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RESISTANCE TO *PHYTOPHTHORA INFESTANS* IN THREE *SOLANUM NIGRUM* F_3 FAMILIES

ABSTRACT

Solanum nigrum is a self-pollinating, hexaploid weed and one of a few *Solanaceae* species native to Europe. It used to be described as a non-host for *Phytophthora infestans*. However, now it is known that, like its distant relatives: potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.), *S. nigrum* can suffer from potato late blight caused by this pathogen. Both susceptible and resistant *S. nigrum* genotypes have been previously identified and inheritance of resistance originating from one accession has been described based on population of F_2 plants and 15 F_3 lines. The goal of this study was to evaluate resistance of three families of F_3 lines, originating from crosses between a susceptible and three different resistant *S. nigrum* accessions followed by two self-pollinations. Parental accessions were tested for the spectrum of late blight resistance against 48 *P. infestans* isolates. The three families consisted of 106, 96 and 115 F_3 lines, respectively, and from each line 20 plants were tested for resistance to *P. infestans*. Laboratory detached leaf assays were performed in two dates and two replications of three leaves each. Segregation of the trait within the line allowed us to distinguish hetero- and homozygous lines. In one F_3 family, the ratio of resistant homozygotes: heterozygotes: susceptible homozygotes was 1:2:1, indicating that a single gene is most likely underlying the late blight resistance in this case. In the other two, observed segregations of the trait significantly deviated from this model suggesting more complex inheritance patterns.

Key words: black nightshade, inheritance, late blight, potato, *Solanum tuberosum*, virulence

INTRODUCTION

Phytophthora infestans (Mont.) de Bary that causes potato late blight, is economically the most important pathogen of this staple crop worldwide. In

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Europe the total annual cost of crop losses, as well as cost of fungicides used for crop protection against late blight of potato and tomato is estimated to be around € 1 000 000 000 (Haverkort *et al.* 2008). Breeding potatoes resistant to *P. infestans* has long been the objective of breeders' efforts (Świeżyński and Zimnoch-Guzowska 2001). However, the first introduction of resistance genes (R genes) against *P. infestans* from the wild species *S. demissum* early in the 20th century brought disappointment, due to the rapid spread of *P. infestans* strains with the corresponding virulence factors. Potato cultivars containing these genes quickly became susceptible (Malcolmson 1969). With the gain of knowledge on plants' disease resistance mechanisms and with the development of genetic engineering techniques, the interest of scientist turned to alternative ways of obtaining effective and more durable resistance. One such idea was to exploit the non-host resistance of distantly related plant species.

Solanum nigrum L., black nightshade, is a hexaploid species originating most likely from spontaneous hybridization between tetraploid *S. villosum* and diploid *S. americanum*. This common weed used to be regarded as a non-host species for *P. infestans* (Colon *et al.* 1993, Platt 1999). Later, infected plants of *S. nigrum* were found in The Netherlands (Flier *et al.* 2003) and in Wales (Deahl *et al.* 2004). Lebecka (2008) has tested 38 accessions of *S. nigrum* of diverse origins by laboratory tests for late blight resistance. She has used two *P. infestans* isolates and 15 *S. nigrum* accessions have been scored as highly resistant to both of them showing either no symptoms of disease or necroses caused by a hypersensitivity response. One accession contained both a resistant and a susceptible plant, while the remaining 22 accessions have been infected with at least one of the two *P. infestans* isolates (Lebecka 2008). A resistant (N19) and a susceptible plant (#13) have been selected from this pool of *S. nigrum* accessions and crossed (Lebecka 2009). F_1 (50 plants), BC_1 (66 plants), F_2 (180 plants) and F_3 (15 lines, 20 plants from each line) have been tested for resistance to *P. infestans* in detached leaf assays. The obtained results of resistance tests supported a model in which the resistant parent had a single dominant resistance gene in a homozygous state in two of the six chromosomes (AAaaaa). The resistant alleles segregated in the tested generations as in a diploid organism, most likely due to the allohexaploid nature of *S. nigrum* and meiotic separation of the ancestral genomes (Lebecka 2009).

S. nigrum cannot be efficiently crossed with a cultivated potato and the transfer of its late blight resistance to potato cultivars has been severely limited by this fact. A few infertile sexual hybrids between these species have been obtained with the use of embryo rescue technique (Eijlander and Stiekema 1994). Somatic hybridization has also been used to enable an introgression of *S. nigrum* resistance into *S. tuberosum* gene pool, with very limited success (Horsman *et al.* 1997, Szczerbakowa *et al.* 2003, Zimnoch-

Guzowska *et al.* 2003). The complexity of the obtained somatic hybrids' genomes that can reach up to decaploid level seems to negatively affect their viability and fertility, hampering further backcrossing to potato. An alternative approach would be based on cloning of the genes underlying *S. nigrum* resistance to *P. infestans* followed by their direct transfer to potato. That way the problems with ploidy, fertility and linkage drag of unwanted wild alleles could be avoided. Our study focused on the first step towards identification and cloning of the late blight resistance genes from *S. nigrum*, that is on developing of material suitable for this purpose.

The goal of this study was to evaluate resistance of three families of F_3 lines, originating from crosses between a susceptible and three different resistant *S. nigrum* accessions from the group described by Lebecka (2008). We included an F_3 family described earlier (Lebecka 2009), that was extended in number of lines from 15 to 106. Parental accessions were tested for the spectrum of late blight resistance against 48 *P. infestans* isolates in order to evaluate their usefulness as the resistance sources.

MATERIAL AND METHODS

Plant material

A susceptible to *P. infestans* and three resistant *S. nigrum* accessions (Table 1) were selected as the parental forms from the work of Lebecka (2008). They were of diverse origins and they differed qualitatively in late blight resistance. The susceptible accession (13) was used as a seed parent, its flowers were emasculated and pollinated with pollen gathered from the resistant parents (N19, N9 and 24). These three crosses were named A, B and C, respectively, and they are described further in Table 2. F_1 plant from each cross was self-pollinated and so were the resulting F_2 plants. To prevent cross-pollinations these plants were isolated by plastic bags with micro perforations that allowed air circulation. Each F_2 plant was given a number that later served as a line number. Seeds of 106, 96 and 115 lines from families A, B and C, respectively, were sown and 20 plants per line were grown in the greenhouse and tested for late blight resistance. In total, 6340 plants were tested, divided into groups of 50 to 60 lines (1000-1200 plants) that were sown twice in each vegetation season of 2009, 2010 and 2011.

Table 1

Accessions of *S. nigrum* used in this study: origin, late blight resistance and source collection (Lebecka 2008)

Name	Accession	Origin	Late blight resistance	Source collection
13	-	Poland, Warszawa	susceptible	Warsaw University of Life Sciences, Poland
N19	984750019	Germany, Karlsruhe	resistant	Botanical Garden of Nijmegen, The Netherlands
N9	954750317	United Kingdom, Birmingham	resistant	Botanical Garden of Nijmegen, The Netherlands
24	SOL35/79	Sweden, Lund	resistant	Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany

P. infestans isolate

An isolate MP324 from the pathogen collection of IHAR-PIB Młochów (Śliwka *et al.* 2006) was used in all resistance tests. The isolate, collected in 1997 in Poland, was of A1 mating type, highly aggressive, metalaxyl resistant and of complex race (1.2.3.4.5.6.7.8.10.11) defined on a set of Black's differentials (Scottish Agricultural Science Agency, Edinburgh, UK). Before each resistance test, the isolate was propagated on susceptible potato tissue at least twice.

Detached leaf assay for resistance to P. infestans

Detached leaves were inoculated with a 30 µl droplet of *P. infestans* sporangia/zoospore suspension (50 sporangia/µl). After incubation of 6 days at 16ºC, in high humidity and under constant light of about 1600 lx, they were scored using a 1-9 scale, where 9 is maximum resistant (Śliwka *et al.* 2006). The detached leaf tests were replicated as follows: 3 leaves/plant × 2 replications × 2 dates, that is 12 leaves per plant were tested in total. A plant from family A or B was considered resistant, when its mean resistance score in detached leaf test was ≥ 8.0, i.e. it showed no symptoms of disease or necroses of hypersensitivity response. In family C, the cut-off value was shifted to 7.5, because we observed some expanded necroses that were scored as 7 but still could be regarded as hypersensitivity reaction. Lines that contained only resistant or susceptible plants were classified as homozygous, while the ones segregating for the trait were regarded as heterozygous. Within the three F_3 families, the fit of segregation to the expected ratio of resistant homozygotes: heterozygotes: susceptible homozygotes, 1: 2: 1, under the assumption of a single dominant gene as described by Lebecka (2009), was checked by a χ^2 test.

Resistance spectrum evaluation

The parental accessions of *S. nigrum*: 13, N19, N9 and 24 were tested for resistance spectrum in detached leaf assays performed with 48 *P. infestans* isolates. These isolates were all obtained from Poland in 2010, from two regions (mazowieckie and podkarpackie), different potato cultivars and various fields (commercial potato production, organic fields, home gardens, protected or unprotected against potato late blight). They differed in mating type, mitochondrial haplotype and metalaxyl sensitivity. Each isolate was used for inoculation of six leaves of each of the *S. nigrum* accessions and Black's differentials with 11 resistance genes against late blight (*R1-R11*) (SASA, Edinburgh, UK). After incubation in the same condition as for detached leaf assay described above, the interaction between plants and *P. infestans* isolates was scored as compatible (disease lesions present, the isolate virulent) or incompatible (either no symptoms of disease or small non-sporulating necroses of hypersensitivity response, the isolate avirulent).

RESULTS AND DISCUSSION

S. nigrum line 13 described previously as susceptible to *P. infestans* (Lebecka 2008) was also highly susceptible to this pathogen in our study. Out of 48 diverse *P. infestans* isolates tested, only nine were not able to infect the leaves of plants from this line in laboratory detached leaf test (Fig. 1). This suggests that although susceptible in laboratory assays, this *S. nigrum* line still bears some resistance that in certain conditions may be effective. In contrary, none of the tested isolates infected leaves of *S. nigrum* lines N9 and N19, and only one was capable to overcome the resistance of line 24 (Fig. 1). That is in agreement with results of Lebecka (2008) who tested the lines N9, N19 and 24 and noted that they were not infected with any of the two applied *P. infestans* isolates (MP324 and MP637). The lack or small number of *P. infestans* isolates virulent on *S. nigrum* N9, N19 and 24 indicate that those lines can be sources of valuable resistance with a high potential for application in obtaining late blight resistant potatoes and tomatoes. The virulence patterns of the 48 tested *P. infestans* isolates on *S. nigrum* lines do not resemble the patterns obtained on 11 Black's differentials (R1-R11) (Fig. 1) which shows that resistance spectra of *S. nigrum* lines are different from those provided by *S. demissum* resistance genes *R1-R11* and most likely are based on recognition of different effectors produced by the pathogen.

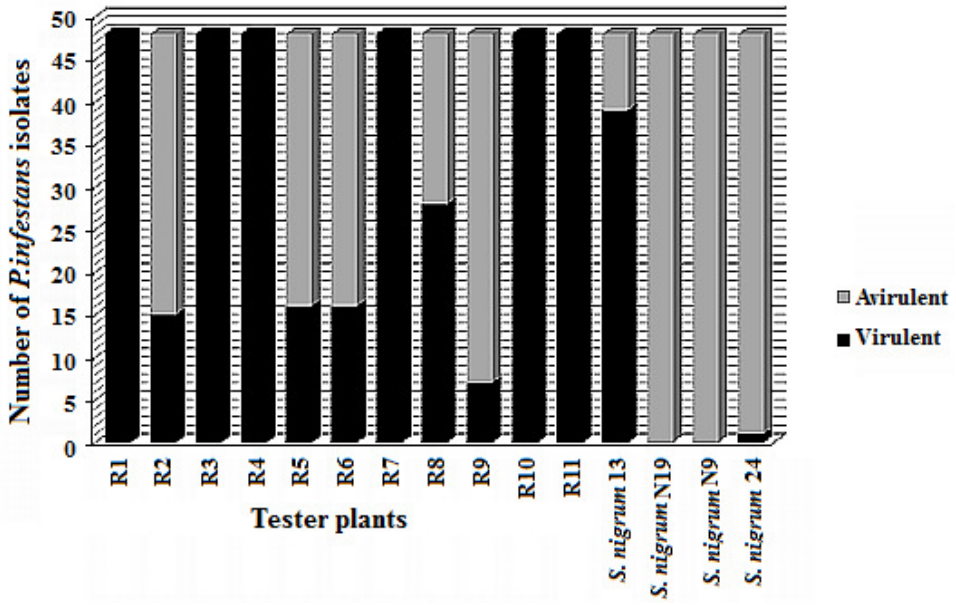

Fig. 1. Virulence of 48 *P. infestans* isolates on a set of Black's differentials (R1-R11) and the accessions of *S. nigrum* used in this study (13, N19, N9 and 24)

Table 2

Results of tests for resistance to *P. infestans* in the F_3 families obtained from crosses between a susceptible (S) and three resistant (R) *S. nigrum* accessions

Family	A	B	C
Cross	13 (S) × N19 (R)	13 (S) × N9 (R)	13 (S) × 24 (R)
Number of tested F_3 lines	106	96	115
Susceptible homozygotes	23	12	20
Heterozygotes	57	57	53
Resistant homozygotes	26	27	42
Expected segregation ratio homozygotes (R): heterozygotes: homozygotes (S) — 1:2:1	χ^2 =0.773, df=2; p < 0.679	χ^2=8.062, df=2; p < 0.018	χ^2=9.12, df=2; p < 0.02

In general, *S. nigrum* is not as susceptible to *P. infestans* as potato or tomato. Although, in our tests some leaves were scored with the most susceptible score 1, the mean resistance of the 317 tested lines from all three *S. nigrum* families A, B and C ranged from 5.4 to 8.9. The families differed

slightly in their size: family A contained 106 lines, B – 96 and C – 115 lines (Table 2). The mean late blight resistances in families A, B and C were 7.9, 8.3 and 7.3, respectively.

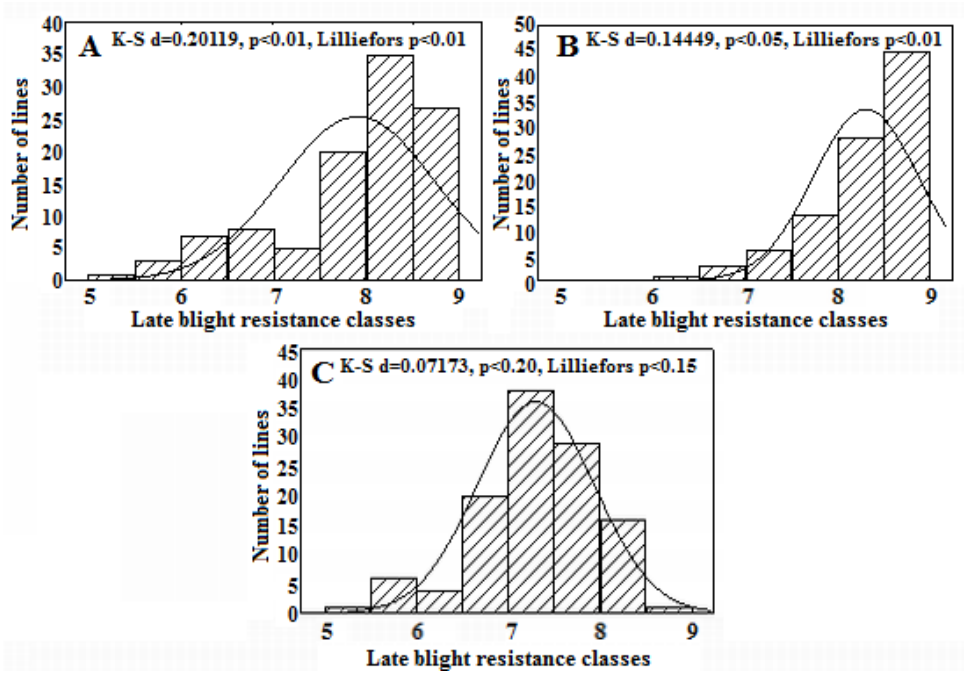

Fig. 2. Distributions of mean leaf resistance to *P. infestans* in three *S. nigrum* families: A, B and C, on 1-9 scale, where 9 is the most resistant. The fitness to the normal curve: K-S – Kolmogorov-Smirnov test (Smirnov 1944), d – coefficient calculated for this test, p – probability, Lilliefors test (Lilliefors 1967), p – probability, the line indicates the normal curve

In the family A, originating from a cross 13 × N19, the plants of 23 lines were all susceptible and therefore we classified these lines as susceptible homozygous ones (Table 2). Within other 26 lines all plants were resistant, which allowed us to recognize them as resistant homozygotes. The remaining 57 lines segregated for resistance to different extends which could be ascribed to the random effects caused by a small sample size (20 plants per line). The observed proportion of the homo- and heterozygous lines in family A was in agreement with the expected ratio of resistant homozygotes: heterozygotes: susceptible homozygotes, 1: 2: 1, according to the χ^2 test (Table 2). Moreover, the distribution of mean line resistance to *P. infestans* in this family was significantly deviated from the normal distribution and resembled rather a bimodal one (Fig. 2A). Both these findings support the hypothesis that a single dominant resistance gene descending from *S. nigrum* N19 is segregating in family A like in a progeny of a diploid organism, as described by Lebecka (2009). The classes of susceptible and resistant homozygotes and heterozygotes were quite distinct in family A (Fig. 3A), although the classification was based on testing of only 20 F_3 plants per line. While the mean resistance of susceptible homozygotes

ranged from 5.1 to 7.5, the overlap with the heterozygous class was limited to only five lines with mean resistance between 7.1 and 7.5. On the other side, the resistant homozygotes were all in a narrow range of resistance: 8.6 – 9.0 and only one heterozygous line was scored in this interval (Fig. 3A).

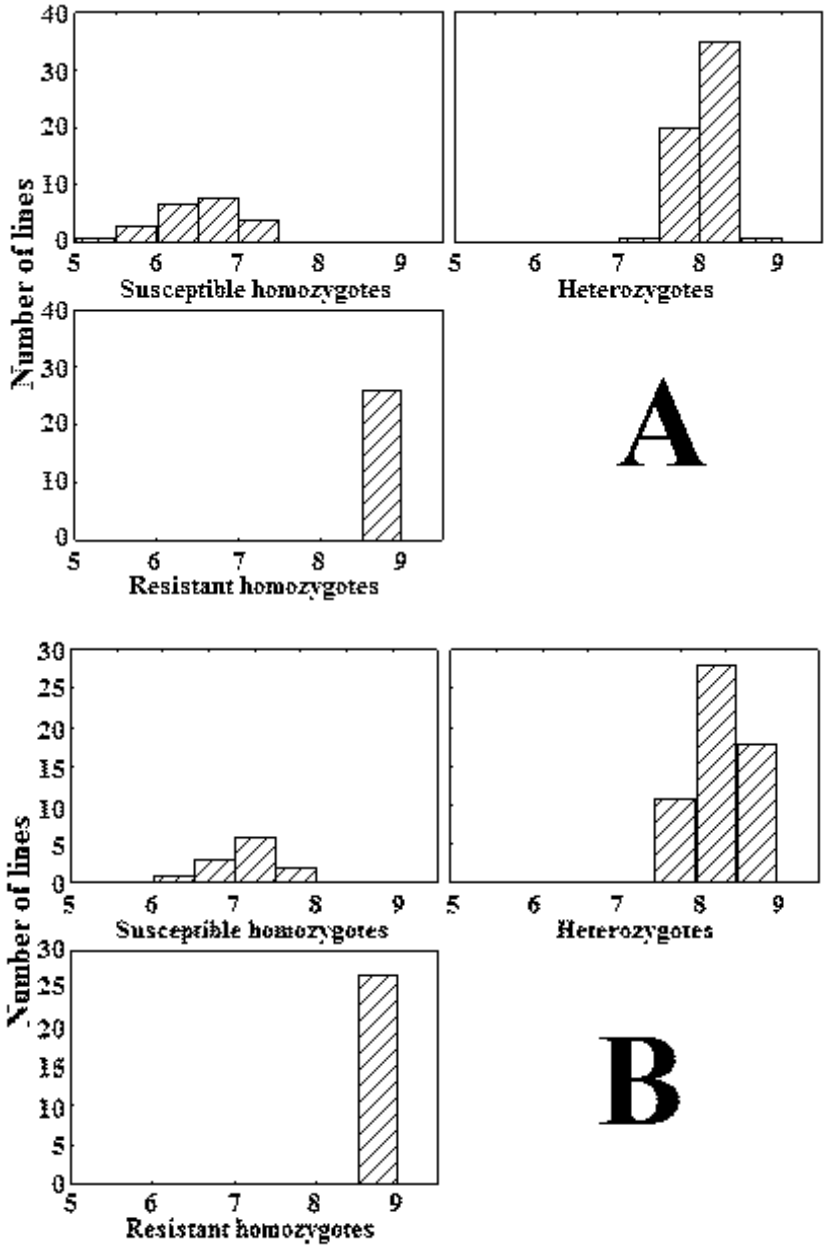

Fig. 3.

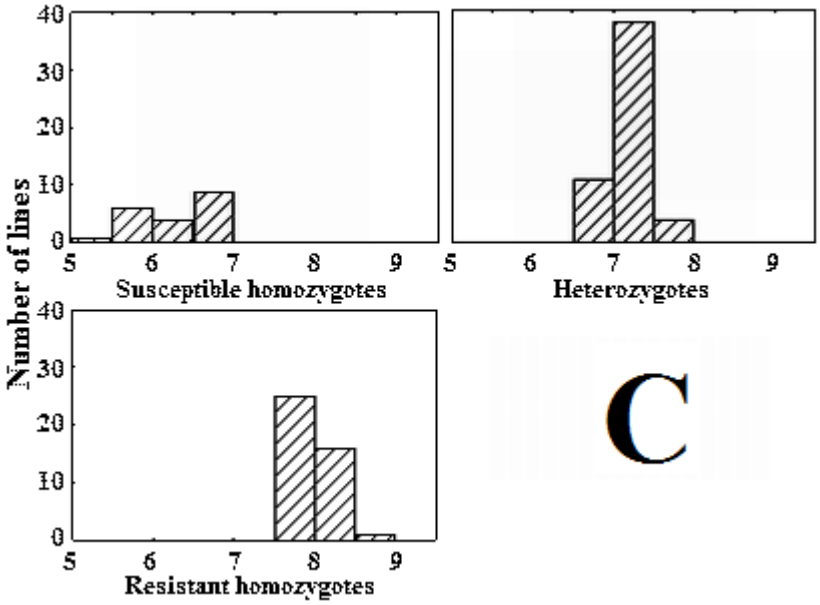

Fig. 3. Frequency histograms of mean leaf resistance to *P. infestans* in three *S. nigrum* families: A, B and C, on 1-9 scale, where 9 is the most resistant. *S. nigrum* lines were categorized into three groups: susceptible homozygotes, heterozygotes and resistant homozygotes within each family on the basis of segregation of late blight resistance in 20 F_3 plants from each line (homozygous lines: all 20 plants resistant or susceptible; heterozygous lines: both susceptible and resistant lines present)

In family B, from a cross 13 × N9, 12 lines were classified as susceptible and 27 as resistant homozygotes, while 57 lines were included into heterozygous group (Table 2), after applying the same procedure as described above for the family A. The observed segregation of late blight resistance was deviated in this case from the expected 1:2:1 ratio according to the χ^2 test (Table 2). The distribution of mean line resistance in family B was significantly deviated from the normal distribution and skewed towards higher resistance (Fig. 2B). Mean resistance scores of two susceptible homozygotes and 11 heterozygous lines were in the same interval of resistance scores 7.6 - 8.0. All 27 resistant homozygotes overlapped with heterozygotes, of which 18 were scored between 8.6 and 9.0 (Fig. 3B). These data suggest that most likely more than one gene is underlying the late blight resistance of *S. nigrum* line N9.

Family C from a cross 13 × 24 had the biggest number of lines tested (115) and the lowest mean resistance to *P. infestans* (7.3). The observed segregation of this trait in family C was deviated from the expected 1:2:1 ratio according to the χ^2 test (Table 2). The distribution of mean line resistance in this case was normal which was confirmed by Kolmogorov-Smirnov (Smirnov 1944) and Lilliefors (Lilliefors 1967) tests (Fig. 2C). There was an overlap of 20 lines with mean resistance 6.6 – 7.0 between

a susceptible homozygous class and a heterozygous one and of 29 lines with mean resistance 7.6 – 8.0 between heterozygous and resistant homozygous class (Fig. 3B). The lack of distinct classes i.e. big overlap between the alleged homo- and heterozygotes as well as normal distribution of the trait suggest that the late blight resistance of the *S. nigrum* line 24 is rather a quantitative character encoded by a number of Quantitative Trait Loci (QTL).

S. nigrum appears to be an interesting and rich source of resistance to *P. infestans*. In this study it was shown that some black nightshade accessions can rely on single dominant resistance gene (N19, family A), some can have most likely more than one effective R gene (N9, family B) or even quantitative resistance (24, family C). In any case the late blight resistance of *S. nigrum* was high and broad-spectrum. An important output of this study was obtaining the F_4 seeds of the lines from all three families. That was a step towards development of Recombinant Inbred Lines (RILs) that may serve as material for mapping and cloning of the genes underlying the late blight resistance. Cloning and cis- or transgenesis might be the only possible way of transfer of the resistance genes from *S. nigrum* to *S. tuberosum* or *S. lycopersicum*. Such approach has been applied in another distant *Solanum* relative – *S. dulcamara* that cannot be crossed with potato. Using intraspecific crosses two late blight resistance genes of this species have been mapped: *Rpi-dlc1* to chromosome IX (Golas *et al.* 2010) and *Rpi-dlc2* to chromosome X (Golas *et al.* 2012). What is interesting, mapping of *Rpi-dlc2* has been difficult due to the fact that in the mapping population both *Rpi-dlc1* and *Rpi-dlc2* were segregating and their phenotypic effects have been overlapping which is similar to the situation in our population B.

CONCLUSIONS

1. Results obtained with 48 diverse *P. infestans* isolates show that the late blight resistance of *S. nigrum* is high and broad-spectrum.
2. Testing inheritance of resistance in three F_3 families indicated that while some black nightshade accessions can have a single dominant resistance gene (N19, family A), some can have most likely more than one effective R gene (N9, family B) or even quantitative resistance (24, family C).
3. The F_4 seeds of the lines from three *S. nigrum* families were obtained in this study and can be used in future for mapping and cloning of the genes underlying the late blight resistance.

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