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ORIGINAL PAPER

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Transfer of tuber soft rot and early blight resistances from *Solanum brevidens* into cultivated potato

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Abstract Tuber soft rot and early blight are serious potato diseases. Development of potato varieties resistant to these diseases has been hindered by the scarcity of resistant germplasm. A diploid wild species, *Solanum brevidens*, shows significant resistance to both diseases. Numerous potato breeding lines have been developed from a potato-*S. brevidens* somatic hybrid, A206. A BC_3 clone, C75-5+297, derived from this somatic hybrid as well as its BC_1 and BC_2 parental lines showed resistance to both tuber soft rot and early blight. Clone C75-5+297 has consistently out-yielded common varieties under disease stress. Using both molecular and cytogenetic approaches we demonstrated that a single copy of chromosome 8 from *S. brevidens* replaced a potato chromosome 8 in C75-5+297. Thus, C75-5+297 represents a potato-*S. brevidens* chromosome substitution line. Our results suggest that the presence of a single chromosome from *S. brevidens* may significantly impact the resistance to multiple potato diseases. The high yield potential of C75-5+297 makes it an excellent parent for developing potato varieties with resistances to both tuber soft rot and early blight.

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Introduction

Potato (*Solanum tuberosum*) encounters a number of serious diseases. Most modern potato cultivars are susceptible to multiple diseases. Wild *Solanum* species have provided invaluable genetic diversity for disease resistance and other agronomic traits for potato breeders (Jansky 2000; Pavek and Corsini 2001). One of the most important potato diseases is late blight, caused by the oomycete *Phytophthora infestans*. At least 11 late blight resistance genes have been introduced from *Solanum demissum* ($2n=6x=72$) into potato (Umaerus and Umaerus 1994). Durable and broad-spectrum resistance against all known races of *P. infestans* was found in a wild diploid species *Solanum bulbocastanum* (Helgeson et al. 1998; Naess et al. 2000, 2001). A major resistance gene to late blight, *RB*, has been successfully cloned from *S. bulbocastanum* and is expected to become an important resource for late blight resistance breeding (Song et al. 2003).

Both tuber soft rot and early blight are serious potato diseases. Resistance to potato tuber soft rot, caused by *Erwinia carotovora*, has not been fully achieved in modern potato cultivars, though some low levels of tolerance have been found (Zimnoch-Guzowska et al. 2000; McGrath et al. 2002). A high level of resistance has been observed only in the exotic potato germplasm. Zimnoch-Guzowska et al. (2000) demonstrated that resistance to tuber soft rot is involved in a complex inheritance pattern and is associated with a number of genes located on all 12 potato chromosomes. These factors have increased the difficulty of breeding resistant varieties.

Potato early blight is caused by the fungus *Alternaria solani* and is an important potato disease worldwide (Jansky 2000). The estimated cost of fungicide application to control early blight exceeds $44 million during a typical potato growing season in North America (Stevenson 1994). One of the drawbacks of searching for resistance to early blight is the strong correlation with late vegetative maturity (Boiteux et al. 1995). Currently, strategies for early blight management include a combination of cultural and chemical practices to avoid

the impact of the disease on yield and tuber quality (Stevenson 1994).

S. brevidens is a non-tuber-bearing wild diploid species that shows a high level of resistance to a number of potato diseases (Helgeson et al. 1985; 1993). A somatic hybrid, A206, between potato and *S. brevidens* was developed by protoplast fusion (Austin et al. 1986). Here we report the tuber soft rot and early blight resistance of a BC_3 clone and its parental lines, which were derived from the somatic hybrid A206. We demonstrated that the *S. brevidens*-derived materials are valuable germplasm resources for breeding resistance to tuber soft rot and early blight.

Materials and methods

Plant materials

Somatic hybrid A206 was generated through protoplast fusion between the cultivated potato clone PI 203900 (2*n*=4*x*=48) and *S. brevidens* PI 218228 (2*n*=2*x*=24) (Austin et al. 1986). Plants C75, C75-5 and C75-5+297 are backcrossed progenies derived from A206 (Fig. 1). Potato varieties Katahdin and Atlantic as well as *S. brevidens* clone PI 218228 were used in DNA analysis.

Disease resistance evaluation

Soft rot resistance was assayed as described by McGrath et al. (2002). In brief, tuber plugs were inoculated individually with *E. carotovora* spp. *carotovora*. After 2–3 days, visual and physical inspections were performed on the plugs to evaluate the bacterial progression. Visual inspections were based on rot development, spreading and deformation of the plugs. The plugs were split and the length (mm) of the rotted tissue was also measured to evaluate the disease development. Resistance was rated as RR, R, R-, S and SS, from highly resistant to highly susceptible.

A special field trial is conducted annually at the Hancock Agricultural Research Station, Hancock, Wisconsin, to evaluate the early blight resistance of potato breeding lines. In tests of the materials under study here, small whole tubers or hand-cut seed pieces were mechanically planted in a randomized complete block design with three replicates. Each replication consisted of a 150-cm section of row for every clone. Test plots were separated within the row by 120-cm sections of Red Norland. Red Norland, a variety highly susceptible to early blight, was used to provide a uniform source of inoculum throughout the trial. Spacing was 30 cm within the row and 90 cm between the rows. Plots were rated weekly during the growing season for early blight severity, using a Horsfall-Barratt 0–11 scale where 0= no disease and 11=100% of foliage exhibiting symptoms. Relative area under the disease progress curve (relative AUDPC) is used as a measure of the relative severity of disease throughout the growing season and calculated from the data obtained weekly during the growing season. A relative AUDPC value of 1.0 indicates 100% foliage infection.

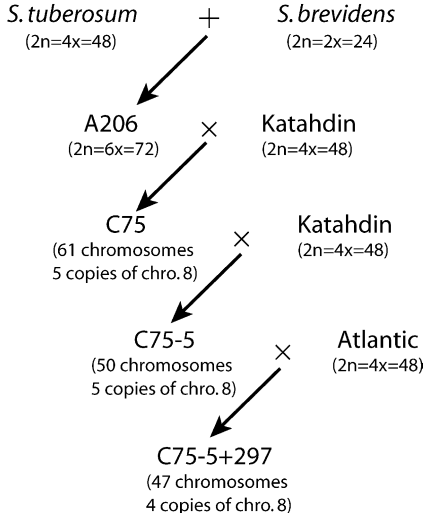

Fig. 1 The pedigree of tuber soft rot and early blight resistant clone C75-5+297 and its parental lines. Potato varieties Katahdin and Atlantic were used as backcross parents of the somatic hybrid A206. The total chromosome number and the copy numbers of chromosome 8 are indicated. + Somatic hybridization, *x* sexual hybridization

Restriction fragment length polymorphism analysis

Restriction fragment length polymorphism (RFLP) markers GP245 and GP301 were mapped to the short and long arms, respectively, on potato chromosome 8 (Gebhardt et al. 2001). These two probes were used to verify the presence of *S. brevidens* chromosome 8. Genomic DNAs were isolated from leaf tissue and digested with restriction enzymes *Eco*RV or *Hin*dIII, run on a 0.8% agarose gel and transferred onto Hybond-N^+ membrane (Amersham Biosciences, Piscataway, N.J.). Prehybridization and hybridization reactions were carried out in 5× SSC, 0.5% SDS, 0.02 M $NaPO_4$ (pH 6.5), 2 mM EDTA, 10 mM Tris (pH 7.4) and 0.02% denatured salmon sperm DNA. The probe was labeled with ^{32}P and hybridized overnight at 65°C. Two washes of 15 min each were sequentially performed at 2× SSC and 1× SSC stringencies. Membranes were exposed to X-ray film.

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) using BAC and genomic DNA probes was performed essentially as described by Dong et al. (2000, 2001). Bacterial artificial chromosome (BAC) clone 220C03, which was mapped to the long arm of potato chromosome 8 (Bradeen et al. 2003), was used as a chromosome-specific cytogenetic DNA marker for the identification of chromosome 8. In brief, root tips were harvested from young plants grown in the greenhouse and pretreated in ice-cold water at 4°C overnight. After fixation in methanol: acetic acid (3:1), the root tips were digested in 2% cellulose and 1% pectolyase at 37°C for 1 h. Squashes of the metaphase chromosomes were prepared in the same fixative and flame dried. DNA probes were labeled with biotin-dUTP (Boehringer Mannheim, Indianapolis, Ind.) and detected by a fluorescein isothiocyanate-conjugated antibiotin antibody (Vector Laboratories, Burlingame, Calif.). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) in a Vectashield antifade solution (Vector Laboratories) and pseudocolored in red. A sequential FISH and GISH (genomic in situ hybridization) procedure (Dong et al. 2001) was used to reveal the specificity of the introgressed *S. brevidens* chromosome in potato background. The order of the hybridizations (first FISH and then GISH) was reversed compared to our published protocols.

Results and discussion

Resistance to tuber soft rot and early blight of clone C-75-5+297 and its parental lines

A number of potato breeding lines derived from the somatic hybrid A206 showed resistance to several potato diseases, including tuber soft rot and early blight. Tuber

soft rot assays indicated that all the parental plants used in the somatic hybridization and backcrosses, with the exception of the wild species *S. brevidens*, are susceptible to soft rot (Austin et al. 1988; Helgeson et al. 1993). Thus the tuber soft rot resistance in the somatic hybrid A206 and its derivative is presumed to be transmitted from *S. brevidens*.

One of the BC_3 clones, C75-5+297, showed high levels of tuber soft rot resistance (Table 1). The parental line of C75-5+297, clone C75-5 (BC_2), showed a medium level of resistance. However, clone C75 (BC_1), as well as A206, showed resistance similar to or better than that of C75-5+297 (Table 1). A transmission study of the tuber soft rot resistance in the backcrossed progenies derived from A206 revealed that several different *S. brevidens* chromosomes may harbor resistance genes against this disease (McGrath et al. 2002). Thus, it will be a challenge to develop advanced backcross lines that maintain the same level of resistance as the somatic hybrid A206.

Clone C75-5+297 and its parental lines also showed considerably better field resistance to early blight than most common potato varieties, including Atlantic, Norland, and Russet Burbank. The early blight resistance of these clones was first observed in 1993 (Table 2) and the resistance of C75-5+297 was confirmed in the following years (Table 3). C75 showed outstanding early blight

Table 1 Mean and standard deviations of visual and physical evaluations of resistance to tuber soft rot. Numbers in parenthesis indicate the number of tuber plugs examined. Visual inspection scores were as follows: *0* no rot, *1* rot limited to the surface, *2* limited to the surface and one side, *3* rot on the surface and sides, *4* wet plug, *5* wet and deformed plug. The penetration scores were as follows: *0* No penetration, *1* less than 5 mm, *2* less than 10 mm, *3* less than 15 mm, *4* less than 20 mm. The plug hardness scores were as follows: *0* Completely soft plug, *1* less than 25% hard, *2* less than 50% hard, *3* less than 75% hard, *4* 100% hard

Clones	Visual inspection (day 2)	Visual inspection (day 3)	Penetration (mm)	Hardness (%)	Score
Katahdin	2.6±0.96 (25)	3.4±1.15 (25)	2.8±1.39 (27)	1.2±1.24 (23)	SS
Atlantic	3.4±0.91 (25)	4.0±0.73 (25)	3.3±1.10 (27)	0.9±0.92 (23)	S
A206	0.3±0.45 (19)	0.2±0.60 (13)	0.0±0.00 (9)	4.0±0.00 (25)	RR
C75	0.8±0.91 (16)	0.9±0.95 (14)	0.5±0.73 (16)	3.7±1.01 (16)	RR
C75-5	2.2±0.96 (19)	2.6±1.16 (19)	2.2±1.33 (21)	2.2±1.36 (19)	R/S
C75-5+297	1.3±1.00 (9)	1.1±0.93 (9)	0.6±0.79 (7)	3.8±0.67 (9)	RR

Table 2 Yield and early blight resistance of backcross clones are contrasted with the commercial varieties. Resistance is based on the percent foliage infection and relative AUDPC in 1993 and 1994. The percent foliage infections in 1993 were measured weekly from 19 July through to 30 August, and a single measurement made on 18 August 2003 is presented for each clone. The percent foliage infections in 1994 were measured weekly from 26 July through to 22 August, and a single measurement made on 26 July is presented for each clone. The relative AUDPC data for 1994 is based on the early season data (26 July), before late blight was seen. *NA* Data not available

Clones	Year 1993			Year 1994		
	Foliage infection (%)	Relative AUDPC	Average yield (ton/ha)	Foliage infection (%)	Relative AUDPC	Average yield (ton/ha)
Russet Burbank	83.0	0.38	39.06	9.1	0.03	31.74
Red Norland	99.8	0.59	26.37	100.0	0.38	NA
Katahdin	82.5	0.39	43.94	NA	NA	NA
Atlantic	90.0	0.38	43.94	15.0	0.04	33.81
A206	66.5	0.28	3.91	NA	NA	NA
C75	23.1	0.14	18.23	11.1	0.03	6.67
C75-5	60.0	0.28	27.99	10.6	0.03	22.87
C75-5+297	60.8	0.27	55.33	8.1	0.04	45.78

Table 3 Yield and early blight resistance of clone C75-5+297 are contrasted with the commercial varieties. Resistance is based on the percent foliage infection and relative AUDPC in 1999 and 2000. The percent foliage infections in 1999 were measured weekly from 25 June through to 8 September 8; a single measurement taken on 23 August is presented for each clone. The percent foliage infections in 2000 were measured weekly from 27 June through to 5 September. A single measurement taken 31 on July is presented for each clone. *NA* Data not available

Clones	Year 1999			Year 2000		
	Foliage infection (%)	Relative AUDPC	Average yield (ton/ha)	Foliage infection (%)	Relative AUDPC	Average yield (ton/ha)
Russet Burbank	65.0	0.28	25.06	100	0.014	5.86
Red Norland	97.5	0.60	24.85	100	0.032	18.23
Atlantic	80.6	0.40	41.23	100	0.014	19.75
C75-5+297	41.9	0.18	84.74	25.8	0.029	32.01

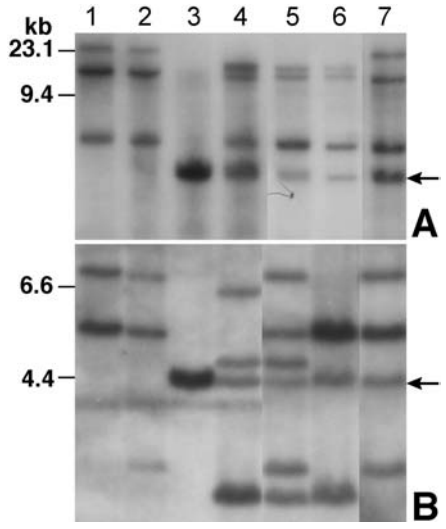

Fig. 2A, B Identification of *Solanum brevidens* chromosome 8 using RFLP markers. **A** Genomic DNAs were digested with *Eco*RV, and probed with GP245, a short arm marker of chromosome 8. **B** Genomic DNAs were digested with *Hin*dIII and probed with GP301, a long arm marker of chromosome 8. *Lane 1 S. tuberosum* cv. Atlantic, *lane 2 S. tuberosum* cv. Katahdin, *lane 3 S. brevidens* (PI 218228), *lane 4* somatic hybrid A206, *lane 5* C75, *lane 6* C75-5, *lane 7* C75-5+297. *Arrows* point to the *S. brevidens*-specific bands

tolerance. C75-5 and C75-5+297 also had markedly lower AUDPC values than all control varieties.

Most of the BC_3 breeding lines derived from A206 have poor overall agronomic performance. However, C75-5+297 consistently showed good yield potential. It out-yielded most common varieties in field trials with high disease pressure (Tables 2, 3). The high-level resistance to multiple diseases may give the yield advantage to clone C75-5+297.

RFLP analysis of clone C-75-5+297 and its parental lines

The somatic hybrid A206 carried the expected two sets of *S. brevidens* chromosomes and four sets of potato chromosomes (Williams et al. 1990). Molecular marker analysis indicated that the BC_2 clone C75-5 may retain a complete chromosome 8 and part of chromosomes 2, 4 and 5 from *S. brevidens* (McGrath et al. 1994, 1996). We used RFLP analysis to test if chromosome 8 of *S. brevidens* in C75-5 has been transmitted into C75-5+297. Southern blot analysis using RFLP markers specific to the short and long arm of potato chromosome 8 showed that C75-5+297 may contain an intact copy of chromosome 8 derived from *S. brevidens* (Fig. 2).

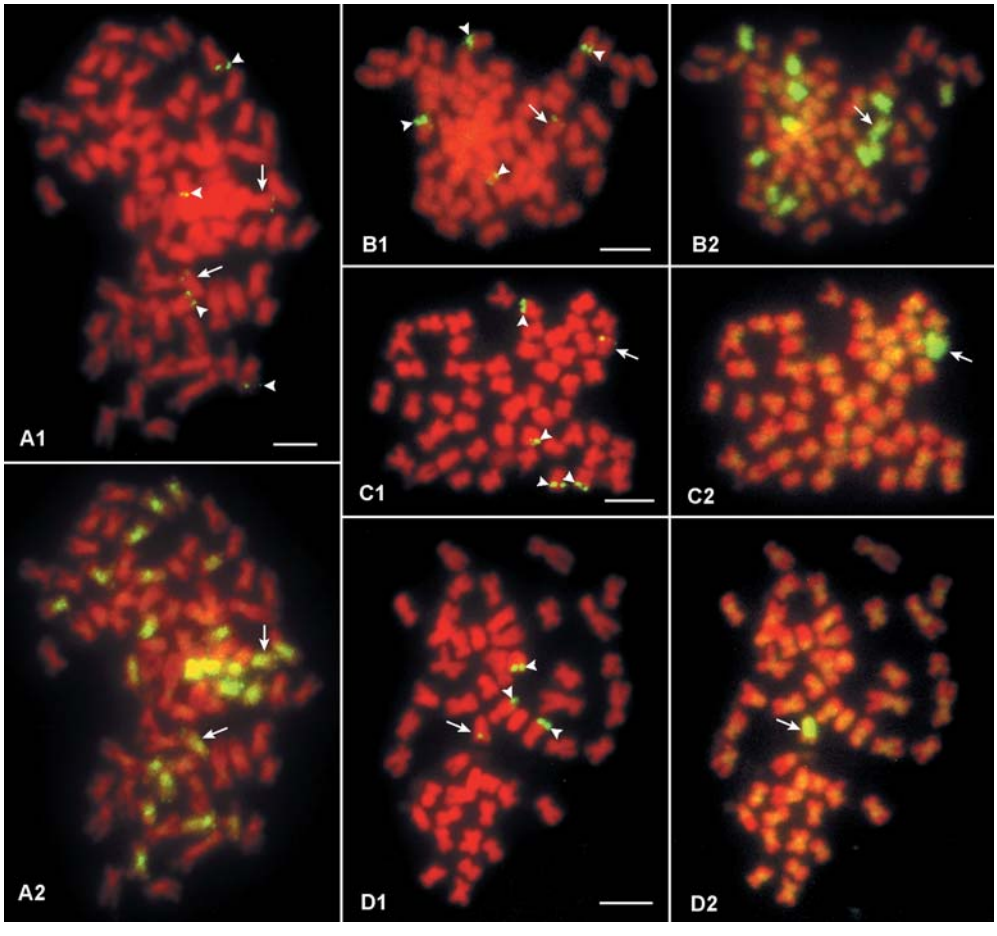

Fig. 3A–D Identification of chromosome 8 from both potato and *S. brevidens* using sequential FISH and GISH analysis. Somatic metaphase cells from A206 (**A1**), C75 (**B1**), C75-5 (**C1**), and C75-5+297 (**D1**) were hybridized with chromosome 8-specific BAC 220C03. The same metaphase cells then were analyzed by GISH (**A2**, **B2**, **C2**, and **D2**, respectively). The chromosomes 8 from *S. brevidens* are indicated by *arrows*. The chromosomes 8 from potato are indicated by *arrowheads*. *Bars* 5 μm

Cytogenetic characterization of clone C-75-5+297 and its parental lines

Although RFLP analysis confirmed the presence of chromosome 8 of *S. brevidens* in clone C75-5+297, it is unclear if the *S. brevidens* chromosome 8 replaces one of the potato chromosomes 8 or is present as an additional chromosome in the potato background. This question would be difficult to address using a DNA marker-based approach due to the tetraploidy and high heterozygosity of the potato genome. Dong et al. (2001) recently developed a sequential GISH and FISH approach to characterize potato germplasm containing chromosomes derived from wild species. In this approach, GISH was first used to reveal the number of chromosomes from the wild species. The same cytological preparations were then re-probed using BAC markers specific to individual potato chromosomes. These markers are called chromosome-specific cytogenetic DNA markers (CSCDM) (Dong et al. 2000, 2001). This sequential approach can reveal the number and identity of the alien chromosomes and their potato homologues in the breeding lines.

The somatic hybrid A206 has 72 chromosomes, 48 from potato and 24 from *S. brevidens* (Fig. 3, A2) as revealed by GISH. The four copies of chromosome 8 from potato and two copies of chromosome 8 from *S. brevidens* are identified by the following FISH using a chromosome 8-specific BAC marker 220C03 (Fig. 3, A1). The BC_1 plant C75 contains 61 chromosomes, instead of the expected 60 chromosomes (48 from potato and 12 from *S. brevidens*). C75 has 12 *S. brevidens* chromosomes, including one copy of chromosome 8 (Fig. 3, B1, B2).

The BC_2 clone C75-5 has 50 chromosomes, including four copies of chromosome 8 from potato and one from *S. brevidens* (Fig. 3, C1, C2). This result suggests that another potato chromosome, in addition to chromosome 8, is in aneuploidic state (five copies). Previous RFLP marker analysis indicated that C75-5 may contain parts of chromosomes 2, 4 and 5 from *S. brevidens* (McGrath et al. 1994, 1996). However, our GISH analysis only revealed the chromosome 8 from *S. brevidens*. The BC_3 clone C75-5+297 has 47 chromosomes, including four copies of chromosome 8, three from potato and one from *S. brevidens* (Fig. 3, D1, D2). Thus, although chromosome 8 is euploid in this plant, another potato chromosome has only three copies. It is interesting to note that C75-5 showed a poor yield compared to C75-5+297 although it has a similar level of early blight resistance as C75-5+297 (Table 2). C75-5 has at least two chromosomes that are in aneuploidy compared to a single chromosome in aneuploidy in C75-5+297. The increased aneuploidy of C75-5 may contribute to its poor yield performance. The high yield potential and resistance to both tuber soft rot and early blight of C75-5+297 make it an excellent germplasm line for developing potato varieties with multiple disease resistances.

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References

Austin S, Ehlenfeldt MK, Baer MA, Helgeson JP (1986) Somatic hybrids produced by protoplast fusion between *Solanum tuberosum* and *Solanum brevidens*: phenotypic variation under field conditions. Theor Appl Genet 71:682–690

Austin S, Lojkowska E, Ehlenfeldt MK, Kelman A, Helgeson JP (1988) Fertile interspecific somatic hybrids of *Solanum*: a novel source of resistance to *Erwinia* soft rot. Phytopathology 78:1216–1220

Boiteux LS, Reifschneider FJB, Fonseca MEN, Buso JA (1995) Search for sources of early blight (*Alternaria solani*) field-resistance not associated with vegetative late maturity in tetraploid potato germplasm. Euphytica 83:63–70

Bradeen JM, Naess SK, Song J, Haberlach GT, Wielgus SM, Buell CR, Jiang J, Helgeson JP (2003) Concomitant reiterative BAC walking and fine genetic mapping enable physical map development for the broad-spectrum late blight resistance region, *RB*. Mol Genet Genomics 269:603–611

Dong F, Song J, Naess SK, Helgeson JP, Gebhardt G, Jiang J (2000) Development and applications of a set of chromosome-specific cytogenetic DNA markers in potato. Theor Appl Genet 101:1001–1007

Dong F, McGrath JM, Helgeson JP, Jiang J (2001) The genetic identity of alien chromosomes in potato breeding lines revealed by sequential GISH and FISH analyses using chromosome-specific cytogenetic DNA markers. Genome 44:729–734

Gebhardt C, Ritter E, Salamini F (2001) RFLP map of the potato. In: Phillips RL, Vasil IK (eds) DNA-based markers in plants, 2nd edn. Kluwer, Dordrecht, pp 319–336

Helgeson JP, Hunt GJ, Haberlach GT, Austin S (1985) Somatic hybrids between *Solanum brevidens* and *Solanum tuberosum*: expression of a late blight resistance gene and potato leaf roll resistance. Plant Cell Rep 3:212–214

Helgeson JP, Haberlach GT, Ehlenfeldt MK, Hunt G, Pohlman JD, Austin S (1993) Sexual progeny of somatic hybrids between potato and *Solanum brevidens*: potential for use in breeding programs. Am Potato J 70:437–452

Helgeson JP, Pohlman JD, Austin S, Haberlach GT, Wielgus SM, Ronis D, Zambolim L, Tooley P, McGrath JM, James RV, Stevenson WR (1998) Somatic hybrids between *Solanum bulbocastanum* and potato: a new source of resistance to late blight. Theor Appl Genet 96:738–742

Jansky S (2000) Breeding for disease resistance in potato. In: Janick J (ed) Plant breeding reviews. Wiley, New York, pp 69–155

McGrath JM, Wielgus SM, Uchytil TF, Kim-Lee H, Haberlach GT, Williams CE, Helgeson JP (1994) Recombination of *Solanum brevidens* chromosomes in the second backcross generation from a somatic hybrid with *S. tuberosum*. Theor Appl Genet 88:917–924

McGrath JM, Wielgus SM, Helgeson JP (1996) Segregation and recombination of *Solanum brevidens* synteny groups in progeny of somatic hybrids with *S. tuberosum*: Intragenomic equals or exceeds intergenomic recombination. Genetics 142:1335–1348

McGrath JM, Williams CE, Haberlach GT, Wielgus SM, Uchytil TF, Helgeson JP (2002) Introgression and stabilization of *Erwinia* tuber soft rot resistance into potato after somatic hybridization of *Solanum tuberosum* and *S. brevidens*. Am J Potato Res 79:19–24

Naess SK, Bradeen JM, Wielgus SM, Haberlach GT, McGrath JM, Helgeson JP (2000) Resistance to late blight in *Solanum*

bulbocastanum is mapped to chromosome 8. Theor Appl Genet 101:697–704

Naess SK, Bradeen JM, Wielgus SM, Haberlach GT, McGrath JM, Helgeson JP (2001) Analysis of introgression of *Solanum bulbocastanum* DNA into potato breeding lines. Mol Gen Genomics 265:694–704

Pavek JJ, Corsini DL (2001) Utilization of potato genetic resources in variety development. Am J Potato Res 78:433–441

Song J, Bradeen JM, Naess SK, Raasch JA, Wielgus SW, Haberlach GT, Liu J, Kuang H, Austin-Phillips S, Buell CR, Helgeson JP, Jiang J (2003) Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proc Natl Acad Sci USA 100:9128–9133

Stevenson WR (1994) The potential impact of field-resistance to early blight on fungicide inputs. Am Potato J 71:317–324

Umaerus V, Umaerus M (1994) Inheritance of resistance to late blight. In: Bradshaw JE, Mackay GR (eds) Potato genetics. CAB International, Wallingford, pp 365–401

Williams CE, Hunt GJ, Helgeson JP (1990) Fertile somatic hybrids of *Solanum* spp.: RFLP analysis of a hybrid and its sexual progeny from crosses with potato. Theor Appl Genet 80:545–551

Zimnoch-Guzowska E, Marczewski W, Lebecka R, Flis B, Schafer-Pregl R, Salamini F, Gebhardt C (2000) QTL analysis of new sources of resistance to *Erwinia carotovora* ssp. *atroseptica* in potato done by AFLP, RFLP, and resistance-gene-like markers. Crop Sci 40:1156–1167