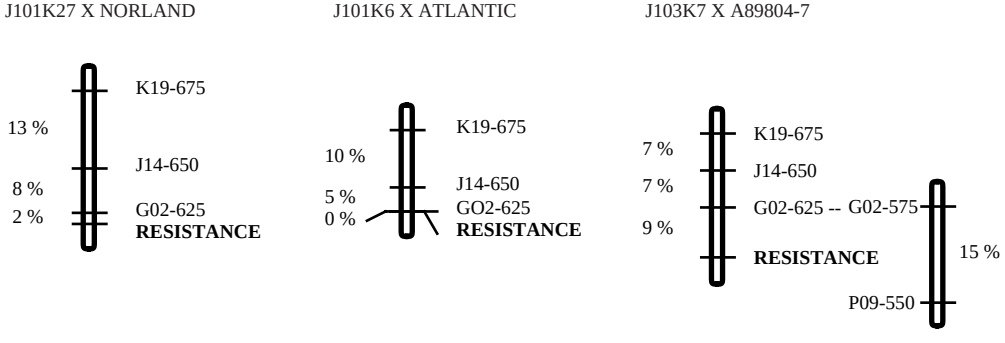

Fig. 2 Association of late blight resistance with RAPD marker G02-625 on chromosome 8 in three different BC_2 progenies. Marker name given by Operon primer followed by estimated marker size in base pairs. Presumed allelic markers are *linked* with a *dotted line*

markers previously mapped in the three BC_2 populations.

For initial considerations, we have assumed that the 3 somatic hybrids are identical with respect to chromosome 8 and have combined data from all BC_1 progeny. In this combined group of 103 individuals, recombination between resistance and G02-625 is 11.5%. P09-550 is linked in repulsion to resistance and, when recoded, maps to the other side of resistance as it does in the LB1s (Fig. 4). Recombination between K19-675 and G02-625, 2 RAPD markers that are common to all populations examined, is 28% in the BC_1 but only 26%, 17%, and 13% in the BC_2 1K27, 1K6, and LB1 populations. Thus, there appears to be some repression of recombination in the BC_2 populations relative to the BC_1 populations.

Expansion of the chromosome 8 molecular map with RFLP markers

Molecular marker coverage of *S. bulbocastanum* chromosome 8 was expanded through RFLP analysis of 64 BC_2 individuals in the 1K6 population. For this, chromo-

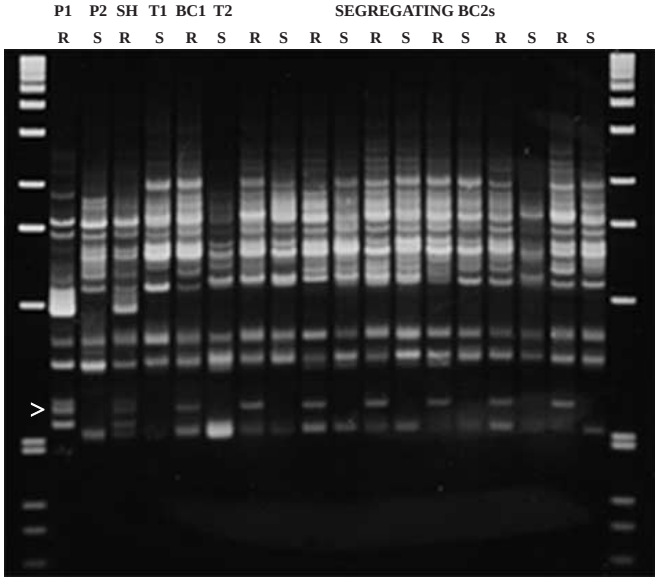

Fig. 3 Cosegregation of RAPD marker G02-625 with late blight resistance in the 1K6 BC_2 population. *R* and *S* Respectively late blight resistant and susceptible individuals, *P1 Solanum bulbocastanum* fusion parent PI 243510 clone PT29, *P2 S. tuberosum* fusion parent PI 23900 clone R4, SH somatic hybrid J101, *T1 S. tuberosum* backcross parent Katahdin, *BC1* BC_1 parent J101K6, *T2 S. tuberosum* backcross parent Atlantic

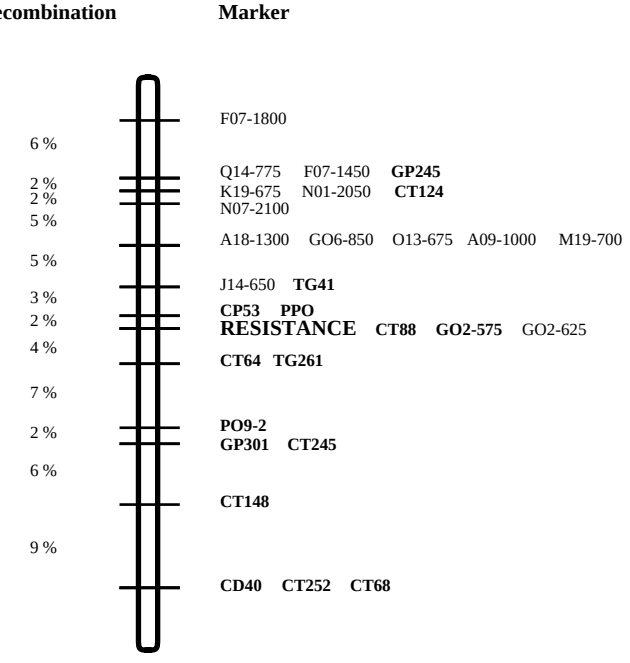

Fig. 5 Combined RAPD, RFLP marker coverage of chromosome 8 based on 64 BC_2 individuals from the cross between J101K6 and Atlantic. RFLP markers and resistance are in *bold*

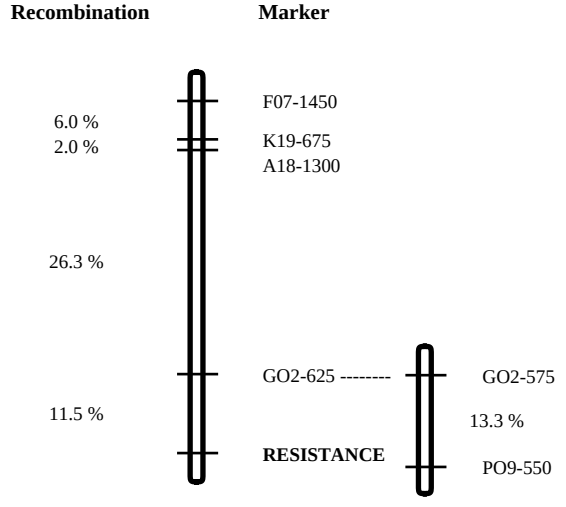

Fig. 4 Association of late blight resistance and RAPD marker G02-625 on chromosome 8 in the combined BC_1 progeny. Marker name given by Operon primer followed by estimated marker size in base pairs. Presumed allelic markers are *linked* with a *dotted line*

some 8-specific tomato cDNA and genomic probes, potato genomic probes, and probes derived from RAPD fragments were used. In this population late blight resistance cosegregates with RFLP marker CT88 and is flanked by RFLP markers CT64 and CP53 (Fig. 5).

RAPD markers G02-575 and P09-550 from the LB1 population were cloned and converted to RFLP probes which hybridize to informative fragments in the 1K6 population. RFLP marker G02-575 cosegregates with late blight resistance in the 1K6 population, as has been previously noted for RAPD marker G02-625. RFLP marker P09 maps distal to resistance, as anticipated from the position of the recoded RAPD marker in the BC_1 and LB1 population. Recombination between resistance and RFLP marker P09 in this population is 10%.

Most of the RFLP probes used in this study are tomato cDNA clones and have not been included on previously published potato maps. Therefore, we turned to the tomato map (Tanksley et al. 1992) in addition to potato maps for useful comparisons. Colinearity between tomato chromosome 8 and *S. bulbocastanum* chromosome 8 appears to be conserved with only a few differences. TG176 which maps distal to TG41 in both the tomato and potato chromosome 8 maps, maps to chromosome 1 in the 1K6 population as it does in another BC_2 population derived from a different *S. bulbocastanum* accession (Brown et al. 1996). CT245 maps to two different locations on the tomato chromosome 8; 1 marker cosegregates with TG176 and the other maps between TG41 and PPO. In the 1K6 population, CT245 cosegregates with RFLP marker GP301 and is flanked by markers P09-2 and CT148. CD40, which maps between RFLP markers TG176 and TG41 in tomato, cosegregates with CT252 and CT68 at the other end of the chromosome in the 1K6 population. Finally, CT124, a probe specific for chromosome 10 in tomato, hybridizes to several restriction fragments in the 1K6 population, one of which maps to chromosome 10 and the other to chromosome 8. None of the discrepancies between marker synteny or order involved more than 1 consecutive marker. As all of these probes hybridize to several restriction fragments in the 1K6

population it is possible that the fragments scored are non-orthologous loci and do not represent actual differences in colinearity between tomato and *S. bulbocastanum*.

Recombination rates between markers common to the tomato, potato, and 1K6 populations are lower in the 1K6 population than in tomato but are comparable to those in the potato map (Tanksley et al. 1992). The genetic distances between markers PPO and TG261, which flank late blight resistance in the 1K6 population, are 12.6 cM, 5.8 cM, and 2.6 cM, respectively, in the tomato, 1K6, and potato maps. In the 1K6 population the distance between RFLP markers CT88 and CT64 is inflated relative to the tomato map (3.9 cM vs 1.4 cM), perhaps due to the small size of the population examined. In an expanded BC_2 population (J101K6×NOR) of 273 individuals with the same maternal parent, recombintion between CT88 and CT64 is only 0.8% (data not shown).

With the addition of RFLP markers to the RAPD map it becomes clear that the RAPD markers are not randomly distributed along the chromosome. Of the 14 RAPD markers mapped to chromosome 8, 11 map to an area flanked by RFLP markers GP245 and TG41, an area of only 13 cM in our map. Only 3 RAPD or RAPD-derived markers map to the region of chromosome 8 stretching from RFLP marker TG41 to CT68, an area of 33 cM in our map.

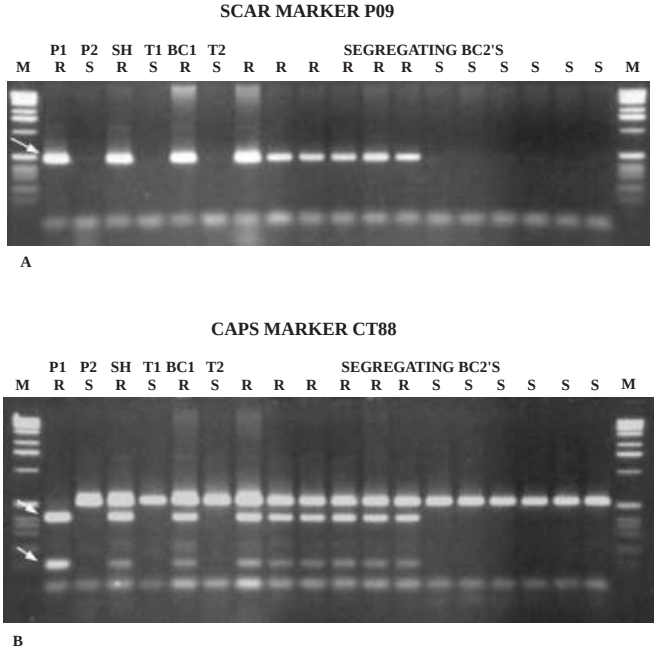

Fig. 6 PCR-based markers generated from RAPD marker P09 and RFLP marker CT88. In both panels P1 is the resistant (*R*) fusion parent *S. bulbocastanum* PI243510 clone PT29, and P2 is the susceptible (*S*) fusion parent *S. tuberosum* PI203900 clone R4, SH somatic hybrid, *T1* and *T2* are, respectively, the *S. tuberosum* backcross parents Katahdin and Atlantic, BC_1 resistant backcross parent J101K6. **A** SCAR marker P09, **B** CAPS marker CT88. *Arrows* mark the restriction products generated following digestion of the PCR product with *Taq*I

SCAR and CAPS markers

PCR-based markers were designed from a RAPD marker linked in repulsion to late blight resistance and from 2 RFLP markers linked to resistance. Primers complementary to the sequence of RAPD marker P09-550 amplified a single fragment 478 bp in length which was specific to *S. bulbocastanum* (Fig. 6). These primers generate informative markers in the 1K6 population, which lacks RAPD marker P09-550. They can be used to detect recombination between RAPD marker G02-625 and SCAR marker P09 which flank resistance in other populations.

Primers designed from the end sequences of tomato probes CT64 and CT88 amplified monomorphic fragments from *S. bulbocastanum* and the susceptible potato fusion parent. The CT88 *S. bulbocastanum* fragment includes a *Taq*I restriction site absent in the potato fragment. Primers designed around nucleotide differences in the two sequences were not specific enough for the generation of informative SCAR markers. We were much more successful with CAPS for this marker, using the primers designed from the end sequences of the tomato probe, which flank the polymorphic *Taq*I site, to amplify monomorphic fragments from all samples and cleaving the resulting amplification product with *Taq*I (Fig. 6).

The CT64 fragments also contained a polymorphic *Taq*I restriction site. However, in this case the *S. bulbocastanum* fragment lacks a site present in the *S. tuberosum* fusion and backcross parents. To take advantage of this polymophism we included *Taq*I, at 1 U per 25 µl of reaction, in the PCR reaction mix, and the tubes were incubated at 65°C for 15 min prior to amplification. Only the fragment specific to *S. bulbocastanum* is amplified following digestion with *Taq*I.

Discussion

The development of late blight-resistant potato varieties has re-emerged as a major objective of potato breeders around the world. Obstacles to sexual hybridization between potato and the late blight resistant wild species *Solanum bulbocastanum* have been overcome through somatic hybridization, and late blight resistance from this species has been recovered in somatic hybrids, BC_1, BC_2, and BC_3 progeny. This resistance appears to be race non-specific; it provides sufficient resistance not only to the US8 genotype of the pathogen but also to many races of *Phytophthora infestans* present in Toluca, Mexico (Fig. 1) where numerous genotypes of the pathogen have been isolated from resistant plants (Helgeson et al. 1998; Grunwald personal communication). Nevertheless, much work remains to be done before introgression of late blight resistance from *S. bulbocastanum* into the potato genome is complete, and the goal of late blight resistant potato cultivars suitable to industry is realized.

In this study, genome coverage was sufficient to find RAPD markers tightly linked to late blight resistance. By mapping resistance in several different populations and recoding unassigned markers to detect possible repulsion phase linkages we were able to find 2 RAPD markers, G02-625 and P09-550, which flank the resistance locus in *S. bulbocastanum*.

Late blight resistance mapped to the RAPD synteny group assigned to chromosome 8. Further expansion of the map with RFLP markers places resistance between RFLP markers CT64 and CP53. This area of chromosome 8 has not previously been associated with late blight resistance in potato or tomato. Many mapping studies on late blight resistance in potato have been conducted on *S. demissum*-derived materials, and these have indicated that a number of chromosomes are involved. Several of the R genes from this species have been mapped, including R1 on chromosome 5 (Leonards-Schippers et al. 1992), R2 on chromosome 4 (Li et al. 1998) and R3, -6 and -7 on chromosome 11 (El Kharbotly et al. 1996).

Quantitative trait loci (QTLs) associated with late blight resistance have also been mapped in potato. These may be more relevant to late blight resistance from *S. bulbocastanum*, which is not governed by R genes (Helgeson et al. 1998). Leonards-Schippers et al. (1994) found QTLs associated with late blight resistance on 9 of 12 chromosomes in a potato mapping population segregating for race-non-specific resistance. None were found on chromosome 8. Meyer et al. (1998) have also mapped QTLs associated with late blight resistance in a potato population segregating for race non-specific resistance. Late blight resistance in their population was previously reported to be located opposite the waxy locus on chromosome 8 but has now been mapped to the short arm of chromosome 4 near microsatellite STM 3016 (De Jong, personal communication). This marker is located between RFLP markers GP180 and GP172 (Milbourne et al. 1998), the same area to which a major QTL for late blight resistance, *Pi1a*, maps in the Leonard-Schippers et al. (1994) population. The race-non-specific tomato late blight resistance gene, *Ph-2*, maps to a single location on chromosome 10 (Moreau et al. 1998).

Late blight resistance in *S. bulbocastanum* does not appear to be due to a large number of QTLs on different chromosomes. Quantitative resistance data from the J101K6×ATL BC_2 population (Table 1) have been analyzed using MAPMAKER QTL (Unix version 1.9) and a nonparametric procedure (Mann-Whitney U test). These analyses identified only one major chromosomal region associated with late blight resistance, the same area on chromosome 8 previously identified by MAPMAKER (data not shown). Sixty-two percent of the observed variability in disease resistance is explained by this chromosomal region.

Several plant defense-related genes have been located on chromosome 8, including the PPO gene which is involved in Colorado potato beetle resistance in *S. berthaultii* (Bonierbale et al. 1994). In our populations, however, PPO from *S. bulbocastanum* cannot be responsible for late blight resistance. We have found resistant individuals lacking *S. bulbocastanum*-derived PPO genes and susceptible individuals with these genes. Leister *et al.* (1996) mapped numerous PCR-derived resistance gene analogs obtained from genomic potato DNA to the 12 potato chromosomes, including chromosome 8. *Solanum tuberosum* fragment St3.4e maps to the same 25-cM region, flanked by markers GP40 and GP36a, as the *S. bulbocastanum*-derived late blight resistance studied here. Although the sequence of many of the PCR-derived probes obtained were related to sequences of known plant resistance genes, St3.4 did not show homologies to known genes (Leister et al. 1996). The St3.4 fragment is multicopy in the potato genome and has as yet not been mapped in our populations. Whether or not St 3.4 is related to late blight resistance in *S. bulbocastanum* remains to be seen.

Suppression of recombination in introgression segments can lead to linkage drag persisting through many backcross generations (Young and Tanksley 1989). There is evidence of suppression of recombination in the BC_2 populations examined as well as in a larger BC_3 population (preliminary data not shown). Marker-assisted selection of parental material will therefore be crucial to the success of the backcross program. Several late blight-resistant individuals from the BC_2 populations are missing all markers from 6 or more *S. bulbocastanum* chromosomes, a reduction in the wild species genome similar to that obtained through asymmetric somatic hybridizations (Oberwalder et al. 1998). These individuals, as well as those resistant individuals recombinant for chromosome 8, should be used to advantage as parental materials for future BC_3 progenies.

The generation of larger progeny populations will be necessary to compensate for the suppression of recombination in the introgressed segments. Locus-specific PCR-based markers are needed to increase the efficiency of screening these progeny for those rare late blight-resistant individuals recombinant in the introgressed segment. SCAR and CAPS markers linked to late blight resistance in *S. bulbocastanum* have been developed for this purpose from 3 markers, P09–550, CT64 and CT88.

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