Theor Appl Genet (1998) 96: 738–742

**J. P. Helgeson · J. D. Pohlman · S. Austin
G. T. Haberlach · S. M. Wielgus · D. Ronis
L. Zambolim · P. Tooley · J. M. McGrath
R. V. James · W. R. Stevenson**

Somatic hybrids between *Solanum bulbocastanum* and potato: a new source of resistance to late blight

Received: 27 October 1997 / Accepted: 11 November 1997

Abstract *Solanum bulbocastanum*, a wild, diploid (2n = 2*x* = 24) Mexican species, is highly resistant to *Phytophthora infestans*, the fungus that causes late blight of potato. However this 1 EBN species is virtually impossible to cross directly with potato. PEG-mediated fusion of leaf cells of *S. bulbocastanum* PI 245310 and the tetraploid potato line *S. tuberosum* PI 203900 (2n = 4*x* = 48) yielded hexaploid (2n = 6*x* = 72) somatic hybrids that retained the high resistance of the *S. bulbocastanum* parent. RFLP and RAPD analyses confirmed the hybridity of the materials. Four of the somatic hybrids were crossed with potato cultivars Katahdin or Atlantic. The BC_1 progeny segregated for resistance to the US8 genotype (A-2 mating type) of *P. Infestans*. Resistant BC_1 lines crossed with susceptible cultivars again yielded populations that segregated for resistance to the fungus. In a 1996 field-plot in Wisconsin, to which no fungicide was applied, two of the BC_1 lines, from two different somatic hybrids, yielded 1.36 and 1.32 kg/plant under a severe late-blight epidemic. In contrast, under these same conditions the cultivar Russet Burbank yielded only 0.86 kg/plant. These results indicate that effective resistance to the late-blight fungus in a sexually incompatible *Solanum* species can be transferred into potato breeding lines by somatic hybridization and that this resistance can then be further transmitted into potato breeding lines by sexual crossing.

Communicated by G. Wenzel

J. P. Helgeson (✉) · J. D. Pohlman · S. Austin
G. T. Haberlach · S. M. Wielgus · D. Ronis · L. Zambolim
P. Tooley · J. M. McGrath · R. V. James · W. R. Stevension
USDA/ARS Plant Disease Resistance Research Unit,
Department of Plant Pathology, University of Wisconsin,
Madison, WI 53706, USA
Fax: +1-608 262-1541
E-mail: jph@plantpath.wisc.edu

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

Introduction

Due to the general susceptibility of potato cultivars to *Phytophthora infestans*, the cause of late blight of potatoes, this disease is a major world-wide problem for potato production. It is not surprising, then, that a source of genetic resistance to this devastating disease has been long sought by plant breeders. Much of this effort has involved the examination of wild species related to potato. For example, Reddick (1930) reported that the mexican species *Solanum demissum* was virtually immune to late blight. Unfortunately, the hypersensitive resistance from this species is not generally effective against all races of the fungus (Black and Gallegly 1957). Another Mexican species, *Solanum bulbocastanum*, is also highly resistant to late blight, even under the rigorous tests made in Toluca, Mexico (Niederhauser and Mills 1953). However, *S. bulbocastanum* is a 1EBN species (Hanneman and Bamberg 1986) and thus extremely difficult to cross directly with potato. Limited success has been obtained by utilizing bridging species (Hermsen 1966; Hermsen and Ramana 1973) but the incompatibility of *S. bulbocastanum* has generally prevented the use of this particularly valuable trait.

Somatic hybridization can provide a means of bypassing sexual incompatibility between *Solanum* species, leading to fertile plants that can be used directly in breeding programs (Helgeson 1992). Resistance to potato leaf roll virus (Austin et al. 1985), the nematode, *Meloidogyne chitwoodi* (Austin et al. 1993), *Erwinia* tuber soft rot (Austin et al. 1988) and hypersensitive resistance to race 0 of *Phytophthora infestans* (Helgeson et al. 1993) has been achieved by this procedure. We

now report that the resistance in *S. bulbocastanum* can be captured and passed on to potato breeding lines by the use of somatic hybridization.

Materials and methods

Materials used in this study

Potato and related species used for somatic hybridization were obtained from Dr. John Bamberg and his colleagues at the Inter-Regional Potato Introduction Station (NRSP-6), 4312 Highway 42, Sturgeon Bay, Wis. These include *S. bulbocastanum*, PI 243510 and *Solanum tuberosum* PI 23900 (potato). Elite copies of the potato cultivars Katahdin and Atlantic were obtained from the Wisconsin potato certification program. All cultivars and wild species, as well as test materials, were routinely maintained clonally in vitro as described by Haberlach et al (1985). The individual clones were multiplied in vitro for analyses.

Protoplast isolation somatic hybridization

Protoplasts were isolated from leaves of in vitro shoots as described by Haberlach et al. (1985). Somatic hybridizations with the protoplasts were performed using a polyethylene glycol (PEG) protocol. For the most part, the procedure of Austin et al. (1985) was followed. However, after PEG additions, and dilutions and pelleting of the cells after the fusion attempts, the cells were suspended in 0.3 M sucrose rather than 0.6 M mannitol. The cell suspension was gently shaken (40 rpm) for 45 min and then centrifuged (HNII centrifuge, IEC) at 1300 rpm for 10 min in a Babcock bottle. This modification resulted in viable protoplasts and fused cells being concentrated at the surface of the sucrose solution in the bottle, thus separating the viable cells from the pelleted debris.

The resulting fused cells were regenerated into whole plants in a manner similar to that reported by Haberlach et al. (1985). Initially, the cells were plated onto culture medium (CUL, Haberlach et al. 1985) and, after macroscopic calli had appeared, the calli were transferred to differentiation medium (DIF, Haberlach et al. 1985). After 2–3 weeks, the calli were transferred to the differentiation medium developed by Lam (1977). After buds had formed, the calli were transferred to proliferation medium (PM, Haberlach et al. 1985) Shoots that formed were then excised and rooted on standard propagation medium (PROP, Haberlach et al. 1985) and maintained in test tubes in vitro. Clonal copies of the reference copy were made for experiments.

RFLP and RAPD analyses

DNA extractions and restriction fragment length polymorphism (RFLP) analyses were carried out as described by Williams et al. (1990). Chromosome-specific tomato genomic (TG) and cDNA (CD) probes were obtained from Dr. Steve Tanksley, Cornell University. Four putative somatic hybrids were analyzed by this method. To complete the analyses for hybridity, randomly amplified polymorphic DNA (RAPD) analyses were carried out as described by McGrath et al. (1996). A total of 109 primers (from 380 primers tested) were selected that gave clearly scorable polymorphisms between potato and *S. bulbocastanum*. Several of these were used with each of the putative somatic hybrids.

Nomenclature of hybrids and progeny

Three of the hybrids have been used extensively in further experiments. These were designated J101, J103, and J138. In crosses, the potato parent was designated as K (for Katahdin) or A (for Atlantic). Thus, for example, J101K1 was the first seed germinated from a berry obtained from the cross of J101 and Katahdin. Similarly, J101K6 and J101K27 were the seedlings obtained from seed 6 and seed 27 respectively from that cross. The BC_2 progeny were named by adding the seed number and cultivar lines in that cross. Thus the cross designated as J101K6A22 is the 22nd seedling from the cross of line J101K6 with Atlantic. This shortcut avoided use of the longer and less informational term, [(*S. bulbocastanum* + *S. tuberosum*) × Katahdin)] × Atlantic, that could be applied to this individual.

Late-blight ratings

Comparisons were made of susceptible and resistant plants in the field. For these studies, the percentage of leaves showing late-blight lesions was recorded at various times during the growing season. For detailed greenhouse studies, a modified Horsfall-Barret rating scheme was used to estimate the percent leaf infection by *P. infestans*. The ratings and the ranges of % infections associated with these scores were 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% infection.

Results

In total, 80 shoots were obtained from 23 calli. Twenty four of these plants (from five calli) showed obvious morphological differences from either of the parent species. The other 56 plants appeared very similar to potato. Initially, chromosome-specific restriction fragment length polymorphism (RFLP) markers were used to confirm that four of the plants derived from the fusion of *S. bulbocastanum* and potato cells were indeed somatic hybrids. Prominent potato bands were retained by the hybrids in addition to the diagnostic *S. bulbocastanum* bands (Fig. 1). The rest of the potentially hybrid plants were evaluated with RAPD probes (data not shown). A total of 13 further somatic hybrids were confirmed by these techniques. The confirmed hybrids were derived from four different callus pieces, and thus probably from four different fusion events.

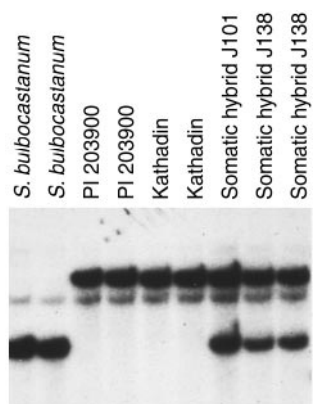

Fig. 1 RFLP analysis of somatic hybrids between *S. bulbocastanum* and potato. The probe used in the analysis was TG310, a tomato genomic probe specific for Chromosome 1 of tomato and potato

2

3

4

Fig. 2 Leaves and stems of *S. bulbocastanum* (left), *S. tuberosum* PI 203900 (right) and the somatic hybrid between these two species (center). Note that the purple color of the *S. bulbocastanum* parent is retained in the somatic hybrid. The leaves of the hybrid are compound, however, as with potato (Photo by Steven Vicen, University of Wisconsin-Madison)

Fig. 3a, b Results of a field test for the resistance of sexual progeny of somatic hybrids between *S. bulbcastanum* and potato. (Photos taken by J. P. Helgeson). **a** The appearance of J101K27, a BC_1 line, in the field at Hancock. Wisconsin. **b** The appeance of a BC_2 line, J101K6A22, in the field at Hancock, Wisconsin

Fig. 4 Resistance in Toluca, Mexico, of a BC_1 line derived from a potato + *S. bulbocastanum* somatic hybrid crossed with the potato cultivar Atlantic. The *arrow* points to a dead plant of the susceptible variety Alpha (Photo taken by J. P. Helgeson)

The appearance of leaves and stems of the parent plants and one of the somatic hybrids is shown in Fig. 2. As has been the case with many of our other hybrids, traits of both of the parent species can be seen in the hybrids. In this case, the purple coloration of the *S. bulbocastanum* stems was expressed in the hybrids. However, leaves of the hybrids were compound rather than single as was the case in the wild species.

Fertility of somatic hybrids

Crosses of four of the somatic hybrids were undertaken with the potato cultivars Katahdin and Atlantic to test for the fertility of the hybrids. Each of the tested hybrids yielded viable seeds and sexual progeny. Further crosses of selected individuals from these progeny lines were also successful. Thus, parental lines and two successive backcross populations were available for the evaluation of disease resistance.

Late-blight resistance tests

Preliminary laboratory tests for resistance to *P. infestans*, made with detached leaves or leaf discs, indicated that the somatic hybrids and some of the progeny retained at least some of the resistance to late blight that was shown by the *S. bulbocastanum* parent.

These preliminary laboratory results were confirmed in field tests, initially carried out in 1994. Somatic hybrids, as well as progeny from somatic hybrids between *S. bulbocastanum* and potato, showed remarkable resistance and were clearly noticeable in test plots as "green islands" in a brown background of dead potato lines. Although the cultivars Atlantic, Russet Burbank and Snowden were killed, 11 different experimental lines showed less than 10% infection. The appearance of one of the BC_1 lines (J101K27) is shown in Fig. 3a and that of one of the BC_2 lines J101K6A22, is shown in Fig. 3b. In each case, the live test plants were surrounded with the susceptible cultivar, Russet Burbank, which had been killed by the fungus by August 9, 1994. In contrast, the % foliage infections on August 15, 1994 were 5.0% and 7.8% for J101K27 and J101K6A22, respectively.

Fourteen BC_1 and BC_2 lines were tested in Toluca, Mexico, in the summer of 1995. Good resistance in the Toluca field test was also obtained with all lines that were resistant in Wisconsin in 1994. For example, Fig. 4 shows the contrast between the susceptible cultivar Alpha with the backcross line J138A12, a line that had been highly resistant in Wisconsin in 1994. All other lines that were resistant in 1994 in Wisconsin were also resistant in Toluca in 1995.

Additional field experiments were carried out at Hancock, Wisconsin, in 1996. Again, a severe natural late-blight epidemic was obtained in test plots, and yields of common cultivars were severely depressed by the late-blight epidemic. For example, in plots where an effective fungicide spray regime was maintained, Russet Burbank yields were as high as 1.7 kg/plant. In the yield trial where no fungicide was used, this was cut almost in half to 0.86 kg/plant. One of the *S. bulbocastanum*-derived lines, J103K7, topped all 90 test lines with a yield of 1.36 kg/plant, and J138A12, the line pictured in Toluca in Fig. 4, ranked fourth at Hancock in 1996 with a yield of 1.32 kg/plant.

Greenhouse tests for the segregation of late-blight resistance

To test the resistance of potentially segregating BC_1 and BC_2 populations, a facility was constructed in the new research greenhouses at the University of Wisconsin Biotron. There, close control of humidity and temperature made uniform epidemics possible. Segregation of resistance and susceptibility was obtained for each of six BC_1 lines from four different somatic hybrids . Representative tests from one of these lines are shown in Table 1. Three of these lines were further crossed to Atlantic or Norland. Representative results on these BC_2 lines are included in Table 2. Again, a clear segregation of resistance and the recovery of both parental extremes of susceptibility and resistance were obtained with these lines.

Discussion

Wild Solanum species related to potato (*S. tuberosum*) have genetic properties that could be valuable if incorporated into commercial cultivars. There is often a problem, however, in crossing these characters into potato breeding lines. Our results indicate that somatic hybrids between *S. bulbocastanum* and potato are sources of highly effective resistance against late blight. Furthermore, this trait can be passed on to potato breeding lines by conventional sexual crossing. The resistance

Table 1 Representative data from Biotron tests of BC_1 lines for late-blight resistance

Plant line	Average blight score[a]		
	5 day	8 day	12 day
J101K09	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0
J101K27	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0
J101K06	8.8 ± 0.5	9.0 ± 0.0	8.8 ± .04
J101K10	9.0 ± 0.4	8.8 ± 0.4	8.6 ± 0.9
J101K30	9.0 ± 0.0	9.0 ± 0.0	8.4 ± 0.9
J101K16	8.8 ± 1.0	8.8 ± 0.5	7.8 ± 1.0
J101K25	8.6 ± 1.1	8.4 ± 0.9	8.2 ± 1.1
J101K02	8.6 ± 0.4	8.0 ± 1.2	7.8 ± 0.4
J101K20	8.6 ± 0.7	7.4 ± 0.9	7.0 ± 0.7
J101K33	7.4 ± 1.8	5.4 ± 0.9	6.2 ± 1.8
J101K19	6.8 ± 2.4	5.6 ± 2.7	5.2 ± 2.4
J101K11	6.0 ± 1.3	4.6 ± 1.1	4.2 ± 1.3
J101K12	7.2 ± 2.5	5.2 ± 2.4	3.8 ± 2.5
J101K18	6.8 ± 1.8	4.6 ± 1.3	3.6 ± 1.8
J101K21	5.2 ± 1.3	2.2 ± 1.3	0.6 ± 1.3
S. bulbocastanum	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0
Somatic hybrid J101	8.6 ± 0.5	7.8 ± 0.8	8.2 ± 0.8
S. tuberosum PI 203900	7.0 ± 0.0	5.8 ± 1.3	5.6 ± 1.9
S. tuberosum cv Kathadin	4.8 ± 0.4	2.0 ± 0.7	1.0 ± 1.2

[a] The ratings and the % infections associated with that 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)

Table 2 Examples of segregation for late-blight resistance in BC_2 progeny of a cross between BC_1 line J101K6 and *S. tuberosum* cv Atlantic

Plant line	Average blight score[a]		
	7 day	10 day	15 day
J101K6A21	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0
J101K6A4	8.8 ± 0.4	8.8 ± 0.4	9.0 ± 0.0
J101K6A22	9.0 ± 0.0	9.0 ± 0.0	8.8 ± 0.4
J101K6A2	9.0 ± 0.0	9.0 ± 0.0	8.8 ± 0.4
J101K6A3	5.2 ± 3.1	5.4 ± 3.6	2.0 ± 3.9
J101K6A10	3.4 ± 2.1	3.0 ± 2.0	1.4 ± 1.9
J101K6A50	5.6 ± 3.4	3.4 ± 3.6	0.0 ± 0.4
J101K6A24	2.6 ± 0.9	1.4 ± 0.5	0.0 ± 0.0
S.bulbocastanum PI 243510[a]	9.0 ± 0.0	8.8 ± 0.4	9.0 ± 0.0
S. tuberosum PI 203900[a]	4.0 ± 1.0	4.0 ± 1.0	0.6 ± 0.9
J101[b]	7.8 ± 1.1	8.6 ± 0.5	8.4 ± 0.9
Kathadin[b]	4.4 ± 0.5	4.2 ± 0.8	3.4 ± 1.8
J101K6[c]	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0
Atlantic[c]	3.6 ± 0.5	3.0 ± 0.0	1.8 ± 1.8

[a] The ratings and the % infections associated with that 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)
[b] Lines crossed to generate BC_1 lines
[c] Lines crossed to generate BC_2 lines

carries over to at least two generations of sexual progeny and, with the 1997 results just obtained, has been stable for four different years in several different locations. As no North American cultivar currently has adequate resistance against this disease, these lines could be very useful for introducing resistance into commercial lines.

The resistance to *P. infestans* from *S. bulbocastanum* appears to be more general than the race-specific resistance derived from *S. demissum*. Nearly every race of the fungus is known to be found in Toluca, Mexico (Hector Lozoya, personal communication), and was actually isolated from fields where the progeny of the somatic hybrids showed good resistance. Observations of the foliage in Toluca in 1995 and 1997 indicated that some lesions actually formed and that limited sporulation also occurred (Helgeson, observations of August 22, 1995 and August 26, 1997).

Although the disease resistance is highly effective we do not know, as yet, the numbers of genes involved. For each of the somatic hybrids tested, the disease resistances of BC_1 lines appeared to segregate. Thus, it follows that the late-blight resistance gene(s) is (are) heterozygous in the clone of *S. bulbocastanum* utilized in the somatic hybridization. We are developing much larger progeny populations to test for segregation parameters and for mapping the disease resistance gene(s) brought into the potato breeding lines by somatic hybridization.

References

Austin S, Baer MA, Helgeson JP (1985) Transfer of resistance to potato leaf roll virus from *Solanum brevidens* into *Solanum tuberosum* by somatic fusion. Plant Sci 39:75–82

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

Austin S, Pohlman JD, Brown CR, Mojtahed H, Santo GS, Douches DS, Helgeson JP (1993) Interspecific somatic hybrids between *Solanum tuberosum* and *S. bulbocastanum*: incorporation of nematode resistance. Am Potato J 70:485–495

Black W, Gallegly ME (1957) Screening of *Solanum* species for resistance to physiologic races of *P. Infestans*. Am Potato J 34:273–281

Hanneman RE Jr, Bamberg JB (1986) Inventory of tuber-bearing *Solanum* species. Bulletin 533, University of Wisconsin

Haberlach G, Cohen BA, Reichert NA, Baer MA, Towill LE, Helgeson JP (1985) Isolation, culture and regeneration of protoplasts from potato and several related *Solanum* species. Plant Sci 39:67–74

Helgeson, JP (1992) New genes for disease resistances through somatic hybridization. Netherlands J Plant Pathol 98 (suppl 2):223–229

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

Hermsen JGTh (1966) Crossibility, fertility, and cytogenetics studies in *Solanum acuale* × *Solanum bulbocastanum*. Euphytica 15:149–155

Hermsen JGTh, Ramana MS (1973) Double-bridge hybrids of *Solanum bulbocastanum* and cultivars of *Solanum tuberosum*. Euphytica 22:457–466

Lam SL (1977) Regeneration of plantlets from single cells in potatoes. Am Potato J 54:575–580

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 recombinations. Genetics 142:1335–1348

Niederhauser JS, Millis WR (1953) Resistance of *Solanum* species to *Phytophthora infestans* in Mexico. Phytopathology 43:456–457

Reddick D (1930) Frost-tolerant and blight-resistant potatoes. Phytopathology 20:987–991

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