

Inheritance of resistance in *Solanum nigrum* to *Phytophthora infestans*

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Abstract *Solanum nigrum*, black nightshade, is a wild non-tuber bearing hexaploid species with a high level of resistance to *Phytophthora infestans* (Colon et al. 1993), the causal agent of potato late blight, the most devastating disease in potato production. However, the genetic mode of resistance in *S. nigrum* is still poorly understood. In the present study, two *S. nigrum* accessions, 984750019 (N19) and #13, resistant (R) and susceptible (S), respectively, to three different isolates of *P. infestans*, were sexually crossed. The various kinds of progeny including F1, F2, F3, and backcross populations (BC₁; F1 × S), as well as two populations produced by self-pollinating the R parent and S parent, were each screened for susceptibility to *P. infestans* isolate MP 324 using detached leaf assays. Fifty seedling plant individuals of the F1 progeny were each resistant to this specific isolate, similarly to the seedling plants resulting from self-pollination of the resistant R parent. Thirty seedling plants obtained from self-pollination of the S parent were susceptible. Among a total of 180 F2 plants, the segregation ratio between resistant and susceptible plants was approximately 3:1. Among the 66 seedling plants of the BC₁ progeny originating from crossing an F1 plant with the

susceptible S parent, there were 26 susceptible and 40 resistant plants to *P. infestans*. The segregation patterns obtained indicated monogenic dominant inheritance of resistance to *P. infestans* isolate MP 324 in *S. nigrum* acc. 984750019. This gene, conferring resistance to *P. infestans*, may be useful for the transformation of potato cultivars susceptible to late blight.

Keywords Leaf assay · Late blight · Breeding

Late blight, one of the most serious diseases of potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) is caused by the oomycete *Phytophthora infestans*. Black nightshade (*Solanum nigrum*), an annual weed plant, has been regarded as a non-host to *P. infestans* for many years, due to the lack of infection resulting from artificial inoculation under laboratory conditions (Colon et al. 1993; Platt 1999), and due to the rare infection under natural conditions in England, Wales, and the Netherlands (Hirst and Steadman 1960; Flier et al. 2003; Deahl et al. 2004). However, Flier et al. (2003) suggested a reconsideration of the non-host status of *S. nigrum* based on the appearance of field infections and on the results of detached leaf inoculations. In additional studies, it was suggested that the resistance to *P. infestans* in *S. nigrum* is conferred by R genes, although the mechanisms of resistance to *P. infestans* in the species are not well known (Colon et al. 1993; Flier et al. 2003; Lebecka 2008).

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Solanum nigrum is a hexaploid species ($2n=2x=72$), most probably a natural hybrid between *Solanum villosum* ($2n=4x=48$) and *Solanum americanum* ($2n=2x=24$). In turn, *S. villosum* is suggested as an allotetraploid species originating from a cross between *S. americanum* and an unknown diploid *Solanum* species, most likely *Solanum sarrachoides* ($2n=2x=24$) (Edmonds 1979). Cytological studies of Rao (1971) showed that the genome of *S. nigrum* is autoallohexaploid with three sets of 12 chromosomes each. Two of these sets are, to a large extent, homologous to each other. *Solanum villosum*, investigated using cytological studies by Sultana and Alam (2007), was shown to be an ancient autotetraploid of *S. americanum*, which in the course of time had started regular meiosis (bivalent formation). The natural hexaploid does not show any quadrivalents between the four homologous genomes; chromosome pairing appears to be restricted to bivalent formation (Rao 1971).

The aim of the present study was to understand the mode of inheritance of resistance to *P. infestans* in *S. nigrum*.

Plant materials used in the present study originated from two accessions of *S. nigrum*: #13 (seeds kindly provided by Agricultural University, Warsaw, Poland) and N19 (seeds of accession *ngr* 984750019, kindly provided by the Botanical and Experimental Garden of the Radboud University, Nijmegen, the Netherlands). The selections of susceptible plants of #13 and resistant plants of N19 for sexual crosses were based on previously obtained results (Lebecka 2008), when two *P. infestans* isolates MP 324 and MP 637 were used for inoculations.

For each sexual cross, undeveloped anthers were removed from the small buds of the maternal plants, and 48 h after emasculation their stigmas were pollinated with freshly collected pollen from paternal plants.

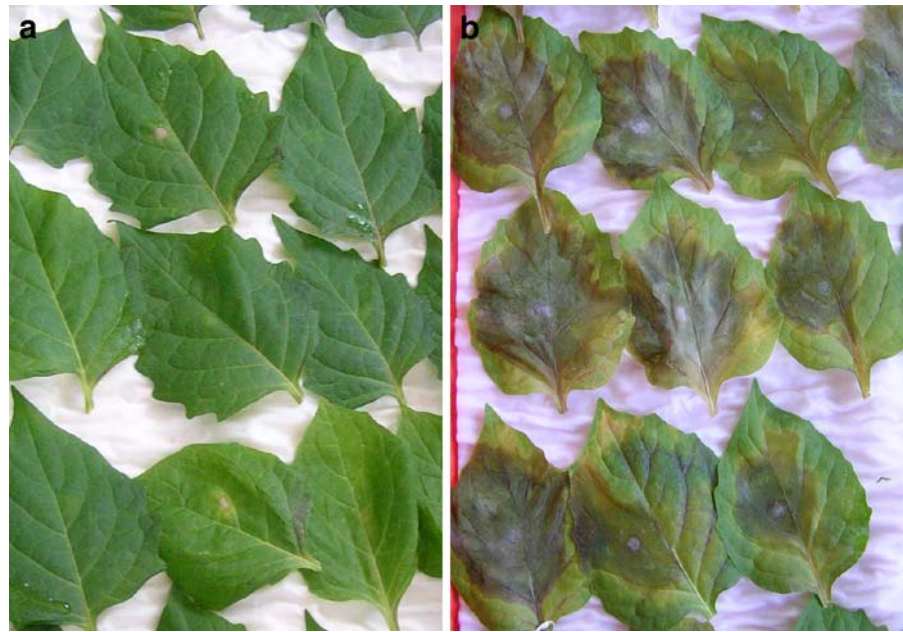
The *P. infestans* isolate MP 324 was used for evaluation of resistance in detached leaf assays. In addition, the isolate MP 853, collected in 2007 from a field grown potato plant, was used for evaluation of susceptibility of parental forms. Inoculum consisted of a sporangial suspension prepared from sporulating lesions of potato leaflets and adjusted using a haemocytometer to a concentration of 50 sporangia μl^{-1} as previously described by Zarzycka (2001). Fully developed leaves were detached from the middle part of the 6 to 12 week-old greenhouse-

grown plants. Leaves were placed on wet cellulose wadding in a plastic tray. Each leaf was inoculated by depositing 30 μl of the inoculum on the abaxial side of the leaf. The trays with leaves were covered with glass. The inoculated leaves were incubated for 7 days at 16°C with a constant illumination of fluorescent light ($11.5 \mu\text{mol m}^{-2}\text{s}^{-1}$). After the first 24 h of incubation, the leaves were turned abaxial side down. Each leaf was scored as resistant when lacking visible infection symptoms or possessing non-sporulating necroses of a size less or equal to the area of the inoculation drop, and as susceptible, when showing sporulating lesions.

Solanum nigrum is a predominantly self-pollinating species. However, natural out- and cross-breeding can still occur (Edmonds and Chweya 1997). It was assumed that the plants originally selected as parental forms for crosses in the present study were highly homozygous. By testing 30 selfed progenies of the resistant parent to *P. infestans*, its homozygosity was confirmed assuming diploid inheritance in *S. nigrum*; all 30 selfed progenies of the susceptible parent were susceptible to MP 324.

The susceptibility and resistance scores in parental lines were confirmed by using both MP 324 and MP 853 isolates (Fig. 1). All 50 individuals from the F1 progeny (crossing #13 \times N19) were resistant to MP 324. Different resistance reactions were observed in leaves from the same plant within and among tests of F2. The differences among tests may have been related to the variation in pathogen aggressiveness; generally, the infection level was not the same in repeated tests. Out of the 180 F2 plants tested, for 80 plants all the leaves tested were resistant in each replicated test, and for 39 plants <5 leaves out of 15 tested were infected. These plants were each ranked as resistant. A total of 33 plants were regarded as susceptible, since more than half of the leaves tested were infected. Plants of 28 genotypes could not be included in any of the classes described, because the results of the detached leaf assays were not consistent. Twenty plants of each of 15 F3 progenies obtained after self-pollination of 15 F2 plants from the non-classified group were also tested. The segregation ratio was verified by a chi-square test; this classified 11 F2 individuals as resistant and four as susceptible. The segregation ratio in the F2 population, between resistant and susceptible plants was approximately 3:1 (Table 1). Among the 66 plants of the BC1 progeny

Fig. 1 The detached leaf assay; symptoms of infection caused by *P. infestans* isolate MP 324 on *S. nigrum* leaves of resistant and susceptible parental forms: **a** accession N19 (resistant, either lack of symptoms, or no spreading lesion, no sporulation), **b** accession #13 (spreading lesions, sporulation)



(crosses between F1 plant and susceptible #13 parent), there were 26 susceptible and 40 resistant plants to *P. infestans* MP 324, a segregation ratio of approximately 1: 1. Based on the results obtained in the detached leaf assay, there was no segregation in the F1 population, and the segregation patterns fitted the theoretical ratios of 1: 1 between resistant and susceptible individuals in the BC₁, and 3: 1 in the F2 progeny. When tetraploid inheritance is taken into consideration there are three possible genotypes of

resistant parent for which the results fit to the expected segregation. When assuming *S. nigrum* as an allohexaploid the resistant parent could be A₁A₁A₂a₂A₃a₃ (and F1 plant A₁a₁a₂a₂a₃a₃), and when *S. nigrum* is autoallohexaploid the resistant parent could be AAAaa₁a₁ (and F1 plant Aaaa₁a₁), or AaaaA₁A₁ (and F1 plant aaaaA₁a₁).

The most probable mode of inheritance is diploid, as quadrivalent formation has not been observed in *S. nigrum*—natural hybrids, or artificially obtained

Table 1 Susceptibility of *Solanum nigrum* parental forms, their F1, F2, BC₁ progeny and their progeny from self-pollination, to *Phytophthora infestans* MP 324, evaluated in detached leaf

assays and verification of hypothesis of monogenic dominant inheritance of resistance in *S. nigrum* accession 984750019-N19

Plant material	Isolate used for testing	No. of plants	No. of leaves/plant	Observed ratio		Hypothesis verification; description or probability for chi-square statistics
				R	S	
Susceptible parent # 13 (S)	MP 324, MP 853	1	20		1	According to expectation
Resistant parent N19 (R)	MP 324, MP 853	1	20	1		According to expectation
Progeny from S self-pollination	MP 324	30	3		30	Homozygosity verified
Progeny from R self-pollination	MP 324	35	3	35		Homozygosity verified
F1 progeny of S × R cross	MP 324	50	3	50		According to expectation
BC1 progeny of F1 individual × S cross	MP 324	66	30	40	26	$\chi^2=2.969; P=0.08$
F2 progeny of S × R cross	MP 324	180	15	130	37	$\chi^2=0.795; P=0.36$
				13 not classified (not included in chi-square test)		

hybrids of *S. villosum* (+) *S. americanum*, and *S. nigrum* possible progenitor, *S. villosum* (Rao 1971; Beg and Khan 1989; Ganapathi and Rao 1985; Sultana and Alam 2007). If the self pollinating status of *S. nigrum* is taken into consideration, the parental lines were most probably homozygous for the resistance, and therefore three putative genotypic models of the resistant parent can be proposed: AAaaaa, assuming that the plant was an autohexaploid with regular meiosis, A₁A₁aaaa—assuming that the plant was an autoallohexaploid, and A₁A₁a₂a₂a₃a₃—when the plant was allohexaploid. In each case, it can be assumed that the resistance in *S. nigrum* N19 is governed by a single dominant gene. The late blight resistance has been transferred from *S. nigrum* to its sexual and somatic hybrids with *S. tuberosum* (Colon et al. 1993; Eijlander and Stiekema 1994; Horsman et al. 1997; Zimnoch-Guzowska et al. 2003). Due to linkage drag, reduction in fitness of obtained hybrids, and infertility, this species is not useful for traditional potato breeding. To avoid the negative linkage drag the late blight resistance from *S. nigrum* may be cloned and introduced into the *S. tuberosum* gene pool.

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