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## Resistance to *Phytophthora infestans* in somatic hybrids of *Solanum nigrum* L. and diploid potato

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**Abstract** In breeding for resistance to late blight, (*Phytophthora infestans* Mont. de Bary), an economically important disease affecting potatoes, the search for new sources of durable resistance includes the non-host wild *Solanum* species. The aim of this work was to evaluate the resistance to *P. infestans* in the somatic hybrids between *S. nigrum* L. and diploid potato clone ZEL-1136. Sixteen somatic hybrids, their fusion parents, and three standard potato cultivars were screened for resistance to *P. infestans* in two types of tests—on whole plants and detached leaves—with two highly aggressive and virulent isolates of *P. infestans*, US8 and MP322. In the whole plant assay, the foliage of the somatic hybrids showed no symptoms of infection, while the foliage of the potato fusion parent and the standard cultivars was infected with *P. infestans*. In the detached leaflet assay, the breaking-down of resistance of the *S. nigrum* L. parent and the variable response of individual hybrid clones were noted. Nine *S. nigrum* L. (+) ZEL-1136 hybrids showed a resistance that was significantly higher than that of *S. nigrum*, while six clones expressed a resistance to *P. infestans* similar to that of *S. nigrum*. The results confirm the effective transfer of late blight resistance of *S. nigrum* into its somatic hybrids with potato.

**Keywords** *Solanum nigrum* L. · Potato · Somatic hybrids · *Phytophthora infestans*

### Introduction

Resistance to late blight (*Phytophthora infestans* Mont. de Bary), the most devastating disease affecting potatoes world-wide, is one of the major traits being selected for in breeding programs. An important element of breeding for resistance is the availability of various sources of resistance. Several resistance sources are currently utilized in potato breeding programs. The resistance of the common potato cultivars originates mainly from *Solanum demissum* and *S. stoloniferum*, but backcrossing programs are also being realized with *S. papita*, *S. polytrichon*, *S. verrucosum* and *S. bulbocastanum* (Świeżyński and Zimnoch-Guzowska 2001). A change in the *P. infestans* population outside Mexico, a place of pathogen origin, has resulted in a new population of the pathogen with certain characters being of concern in resistance breeding. An increased frequency of the A2 mating type as well as an increased frequency of virulence genes and a better fitness of this new population of *P. infestans* relative to its predecessor confirm the necessity of searching for new sources of resistance with a higher probability of durable expression (Umaerus and Umaerus 1994).

*S. nigrum* is a wild non-tuber bearing species with a high level of late blight resistance of a non-host type (Colon et al. 1993). As a non-host to *P. infestans*, this common weed survives in the infected fields and has remained durably resistant since the introduction of *P. infestans* to Europe 150 years ago. Laboratory inoculation of *S. nigrum* with *P. infestans* showed penetration of the leaf epidermis accompanied by rapid cell death (hypersensitive response) of the penetrated plant cells (Kamoun et al. 1999). A few sexual hybrids of *S. nigrum* with *S. tuberosum* were produced by Eijlander and Stiekema (1994) by applying the embryo rescue technique, but the hybrids were highly sterile and thus not useful for potato breeding. Nevertheless, the late blight resistance was proved to be transferred to its sexual hybrids with *S. tuberosum*, where it behaved as a dominant character (Colon et al. 1993). Horsman et al. (1997) obtained numerous somatic hybrids between four species of the *S.*

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*nigrum* and different genotypes of potato. Resistance to late blight of a selected somatic hybrid, its BC<sub>1</sub> clone and eight BC<sub>2</sub> clones was tested with a drop inoculation method. Both the somatic hybrid and the BC<sub>1</sub> clone were as resistant as the *S. nigrum* fusion parent, with some necrotic spots observed at the inoculation site. Out of eight BC<sub>2</sub> clones, six were slightly infected with either necrotic spots or slightly necrotic regions formed, while two clones developed lesions (Horsman et al. 2000).

The aim of the investigation reported here was to evaluate the resistance to *P. infestans* in the somatic hybrids between *S. nigrum* L. and potato diploid clone. The regeneration, identification and verification of the hybrids based on leaf and flower morphology and flow cytometry and RAPD analyses have been described previously (Szczerbakowa et al. 2003). The hybrids produced did not contain alien selectable markers. The most vigorous somatic hybrids were selected for assessment of their late blight resistance.

## Materials and methods

### Plant material

Material was represented by 16 somatic hybrids designated as numbers of NT98, their fusion parents, *Solanum nigrum* L., and the diploid potato clone ZEL-1136, and three potato cultivars used as standards in tests for resistance to *P. infestans*: susceptible Irys and resistant Bzura and Meduza.

The in vitro culture of *S. nigrum* L. was started from seeds received from Warsaw Botanical Garden, Polish Academy of Sciences, Poland.

The diploid clone ZEL-1136, susceptible to *P. infestans*, originated from crosses between dihaploids of *S. tuberosum* and the wild species *S. chacoense* and *S. yungasense*. It was kindly supplied by Dr. J. Jakubiec (Warsaw Agricultural University, Poland).

### Assessment for late blight resistance

Greenhouse-grown plants were assessed for late blight resistance in both leaflet tests and following infection of the whole plants. The leaflet tests were performed according to Świeżyński et al. (1991). The leaflets (five leaflets per clone, in three replicates on three different dates) were detached from the plants and drop-inoculated with two highly aggressive isolates of *P. infestans* characterized by a complex virulence spectrum: (1) US8 (initial virulence factors v1, v2, v3, v4, v6, v7, v8, v10, v11 and A<sub>2</sub> mating type) from Cornell University, USA; (2) MP322 (virulence factors v1, v2, v3, v4, v(5), v6, v7, v(8), v(9), v10, v11 and A<sub>1</sub> mating type) from PIOR Koszalin, Poland. Along with the screening tests, the virulence expression of the isolates applied was evaluated in each test in relation to the widely used Black's differentials carrying *R*-genes from *R1* to *R11* (Black et al. 1953). The concentrations of inoculum applied onto detached leaflets were 50, 100, 200, and 400 sporangia mm<sup>-3</sup>.

The whole plants were assessed for late blight resistance under growth chamber conditions according to the method described by Umaerus (1969) with both of the isolates used for inoculation being applied at an inoculum concentration 50 sporangia mm<sup>-3</sup>.

The rate of infection was expressed on a 1 to 9 scale, where 9 means resistant (Zarzycka 2001). Scoring was carried out after 6 days of incubation in all of the tests with both screening methods used. Based on the official description, susceptible standard cv. Irys has grade 3 resistance, while resistant cvs. Bzura and Meduza have

grade 8. Data collected for leaflet assessment were analyzed in three-factorial analysis of variance, based on replication means and a completely randomized design.

### Crossing experiments

Somatic hybrids expressing the highest level of resistance to late blight in the detached leaflet assay were sexually backcrossed to potato in 2001 and 2002. NT-98 clones nos. 2, 6, 57, 69, 109, 127 and 129 grown in pots in a greenhouse were pollinated in 2001 with two diploid interspecific hybrids (2x) and in 2002 with two *S. phureja* clones (2x), one *S. hougasii* clone (6x) and two cultivars, Accent and Triada (4x). In 2002, an immature seeds rescue technique was applied to supplement natural seed set.

## Results

In all of the tests performed with both methods the inocula of both isolates, US8 and MP322, were found to be highly virulent, expressing 8–10 of the 11 virulence factors tested.

The reaction of standard cultivars to *P. infestans* indicated a strong infection pressure in the detached leaflet assays: the resistant control cv. Bzura (officially scored as 8) was strongly affected, even when the lowest concentration of inoculum (50 sporangia mm<sup>-3</sup>) was applied. In this experiment cv. Bzura was scored as 3.6 with isolate US8 and 2.9 with isolate MP322 (Table 1). This higher susceptibility of cv. Bzura in the detached leaflet assay in relation to its official scoring has also been noted previously (Zimnoch-Guzowska et al. 2000). Susceptible control cv. Irys (officially scored as 3) did react according to the expectations. Our scoring of cv. Meduza (8), the second resistant control, at the lowest inoculum concentration was in agreement with its official resistance level (7.8 and 8.0 for US8 and MP322, respectively). The application of increased concentrations of inoculum evoked a stronger infection of the tested material in general, although this was not always true for the individual plants tested. Significant differences were found using the Tuckey test for general means of concentrations applied for both isolates. In individual tests, the ranking of the genotypes tested was usually the same on different dates and for different inoculum concentrations. Three-factorial analysis of variance revealed a significance of genotypes and concentrations. Since the interactions genotype × isolate, and genotype × inoculum concentration were found to be insignificant, the levels of infection of the tested genotypes were presented by the means of pooled data from all the tests (i.e. all dates and inoculum concentrations). In the detached leaflet assays, the infection data with isolates US8 and MP322 were highly correlated ( $r = 0.99$ ), what allowed us to discuss the resistance level of the somatic hybrids using the overall means for both isolates.

An important result was the breaking-down of resistance of the resistant fusion parent *S. nigrum* L. in detached leaflet assay by both isolates applied at all dates of testing and at all inoculum concentrations (Table 1).

**Table 1** Resistance to *Phytophthora infestans* of the *Solanum nigrum* L. (+) ZEL-1136 resistant). For the detached leaflet assay, the means of three testing dates  $\pm$ SD for the somatic hybrids, their parents and standard cultivars in detached leaflet and whole plant respective isolates and the overall means (compared using Tukey test) are given. For the assays with two isolates, US8 and MP322, and an inoculum concentration of 50 whole plant assay, overall means  $\pm$ SD for two or three plants and the two isolates applied sporangia  $\text{mm}^{-3}$ . In the detached leaflet assay, additional inoculum concentrations of 100, are given. (SD standard deviation)

Genotype	Late blight resistance score $\pm$ SD										Overall mean <sup>a</sup>	Whole plant
	Detached leaflet					MP322 isolate						
	US8 isolate	100	200	400	Mean	50	100	200	400	Mean		
<i>S. nigrum</i> L. <sup>b</sup>	7.7 $\pm$ 0.9	7.0 $\pm$ 0.9	6.6 $\pm$ 1.5	5.8 $\pm$ 0.4	6.8	6.5 $\pm$ 1.5	6.0 $\pm$ 0.8	5.6 $\pm$ 1.4	5.1 $\pm$ 0.1	5.8	6.29 e, f	9.0 $\pm$ 0.0
ZEL-1136 <sup>b</sup>	2.6 $\pm$ 0.2	1.8 $\pm$ 0.8	1.5 $\pm$ 0.9	1.5 $\pm$ 0.9	1.9	1.8 $\pm$ 0.6	1.5 $\pm$ 0.9	1.5 $\pm$ 0.9	1.5 $\pm$ 0.9	1.6	1.72 h	5.7 $\pm$ 1.7
NT98-129	9.0 $\pm$ 0.0	9.0 $\pm$ 0.0	8.9 $\pm$ 0.4	9.0 $\pm$ 0.0	9.0	9.0 $\pm$ 0.0	9.0 $\pm$ 0.0	9.0 $\pm$ 0.0	8.7 $\pm$ 1.0	8.9	8.95 a	9.0 $\pm$ 0.0
NT98-57	9.0 $\pm$ 0.0	8.9 $\pm$ 0.3	9.0 $\pm$ 0.0	9.0 $\pm$ 0.0	9.0	9.0 $\pm$ 0.0	8.8 $\pm$ 0.6	8.9 $\pm$ 0.5	8.4 $\pm$ 1.1	8.8	8.88 a	9.0 $\pm$ 0.0
NT98-6	9.0 $\pm$ 0.0	9.0 $\pm$ 0.0	8.9 $\pm$ 0.3	8.1 $\pm$ 1.4	8.8	9.0 $\pm$ 0.0	8.7 $\pm$ 0.7	9.0 $\pm$ 0.0	8.7 $\pm$ 0.6	8.9	8.82 a	9.0 $\pm$ 0.0
NT98-69 <sup>b</sup>	8.9 $\pm$ 0.2	8.9 $\pm$ 0.2	9.0 $\pm$ 0.0	8.3 $\pm$ 0.2	8.8	8.9 $\pm$ 0.2	9.0 $\pm$ 0.0	8.7 $\pm$ 0.3	8.7 $\pm$ 0.1	8.8	8.79 a	9.0 $\pm$ 0.0
NT98-109	8.6 $\pm$ 0.8	9.0 $\pm$ 0.0	9.0 $\pm$ 0.0	8.1 $\pm$ 1.7	8.7	9.0 $\pm$ 0.0	9.0 $\pm$ 0.0	8.1 $\pm$ 2.0	8.4 $\pm$ 1.3	8.6	8.64 a, b	9.0 $\pm$ 0.0
NT98-2	8.7 $\pm$ 1.0	8.8 $\pm$ 0.4	8.6 $\pm$ 0.5	7.7 $\pm$ 1.6	8.5	8.5 $\pm$ 2.1	9.0 $\pm$ 0.0	8.1 $\pm$ 0.9	8.3 $\pm$ 1.2	8.5	8.47 a, b, c	9.0 $\pm$ 0.0
NT98-118	9.0 $\pm$ 0.0	8.3 $\pm$ 1.0	8.1 $\pm$ 1.0	7.7 $\pm$ 1.0	8.3	9.0 $\pm$ 0.0	8.4 $\pm$ 0.9	7.0 $\pm$ 0.0	7.6 $\pm$ 1.2	8.0	8.13 a, b, c, d	9.0 $\pm$ 0.0
NT98-81	9.0 $\pm$ 0.0	8.7 $\pm$ 0.5	7.8 $\pm$ 1.3	7.3 $\pm$ 1.3	8.2	8.3 $\pm$ 1.0	8.6 $\pm$ 0.6	7.2 $\pm$ 1.1	7.7 $\pm$ 0.6	8.0	8.08 a, b, c, d	9.0 $\pm$ 0.0
NT98-119	8.7 $\pm$ 0.8	8.5 $\pm$ 1.0	7.5 $\pm$ 1.9	7.5 $\pm$ 2.4	8.0	8.7 $\pm$ 0.7	8.6 $\pm$ 0.7	7.8 $\pm$ 1.6	6.8 $\pm$ 1.7	8.0	8.01 a, b, c, d	9.0 $\pm$ 0.0
NT98-117	8.4 $\pm$ 1.4	7.6 $\pm$ 1.9	7.7 $\pm$ 1.9	6.7 $\pm$ 2.2	7.6	7.9 $\pm$ 1.6	7.1 $\pm$ 1.9	7.4 $\pm$ 1.8	6.7 $\pm$ 2.3	7.3	7.45 b, c, d, e	9.0 $\pm$ 0.0
NT98-130	9.0 $\pm$ 0.0	6.5 $\pm$ 2.0	7.1 $\pm$ 2.2	7.3 $\pm$ 1.6	7.5	7.6 $\pm$ 1.7	7.9 $\pm$ 1.0	6.8 $\pm$ 1.6	6.7 $\pm$ 1.8	7.3	7.38 b, c, d, e	9.0 $\pm$ 0.0
NT98-3	9.0 $\pm$ 0.0	7.2 $\pm$ 1.3	6.2 $\pm$ 1.7	6.1 $\pm$ 1.0	7.1	8.2 $\pm$ 1.3	7.1 $\pm$ 0.9	6.7 $\pm$ 0.9	7.0 $\pm$ 1.7	7.3	7.18 c, d, e	9.0 $\pm$ 0.0
NT98-30	7.7 $\pm$ 1.9	6.7 $\pm$ 2.5	7.1 $\pm$ 1.8	5.5 $\pm$ 2.0	6.7	7.7 $\pm$ 1.9	6.3 $\pm$ 2.4	6.3 $\pm$ 2.3	5.1 $\pm$ 0.8	6.4	6.55 e, f	9.0 $\pm$ 0.0
NT98-128	8.1 $\pm$ 1.8	7.1 $\pm$ 2.5	5.7 $\pm$ 0.5	5.0 $\pm$ 1.5	6.5	7.0 $\pm$ 2.2	5.9 $\pm$ 2.2	6.0 $\pm$ 2.0	5.3 $\pm$ 1.8	6.1	6.27 e, f	9.0 $\pm$ 0.0
NT98-115	6.5 $\pm$ 2.2	5.7 $\pm$ 2.8	6.5 $\pm$ 2.6	4.7 $\pm$ 2.4	5.8	6.6 $\pm$ 2.4	4.9 $\pm$ 1.9	5.9 $\pm$ 2.7	4.3 $\pm$ 1.5	5.4	5.63 f, g	9.0 $\pm$ 0.0
NT98-5	6.1 $\pm$ 2.1	5.0 $\pm$ 2.0	5.3 $\pm$ 2.0	4.7 $\pm$ 2.4	5.3	5.5 $\pm$ 1.6	4.0 $\pm$ 1.5	4.2 $\pm$ 1.6	3.9 $\pm$ 1.6	4.4	4.85 g	9.0 $\pm$ 0.0
cv. Meduza	7.8 $\pm$ 1.8	7.7 $\pm$ 0.5	6.2 $\pm$ 1.6	5.8 $\pm$ 1.7	6.9	8.0 $\pm$ 1.1	8.2 $\pm$ 0.8	5.7 $\pm$ 1.0	6.7 $\pm$ 1.5	7.1	7.00 d, e	8.3 $\pm$ 0.4
cv. Bzura <sup>b</sup>	3.6 $\pm$ 0.3	1.8 $\pm$ 0.6	1.9 $\pm$ 1.0	1.6 $\pm$ 0.6	2.2	2.9 $\pm$ 0.1	1.9 $\pm$ 0.8	1.8 $\pm$ 0.7	2.2 $\pm$ 0.6	2.2	2.22 h	5.5 $\pm$ 0.7
cv. Irys <sup>b</sup>	2.7 $\pm$ 0.4	1.9 $\pm$ 0.3	1.5 $\pm$ 0.5	1.5 $\pm$ 0.5	1.9	2.5 $\pm$ 0.2	1.9 $\pm$ 0.3	1.8 $\pm$ 0.8	2.7 $\pm$ 0.2	2.2	2.06 h	2.5 $\pm$ 0.7
Mean <sup>a</sup>	7.6 m	6.9 n	6.7 n	6.1 o	6.8	7.2 m	6.8 m, n	6.4 n, o	6.2 o	6.6	6.75	

<sup>a</sup> Means marked by the same letter are not significantly different at  $P = 0.05$  (Tukey test)

<sup>b</sup> For these genotypes, an additional set of experiments was performed, and the data were pooled over the two sets of experiments



**Fig. 1** Leaflet of *Solanum nigrum* L. infected with *Phytophthora infestans* (with noted sporulation) in a detached leaf assay

The resistant parent was scored as 6.29. As well as the hypersensitive response, a slight sporulation of *P. infestans* on the blight lesions of the *S. nigrum* leaves was also observed. The inoculated *S. nigrum* L. leaves became more red due to anthocyanin accumulation (Fig. 1). Of 16 somatic hybrids tested, nine expressed a significantly higher level of late blight resistance in detached leaflets than the wild parent *S. nigrum* (the NT98 hybrids nos. 2, 6, 57, 69, 81, 109, 118, 119 and 129). Their overall means varied from 8.01 to 8.95. The six hybrids did react in the leaflet assay at the level of resistant parent *S. nigrum* with grades varying from 5.63 to 7.45. The last clone, NT98-5, was intermediate in its resistance reaction between both parents.

In the whole plant tests, no symptoms of infection were developed either by the somatic hybrids or by the *S. nigrum* L. parent (Table 1). The whole set of 16 hybrids tested, as well as their resistant parent, were scored as 9.0 in all individual plants and for both isolates applied. The susceptible parent, clone ZEL-1136, was scored as 5.7. Resistant cv. Meduza and susceptible cv. Irys were assessed as 8.3 and 2.5, respectively, which corresponds

to their official level of resistance, while resistant cv. Bzura was scored as 5.5.

In all the tests with both methods applied, *S. nigrum* as well as all of the somatic hybrids were significantly superior in resistance to clone ZEL-1136 as well as to two of the three standard cultivars, demonstrating the introgression of resistance to late blight disease into the *S. nigrum* L. (+) ZEL-1136 somatic hybrids.

#### Crossing experiments

Seven *S. nigrum* L. (+) ZEL-1136 hybrids, all resistant to late blight, were used in backcross experiments. Since the hybrids represented variable and rather high (up to 10x) ploidy levels, as judged from flow cytometry analysis (Szczerbakowa et al. 2003), the superior diploid pollinators were used to reduce the ploidy level. In addition, the clone of *S. hougasii* (6x) and two cultivars (4x) were used as pollinators. In 2001, 389 berries were formed from over 500 pollinations made. All of the tested hybrids had a relatively high berry set, but all the berries were seedless. Parthenocarpic berries also formed on hybrid plants without pollination. In 2002, over 2,000 berries were collected after 5,600 pollinations, from which 98 immature seeds with a diameter greater than 1 mm were rescued and transferred onto Neal and Topoleski (1983) medium for further development. Seeds were formed when two cultivars (cvs. Accent and Triada) were used as pollinators. This part of experiment is still in progress.

#### Discussion

The *S. nigrum* L. (+) ZEL-1136 somatic hybrids that were generated retained the high resistance of the *S. nigrum* L. parent. In the whole plant assay, the foliage of the somatic hybrids had the same high level of resistance as the wild fusion parent—9 on the scale of 1–9—while the foliage of the potato fusion parent and susceptible control cultivars was infected with *P. infestans*. In this type of testing, *S. nigrum* L. and its hybrids reacted according to the assumed non-host character of resistance to *P. infestans*.

An increasing concentration of inoculum was used in order to check the resistance of the hybrids and the *S. nigrum* fusion parent in the leaflet assay. Of the 16 somatic hybrids tested, nine were found to be significantly more resistant than the resistant fusion parent and set of standards, while six hybrids expressed a resistance that was not different from that of *S. nigrum* (Table 1). Quite unexpectedly, the breaking-down of resistance of the *S. nigrum* L. parent occurred in detached leaflets even with the standard concentration of inoculum (50 sporangia mm<sup>-3</sup>). The inoculum used was highly aggressive and virulent. Leaflets of *S. nigrum* were infected and some sporulation of *P. infestans* on blight lesions was observed in addition to the hypersensitive response expressed as necrotic spots. Colon et al. (1993) also observed the slight production of sporangiophores with sporangia, in addition

to chlorosis and necrotic spots, on leaflets of sexual hybrids of *S. nigrum* ssp. *schultesii* and diploid potato after inoculation with *P. infestans*, while Kamoun et al. (1999) noticed only hypersensitive response of *S. nigrum* after its laboratory inoculation with *P. infestans*. Hirst and Stedman (1960) mentioned the isolation of *P. infestans* from shaded leaves of *Solanum nigrum*.

It is possible that both the interaction of the plasmons and the phenomenon of complementation of nuclear genes are important for expressing the higher level of leaflet resistance in the fusion progenies in comparison to that present in the resistant fusion parent *S. nigrum* L.

Helgeson et al. (1986, 1993) showed the resistance to *P. infestans* race 0 in somatic hybrids of *S. brevidens* (+) R4 Black's differential, although in this case the monogenic resistance from the R4 *S. tuberosum* partner was transferred to its hybrids with *S. brevidens*. Helgeson et al. (1998) subsequently successfully transmitted late blight resistance from *S. bulbocastanum* to tetraploid potato. The hexaploid somatic hybrids that were obtained retained the high resistance of the *S. bulbocastanum* parent.

In our case, the resistance of the *Solanum nigrum* L. (+) ZEL-1136 somatic hybrids to late blight was not correlated with differences in their morphology, presumably because of the strong domination of the wild genotype. Morphologically, the somatic hybrids with the highest resistance level belonged to group II, where the combination of characteristics from both parents was the most conspicuous (Szczerbakowa et al. 2003).

A transfer of late blight resistance incorporated into *Solanum nigrum* L. (+) ZEL-1136 somatic hybrids through sexual generations should be further attempted. The segregation in disease resistance to *P. infestans* would indicate the sexual transfer of the trait retained in the hybrids after protoplast fusion of potato with the wild species. Since the *Solanum nigrum* L. (+) ZEL-1136 hybrids seem to be male-sterile, the anther culture method may be inefficient in reducing chromosome set in the hybrids. The female fertility of the generated *Solanum nigrum* L. (+) ZEL-1136 hybrids would predispose them as the staminate parents in backcross experiments. It has been assumed that sexual crosses of the *Solanum nigrum* L. (+) ZEL-1136 hybrids with diploid potato would possibly reduce the chromosome number in BC<sub>1</sub> progeny, however the 2001 and 2002 crossings to 2x pollinators were not successful. In 2002, pollinations with 4x cultivars yielded 98 immature seeds that were transferred onto artificial medium for further development. The pollination with potato of *S. nigrum* (+) *S. tuberosum* somatic hybrids performed by Horsman et al. (2000) resulted in two BC<sub>1</sub> and one BC<sub>2</sub> clones after over 4,000 pollinations with tetraploids and the embryo rescue procedure. However, no progeny was obtained from BC<sub>2</sub> genotypes due to the still strong sexual crossing barrier. A somatic re-fusion procedure was then recommended for overcoming the backcross problems. An intensive backcrossing program with 4x and 6x pollinators combined with the embryo rescue technique, further

fusion experiments between *S. nigrum* L. and a set of new potato parents, and studies on resistance expressed by *S. nigrum* L. with various screening methods are the objectives of future research. In the case where the high resistance to *P. infestans* is retained following a reduction in chromosome number, the hybrids generated might be valuable for molecular studies on the genetic background of the late blight resistance.

In conclusion, the foliage of all of the tested *S. nigrum* L. (+) ZEL-1136 somatic hybrids retained a high resistance to *P. infestans* that was characteristic of the *S. nigrum* parent, although the resistance of *S. nigrum* was partly broken down in leaflet assay. This might indicate that the resistance of *S. nigrum* L. to *P. infestans* is not a non-host-type exclusively. Of the 16 somatic hybrids tested, nine expressed a resistance in leaflets that was superior to that of the resistant parent *S. nigrum*, which is not fully understood and might be explained by genetic interactions induced by the combination of the two parental genomes. The female fertility of *S. nigrum* L. (+) ZEL-1136 somatic hybrids could enable them to be used as female partners in a backcross program in order to continue studies on transferring the late blight resistance from the hybrids to their BC progeny as well as to introgress this source of resistance into the potato breeding pool.

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