Fertile Interspecific Somatic Hybrids of *Solanum*: A Novel Source of Resistance to *Erwinia* Soft Rot

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ABSTRACT

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Tubers of somatic hybrids produced by protoplast fusion between *Solanum brevidens*, a diploid, nontuber-bearing wild species, and a tetraploid potato (*S. tuberosum*) were screened for resistance to bacterial soft rot caused by *Erwinia* sp. Tubers of the *S. tuberosum* fusion parent, and two potato cultivars, Katahdin and Russet Burbank, were susceptible to bacterial soft rot, whereas tubers of the somatic hybrids were resistant. Furthermore, some of the sexual progeny from crosses between the somatic hybrids and Katahdin had the same high level of resistance as the somatic hybrids. Thus, the resistance incorporated from *S. brevidens* by somatic fusion was sexually transferred. Interspecific somatic hybridization may make it possible to use new sources of disease resistance and other important agronomic traits that were previously unavailable because of sexual incompatibilities between species.

Additional keywords: *Erwinia carotovora* supsp. *atroseptica*, *E. c.* subsp. *carotovora*, *E. chrysanthemi*, interspecific protoplast fusion, transfer of disease resistance.

Wild *Solanum* species have been valuable as sources of desirable agronomic characteristics for the development of potato cultivars. Full utilization of related species has been limited, however, by sexual incompatibilities between gametic cells. For example, *Solanum brevidens*, the diploid, nontuber-bearing wild species used in this study, has resistance to several potato virus diseases, but is very difficult to cross sexually with *Solanum tuberosum*, the cultivated potato (13,28). A potential alternative hybridization pathway is the direct fusion and culture of isolated somatic cells (14). Agronomically important traits of wild species could be incorporated into breeding programs for crop improvement by means of protoplast fusions between sexually incompatible species. Full expression of the traits in the original somatic hybrids is essential and, more importantly, the hybrids must be fertile to allow the subsequent sexual transfer of the desirable traits.

Somatic hybrids between *Solanum* species using protoplast fusion have been obtained by several research groups (5,6,8,23,27). Our research has involved an evaluation of protoplast fusion as a means for transferring disease resistance from wild *Solanum* species into breeding lines of *S. tuberosum* Group Tuberosum, the cultivated potato. Inter- and intraspecific hybrids have been produced that clearly show phenotypic characteristics that are in between those of the parental types used in the fusion (1,2,3). One set of hybrids expressed resistance genes (late blight and leafroll resistance) of both of the fusion parents (18). Furthermore, some somatic hybrid plants were crossed with cultivars in conventional sexual crosses (14). Thus, certain desirable characteristics present in wild *Solanum* species and incorporated by protoplast fusion into *S. tuberosum* were transferred in subsequent sexual crosses with a commercial cultivar.

Transmission genetics of somatic hybrids is still unclear, and research has been hampered because somatic hybrids, particularly interspecific hybrids, are usually sterile (15). A low level of fertility was found in two interspecific hybrids of *Nicotiana* produced by protoplast fusion. Resistance to tobacco mosaic virus, derived from the wild species used in the fusion, was expressed in the hybrids and in some of the sexual progeny of the hybrids (16,24).

In this study, screening for bacterial soft rot resistance in tubers was initiated after it was noted that tubers of hybrid material did not decay in storage. In contrast, tubers of the *S. tuberosum* line used in the fusion were susceptible to decay. Furthermore, tubers of the hybrids used as seed pieces in the spring were usually intact at harvest in contrast to those of the parental line or common cultivars that typically decayed under the same field conditions. Among the major causes of storage rot and seed piece decay are the soft rot erwinias, including *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora*, and *E. chrysanthemi* (26). Tubers of potato cultivars vary in their relative susceptibility to bacterial soft rot (7,19,20,22,31), but none of the common cultivars grown in the U.S.A. are considered to be highly resistant, although a range in relative susceptibility has been reported (11,22,29).

The genetic basis of resistance to *Erwinia* soft rot is not known. Some degree of resistance has been found in accessions of wild species and also in *S. tuberosum* Gp. Andigena (30). *S. brevidens* has not been considered as a potential source of tuber resistance to bacterial soft rot because it does not form tubers. However, *S. brevidens* seedlings (4–5 wk old) obtained from true seeds were very resistant to stem rot (unpublished results). A preliminary report on this study of resistance to bacterial soft rot in somatic hybrids has been presented (4).

MATERIALS AND METHODS

Sources of test material. The materials used in this study were obtained from the fusion of diploid ($2n = 2x = 24$) *S. brevidens* (PI 218228) protoplasts with those of tetraploid ($2n = 4x = 48$) *S. tuberosum* (PI 203900). The production and field evaluation of these plants has been described previously (3). Tubers of the

hexaploid somatic hybrids, the fusion parent *S. tuberosum* (PI 203900), and cultivar Russet Burbank were harvested in the fall of 1986 and 1987 from plants derived from 1985 and 1986 field-grown tubers. Russet Burbank, which comprises 40% of the acreage in North America, is considered one of the less susceptible cultivars (22,29) and was thus included in the screen for comparative purposes.

Seeds from the sexual crosses of somatic hybrids with the cultivar Katahdin ($2n = 4x = 48$) were germinated in vitro and clones of the seedlings obtained by nodal sections (17). These pentaploid progeny ($n = 60$) were transplanted into the field for tuber production in 1986 along with transplants of clonally maintained Katahdin. Tubers of pentaploid material crossed again with cultivar Katahdin were derived from seedlings planted directly into the field in 1986. Preliminary chromosome counts show these progeny from second crosses also to be pentaploid. Tubers of the above plants screened in 1986 were replanted in the spring of 1987 and harvested in the fall for further testing. All tubers were stored at 7 C for 3 mo in 1986 before screening for resistance to *Erwinia* sp. In 1987 tubers were tested immediately after harvest.

Resistance screening: Point titration assay. Tubers (free of any obvious mechanical damage or disease) were washed and then immersed in 0.5% sodium hypochlorite (twice for 20 min), rinsed with sterile deionized water, sprayed with 95% ethanol, and allowed to dry in air. Sterile polypropylene pipette tips containing bacterial suspensions ranging from 10^5 to 10^9 cfu/ml were pressed into each tuber to the depth of 10 mm (21). Sterile water was used as a control. Suspensions were prepared from cultures of *E. c. atroseptica* (Van Hall) Dye (Eca-SR 8), *E. c. carotovora* (Jones) Bergey et al (Ecc-SR 394), and *E. chrysanthemi* Burkholder (Echr-SR 325) following established procedures (12). Tubers were incubated at either 22 C (Ecc and Eca) or 35 C (Echr) in a dew chamber with a relative humidity of 92%, or in a chamber flushed with water-saturated nitrogen to provide low oxygen conditions. After 72 hr of incubation, tubers were sliced vertically through the infection points, and the width of decayed tissue was measured.

Resistance screening: Mist chamber assay. Tubers from 1987 field planting were uniformly injured by banging twice at one site per tuber with a pendulum bruiser (9). Tubers were immediately inoculated by placing a 10-μl suspension of bacterial cells (Ecc-SR 394) at a level of 5×10^8 cfu/ml at the bruised site. Ten tubers from each line or cultivar were inoculated and then incubated in a mist chamber at 20 C for 3 days. Tubers were sliced vertically at the inoculation site, and the width of decayed tissue was measured.

Statistical analysis. The data were analyzed by using a Kruskal-Wallis test; multiple comparisons were then performed using a Duncan's procedure on the ranked data ($P = 0.05$) (10).

TABLE 1. Width of decayed tissue following inoculation of *Erwinia carotovora* subsp. *atroseptica*, *Erwinia carotovora* subsp. *carotovora*, and *Erwinia chrysanthemi* into tubers of *Solanum tuberosum* cultivars, parental fusion line (PI 203900), and somatic hybrids of *S. tuberosum* and *S. brevidens*

Test plants (1986)	Decay width (mm)[y]		
	Eca SR 8[z]	Ecc SR 394[z]	Echr SR 325[z]
S. tuberosum 'Russet Burbank'	8.7 a	13.4 a	11.1 a
S. tuberosum 'Katahdin'	10.6 a	11.7 a	
S. tuberosum PI 203900 fusion parent	7.4 a	11.0 a	11.2 a
Individual somatic hybrids			
#206	1.6 bc	2.8 c	
#249			0.6 c
#937	3.4 b	3.7 c	0.8 c
#946	0.2 c	6.1 b	
#1,690	2.6 b	4.3 bc	3.5 b

[y] Measurements were taken after incubation in dew chamber for 72 hr at 22 C (Eca and Ecc) or 35 C (Echr), relative humidity 92%. Tubers were sliced at the inoculation points and width of decayed tissue measured. Values are overall means (five tubers per sample) from all concentrations combined. Means within a column with the same letter are not significantly different ($P = 0.05$).

[z] Injection of 100 μl of bacterial suspension (5×10^4, 5×10^6, and 5×10^8 cfu/ml).

[z] Injection of 25 μl of bacterial suspension (1×10^7, 1×10^8, and 1×10^9 cfu/ml).

RESULTS

Tubers of the somatic fusion hybrids differed significantly from the tuber-bearing parent used in the fusion and cultivars Katahdin and Russet Burbank in the degree of rotting caused by three different species of *Erwinia* (Table 1). Inoculated tubers of cultivars and the fusion parent typically had areas of soft macerated tissue around the inoculation site. In contrast, tubers of somatic hybrids usually had a clearly defined, localized wound response consisting of a dry necrotic lesion at the site of inoculation with intense browning and the production of dry, corklike, suberized tissue at the edges of the inoculation point (Fig. 1). All the *Erwinia* strains used were reisolated during decay evaluation from inoculation sites of both susceptible and resistant

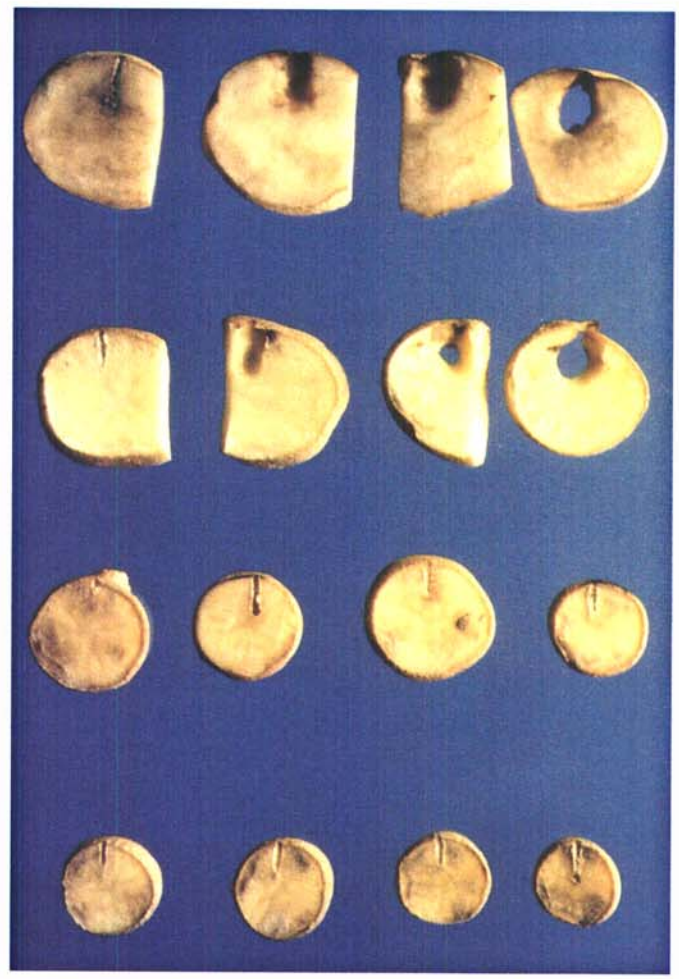

Fig. 1. Lesions in tubers following inoculation with *Erwinia carotovora* subsp. *carotovora* (Ecc): Russet Burbank (first row); *S. tuberosum* PI 203900, fusion parent (second row); somatic hybrid, #1690 (third row); and somatic hybrid, #249 (fourth row). Tubers were injected with 25 μl of sterile water (far left) or with increasing levels of Ecc (1×10^7, 1×10^8, 1×10^9 cfu/ml), (left to right), and incubated in a dew chamber at 22 C for 72 hr. Slices were cut at inoculation site. Slices from the resistant tubers (third [#1,690] and fourth rows [#249]) show clearly delineated inoculation sites in contrast to slices from the susceptible tubers that have large areas around the inoculation site from which the soft macerated tissue was readily removed.

interactions. Of the three bacterial strains, Ecc (SR 394) was the most virulent (Table 1). Therefore, it was used in the inoculation of tubers from the subsequent sexual crosses with different levels of inoculum.

In general, the number of tubers with decay symptoms increased with each increase in level of inoculum (Table 2). The reaction of the tissue at the inoculation site in resistant material was more extensive at the highest level of inoculum than at the intermediate level, but the lesion was characterized by the formation of the corklike layer of tissue localizing the infection. Somatic hybrid tubers were significantly more resistant to infection than those of the fusion parent or the cultivars at all levels of inoculum. When decay was present, the width of the decayed area was usually significantly smaller than lesions in the fusion parent or in cultivars. Thus, the low overall mean decay widths in hybrid tubers (Table 2) do not simply reflect a consistent low level of decay throughout the sample, but are a result of low and zero levels of decay. This is clearly shown in Table 2, in which the number of infected tubers at different inoculum levels of Ecc is given. In this test, tubers from sexual crosses of two somatic hybrids with the cultivar Katahdin were included in the screen. Tubers from four of six lines of the first sexual cross were also highly resistant, indicating that the resistance was transferred sexually (Table 2). Also, progeny from the second sexual cross of a pentaploid with cultivar Katahdin expressed a high level of resistance to bacterial soft rot. Because the tuber-bearing fusion parent is susceptible to tuber soft rot, the source of resistance in the hybrids is presumably the nontuber-bearing parent, *S. brevidens*.

The susceptibility of these lines to bacterial soft rot under low oxygen conditions was also evaluated. Previous studies indicated that maceration of potato tissue by soft rot erwinias is enhanced, and the wound healing response is suppressed under low oxygen conditions (21). Thus, tubers from the test lines harvested in 1986 and 1987 were inoculated with Ecc (SR 394) and incubated either in aerobic (in a dew chamber) or anaerobic conditions (in a chamber flushed with nitrogen). In the dew chamber a high relative humidity is maintained, but no water films are present on surfaces of tubers. As expected, decay widths were generally larger in tubers in a chamber flushed with nitrogen than in tubers held in a dew chamber (Table 3). However, widths of soft rot decay lesions in tubers of the somatic hybrids and their sexual progeny were significantly lower than those in the fusion parent and cultivars under both regimes. The data further indicate that hybrid tubers possess sexually transmissible resistance factors to tuber soft rot

TABLE 3. Width of decayed tissue following inoculation of *Erwinia carotovora* subsp. *carotovora* into tubers of *Solanum tuberosum* cultivars, parental fusion line *S. tuberosum* (PI 203900), somatic hybrids of *S. tuberosum* and *S. brevidens* and their sexual progeny in two environmental conditions

	Decay width (mm)[x]			
	In dew chamber[y]		In nitrogen chamber[z]	
Test plant	1986	1987	1986	1987
S. tuberosum 'Russet Burbank'	7.1 b	5.6 b	17.2 a	10.3 cd
S. tuberosum 'Katahdin' (Kat.)	6.3 b	6.9 b	14.4 ab	18.4 ab
S. tuberosum PI 203900 fusion parent	12.5 a	12.1 a	12.6 b	21.9 a
Somatic hybrids				
# 206	1.6 cd	3.3 c	7.5 d	6.3 d
# 249	0.0 d	1.0 d	9.7 c	5.4 de
# 937	2.7 c	3.0 c	9.0 cd	7.4 d
#1,690	1.4 cd	2.4 cd	7.6 d	6.3 d
#2,018	2.4 c	2.9 c	7.9 d	7.3 d
Sexual progeny				
4,707 (206 × Kat.)	6.8 b	5.3 bc	10.7 c	9.1 cd
4,709 (206 × Kat.)	0.9 d	3.0 c	6.3 d	5.9 de
4,680 (937 × Kat.)	1.2 cd	3.3 c	7.7 d	9.8 cd
M264 (206 × Kat.) × Kat.	0.0 d	1.9 d	6.4 d	9.7 cd
T406 (937 × Kat.) × Kat.	···	2.5 cd	···	10.7 cd
T532 (937 × Kat.) × Kat.	···	6.3 b	···	16.5 b

[x] Measurements were taken after incubation for 72 hr at 22 C; tubers were sliced at the inoculation sites and width of soft rotted tissue measured. Injections were 25 μl of three dilutions of Ecc suspension (1×10^5, 1×10^7, and 1×10^9 cfu/ml) Values are overall means (5 tubers per sample) from all concentrations together. Means within a column with the same letter are not significantly different, $P = 0.05$.

[y] Incubation in dew chamber in relative humidity of 92% (aerobic conditions).

[z] Incubation in atmosphere of water-saturated nitrogen (anaerobic conditions).

TABLE 2. Decay of potato tissue following inoculation of *Erwinia carotovora* subsp. *carotovora* into tubers of *Solanum tuberosum* cultivars, parental fusion line *S. tuberosum* (PI 203900), somatic hybrids of *S. tuberosum*, and *S. brevidens* and their sexual progeny

	Numbers of tubers with symptoms of infection[x]			Average width of rotting tissue (mm)[x]			Mean value (mm)[y]
Test plants (1986)	10^5	10^7	10^9	10^5	10^7	10^9	
S. tuberosum 'Russet Burbank'	1	5	10	11.0	11.1	11.8	6.1 b
S. tuberosum 'Katahdin' (Kat.)	3	6	9	13.0	15.2	13.5	9.8 a
S. tuberosum PI 203900 fusion parent	3	8	9	12.0	13.8	13.5	9.2 a
Somatic hybrids							
#206	0	0	2	0.0	0.0	6.0	0.4 cd
#249	0	0	0	0.0	0.0	0.0	0.0 d
#937	0	0	0	0.0	0.0	0.0	0.0 d
Sexual progeny							
4,707 (206 × Kat.)	2	8	10	7.0	10.5	10.6	6.8 ab
4,708 (206 × Kat.)	6	8	10	9.7	10.3	10.8	8.3 a
4,709 (206 × Kat.)	0	1	3	0.0	7.0	6.0	0.8 cd
4,676 (937 × Kat.)	0	2	7	0.0	8.5	7.5	2.3 c
4,678 (937 × Kat.)	0	0	3	0.0	0.0	6.3	0.6 cd
4,807 (937 × Kat.)	0	0	2	0.0	0.0	6.0	0.4 cd
M264(206 × Kat.) × Kat.	1	0	5	10.0	0.0	9.8	1.9 cd
M261(206 × Kat.) × Kat.	0	0	0	0.0	0.0	0.0	0.0 d

[x] Injection of 25 μl of three dilutions of Ecc suspension (1×10^5, 1×10^7, and 1×10^9 cfu/ml.) Measurements were taken after incubation in dew chamber for 72 hr at 22 C, relative humidity 92%. Tubers were sliced at the inoculation sites and width of the soft rotted tissue measured.

[y] Values are overall means of decay widths (10 tubers per sample) for all concentrations together. Means with the same letter are not significantly different, $P = 0.05$.

caused by *E. carotovora*.

An overall summary of results obtained from inoculation of tubers after mechanical bruising is given in Table 4. As was observed with direct inoculations by the pipette tip point inoculation procedure, the tubers of somatic hybrids and some of their sexual progeny showed greater resistance to *Erwinia* soft rot than the fusion parent and cultivars. Thus, these results provide additional evidence for the sexual transfer of *Erwinia* resistance incorporated by protoplast fusion.

DISCUSSION

The results presented above indicate that tubers from somatic hybrids between *S. tuberosum* and *S. brevidens* possess resistance to bacterial soft rot. Furthermore, tubers were resistant to three different species of *Erwinia* (Table 1). Previous work on several potato cultivars indicates that generally cultivars show a similar level of resistance to both *E. c. carotovora* and *E. c. atroseptica* (20). However, only limited research has been reported on comparative susceptibility of potato tubers to soft rot caused by subspecies of *E. carotovora* and *E. chrysanthemi*.

Tubers from somatic hybrid plants and some of their sexual progeny were significantly more resistant to bacterial soft rot than tubers from the parental line and commercial cultivars under a wide range of inoculum levels (from 10^5 to 10^9 cfu/ml) (Table 2). At the highest inoculum level, the wound response was more marked, and a dry necrotic lesion formed at the injection site. Intense browning was also evident at the edge of the necrotic area. The affected tissue did not show characteristics of typical soft rot maceration. This type of reaction has not been described previously by investigators screening potato tubers for soft rot resistance.

A high resistance to soft rot *Erwinia* was observed in somatic hybrid material (Table 3) even under the low oxygen conditions that are favorable for tissue maceration (21). Tubers harvested both in 1986 and in 1987 were evaluated under ambient and low oxygen levels. The results from both years were generally consistent (Table 3). Resistance in somatic hybrid tubers was also evident when tubers were inoculated after mechanical bruising and incubated in a mist chamber. Mist chamber conditions are highly favorable for disease development because presence of a film of water on tuber surface results in a decrease in availability of oxygen (21) (Table 4).

Thus, we have demonstrated that somatic hybrids show a higher level of resistance to *Erwinia* soft rot than the *S. tuberosum* fusion parent and potato cultivars after different types of injury and after incubation under different conditions. This high level of resistance when compared with the cultivars tested is also present in some of the sexual progeny derived from different somatic hybrids.

Because the presumed source of resistance, the nontuber-bearing *S. brevidens*, cannot be evaluated for tuber resistance directly, we have been unable to determine unequivocally that the wild species possesses a gene (or genes) specific for *Erwinia* resistance in tubers. It is possible that other features of the *S. brevidens* genome interact with those of *S. tuberosum* in the formation of tubers that have physiologically based resistance. For example, among other factors, susceptibility of tubers of a given cultivar has been associated with low levels of calcium in the tissue (22,29) or abnormally high reducing sugar levels (25). However, preliminary data indicate that these two characteristics in somatic hybrids and their progeny fall within expected normal ranges and are similar to those found in the susceptible fusion parent. Furthermore, the fact that some of the sexual progeny are resistant, whereas others are susceptible, indicates that a genetic rather than a strictly physiological or nutritional explanation is more likely.

The genetic basis of the resistance to soft rot is not well known, and some investigators describe it to be complex. Immunity has not been found either in potato cultivars (7,11,19,20,22,29,31) or in accessions of wild *Solanum* species (30,31).

Sexual incompatibility between species often limits the transfer of germplasm in conventional breeding schemes. Our results indicate that somatic fusion could be a useful way to bypass this incompatibility and, thereby, obtain access to many useful genes. The incorporation by protoplast fusion of a useful agronomic character and its transfer through two sexual generations has been achieved. Furthermore, the findings of resistance in *S. brevidens* was quite unexpected and, therefore, protoplast fusion has been useful in uncovering a previously unknown source of resistance. The materials obtained are valuable not only for direct potential use in breeding programs, but also for the examination of the transmission genetics of somatic hybrids. In addition to *Erwinia* resistance, the somatic hybrids have resistance to potato leafroll virus (from *S. brevidens*) and to race 0 of *Phytophthora infestans* (from *S. tuberosum*) (18). Preliminary studies indicate that the latter resistance can also be transferred to sexual progeny of the somatic hybrids.

TABLE 4. Comparison of tubers of *Solanum tuberosum* cultivars, parental fusion line, somatic hybrids and their sexual progeny for the resistance to soft rot *Erwinia* after inoculation at bruised sites

Test plant (1987)	Number of lines screened	Number of resistant lines[a]	Percent of lines resistant	Decay width (mm)[b]
S. tuberosum 'Russet Burbank'	1	0	0	18.5
S. tuberosum 'Katahdin' (Kat.)	1	0	0	19.3
S. tuberosum PI 203900 fusion parent	1	0	0	23.1
Somatic hybrids	8	8	100	8.3
Somatic hybrids × Kat.	19	8	42	14.4
(Somatic hybrids × Kat.) × Kat.[c]	14	3	21	17.5

[a] Lines with mean width of decayed tissue below 13.5 mm are considered resistant to soft rot on the basis of statistical analysis, using a Kruskal-Wallis test and the multiple comparisons, completed for all screened lines together.

[b] Measurements were taken at bruise site after incubation for 72 hr at 22 C in mist chamber; tubers were sliced at the inoculation site and width of soft rotted tissue measured. Bruised site was inoculated with 10 µl of Ecc suspension (5×10^8 cfu/ml). Values are overall means (10 tubers per lines) from all lines in each category together.

[c] Of the 14 lines tested seven were derived from resistant somatic hybrid × Kat. lines and seven from susceptible somatic hybrid × Kat. lines.

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