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Assessment of foliar and tuber resistance in *Solanum neoantipoviczii* Buk. × *S. phureja Juz.* et Buk. hybrid populations using different isolates of *Phytophthora infestans*

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SUMMARY
In an evaluation of resistance to *Phytophthora infestans* in wild potato species from VIR's collection performed in late 90-ies at IHAR-Mlochow Research Center (IHAR-Mlochow), Poland the accession *Solanum neoantipoviczii* Buk. k-8505 was found highly leaf and tuber resistant. In 1999 it was used in direct cross with an accession of *S. phureja* Juz. et Buk DB 254 from SCRI (UK) collection. Resistance to *P. infestans* in the hybrid obtained were assessed in several tests using two (leaflet tests) and three (tuber tests) isolates of *P. infestans* differing by phenotype. Foliar and tuber resistance evaluation were performed in 2001 at IHAR-Mlochow and in 2008, 2010 at Swedish University of Agricultural Sciences (SLU-Alnarp), Sweden. In all tests hybrid progenies were found segregating for foliar and tuber resistance. Lower tuber resistance was observed after application of more virulent isolates. In each test genotypes combining both leaf and tuber resistance were found.

KEYWORDS
Phytophthora infestans, resistance, potato hybrid

INTRODUCTION

Breeding achievements using large scale approaches have not been able to significantly decrease yield losses caused by late blight. New, more virulent *Phytophthora infestans* strains have evolved which overcome the genetic resistance that has been introgressed by conventional breeding from wild and cultivated potato species into commercial varieties. The most effective and environmentally friendly way is the incorporation of genes for resistance to *P. infestans* from newly found natural sources able to cross with cultivated potatoes.

The R genes from *Solanum demissum* Lindl. have been introgressed into potato cultivars. However, their durability proved to be a problem due to the rapid appearance of compatible races of the pathogen after market introduction (Wastie 1991). Several other wild species of the genus *Solanum* beside *S. demissum* are also being considered as possible sources of resistance to late blight (Hermsen and Ramanna 1973). Recently new sources of R-gene resistance have been found in number of wild

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potato species (Gebhardt and Valkonen 2001).
The accession of *S. neoantipoviczii* maintained in VIR's collection was identified as foliar and tuber resistant on evaluation curried out in the late 90-ies (Zoteyeva *et al.* 2004). Mexican wild potato species *S. neoantipoviczii* was described and specified as an independent species by Russian taxonomist Bukasov. This species is closely related to *S. stoloniferum* Schlechtd. and mentioned in Hawkes's monography as its synonym. (Hawkes, 1990).

MATERIAL AND METHODS

Plant material
Two F1 populations of the interspecific hybrid *S. neoantipovichii* × *S. phureja* were evaluated in 2001 at IHAR-Mlochow, Poland and in 2008 at SLU-Alnarp, Sweden. Clones obtained from the seedlings evaluated in 2008 were tested in 2010.

Pathogen material
Three isolates of *P. infestans* were used in tuber and two ones in leaflet tests. The 2001 leaflet test and one of tuber tests were done using isolate MP-324 (1.2.3.4.6.7.10.11.) from IHAR-Mlochow collection of pathogens. The other isolate applied in the 2001 tuber test was American US-8 (1.2.3.4.5.6.7.10.11.) also maintained at IHAR-Mlochow. In the tests performed at SLU-Alnarp Swedish isolate SE 03058 (1.3.4.7.10.11.) was used for leaf and tuber inoculation. The virulence of isolates was examined using a set (R1-R11) of Black's differentials maintained in IHAR-Mlochow's collection of pathogens.

Leaf and tuber resistance assessment
For foliar resistance evaluation three leaflets from each plant in two replications have been inoculated with a drop of inoculum (20 µl) of *P. infestans*. Two isolates of *P. infestans* were used for inoculation: MP-324 and SE 03058. The reading of disease symptoms was done after 6 days of incubation at 16°C. Leaflet disease rating was recorded using 1-9 grade scale, where 9 is the most resistant. General score criteria was a combination of a percent of affected leaf area and mycelia development intensity (Zarzycka 2001). Cultivars Irys (S) and cv. Bintje (S) as well as cultivar Meduza (R) and selection from *S. guerreroense* (R) were used as controls (Table 1).
For tuber resistance evaluation the original method of inoculation of decapitated tubers was applied (Zoteyeva and Zimnoch-Guzowska 2004). Three *P. infestans* isolates were used for inoculums preparation: MP-324, US-8 and SE 03058. From five to 10 tubers per each plant were inoculated. Lesion sizes were scored using grade scale 1-9 where 9 is the most resistant. For mycelia growth 0 - 3 grade scale where 0 means lack of mycelium growth and 3 very intense mycelium growth was used. Cultivars and breeding lines (6 tubers of each) with different resistance levels were included as standards in each test (Table 1). In all tests the inoculums concentration comprised 20000 zoospores /ml.
For statistical analyses the MINITAB 15 Statistical Software was used.

Table 1. Resistance of *S. neoantipoviczii* × *S. phureja* hybrid progenies in laboratory tests using different isolates of *Phytophthora infestans*

P. infestans isolate	Number of tested plants/tubers	Accession tested	Resistance, grade	
			average	min - max
leaflet tests				
2001 (MP -324)	26	nan* x phu	8.6	5.2 – 9.0
2010 (SE 03058)	20	nan x phu	6.9	4.0 – 9.0
Controls:				
2001 (MP 324)		cv. Irys (S**)	1,0	1.0 – 1.0
`` ``		cv. Meduza (R***)	6,2	5.0 – 7.0
2010 (SE 03058)		cv. Bintje (S)	1.4	1.0 – 2.0
`` ``		grr-08-3 (R)	8.7	7.0 – 9.0
tuber tests				
2001 (MP-324)	26/251	nan x phu	5.3	3.0 – 7.5
2001 (US-8)	20/160	nan x phu	4.7	2.8 – 6.5
2008 (SE 03058)	18/78	nan x phu	7.6	6.2 – 9.0
2010 (SE 03058)	20/90	nan x phu	6.2	7.8 – 3.8
Controls:				
2001 (MP 324)		cv. Irys (S)	2.8	2.2 – 3.4
`` ``		cv. Meduza (R)	6.5	5.0 – 7.0
2001 (US-8)		cv. Irys (S)	2.3	1.0 – 3.0
`` ``		cv. Meduza (R)	6.1	5.0 – 7.8
2008 (SE 03058)		cv. Bintje (S)	3.1	3,0 – 3.6
`` ``		cv. Matilda (R)	6.8	6.0 – 7.4
2010 (SE 03058)		cv. Bintje (S)	2.9	2.0 – 4.0
`` ``		cv. Matilda (R)	6.4	5.0 – 9.0

*) – potato species name abbreviations (nan = *S. neoantipoviczii*, phu = *S. phureja*)
**) – susceptible
***) – resistant

RESULTS AND DISCUSSION

The F1 hybrid population assessed in leaflet (using the isolate MP-324 (1.2.3.4.6.7.10.11.)) and tuber (using the isolates MP-324 and US-8 (1.2.3.4.5.6.7.10.11.)) tests was found slightly segregated for leaf resistance shown high percent of highly resistant plants but much stronger segregated for tuber resistance (Table 1). In leaflet test resistance was scored on average 8.6 grades for two replications. Resistance in 24 out of 26 tested plants in both replications was scored on average grades from 8 to 9. The infection pressure in this test was sufficient as could be observed by the reaction of the controls scored with grade 1 (susceptible) and grade 6.2 (resistant). Another population of this hybrid was obtained from original seeds at SLU-Alnarp in 2008. Twenty clones from this population were evaluated in leaflet test in 2010 using an aggressive isolate SE 03058 (1.3.4.7.10.11.). In this test resistance of hybrid progenies was lower than in 2001 when Polish isolate (1.2.3.4.6.7.10.11.) was applied. To classify populations by resistance plants were divised into three groups: resistant (R, grades from 7 to 9), moderately resistant (MR, grades from 5 to 6) and susceptible (S, grades from 1 to 4). Distribution by resistance levels within populations evaluated in 2001 and 2008 tests resulted in: 24R : 2MR : 0S (2001) and 13R : 6MR : 1S (2008). The results obtained showed reduced share of

resistant plants in population tested in 2008. Regardless less virulent isolate applied, the resistance of hybrid progenies tested in 2010 was lower. Nevertheless, about half of the population expressed resistance.

Table 2. Resistance of *S. neoantipoviczii* × *S. phureja* progenies in tuber test applying two isolates of *Phytophthora infestans*

P.infestans Isolate	Nr of plant	Resistance grade		*P.infestans* isolate	Nr of plant	Resistance grade	
		Lesion size	Mycelium growth			Lesion size	Mycelium growth
MP-324	1	6.4	0.4	US-8	1	5.2	1.4
MP-324	2	5.3	1	US-8	2	5.0	1
MP-324	3	5.1	0.8	US-8	3	6.0	1
MP-324	4	3.8	1.4	US-8	4	4.2	1.2
MP-324	5	4.5	1	US-8	5	3.7	1.8
MP-324	6	6.3	0.6	US-8	6	6.0	0.8
MP-324	7	5.3	0.6	US-8	7	4.3	1.2
MP-324	8	3.1	2		8	non tested	
MP-324	9	5.7	0	US-8	9	5.9	1.2
MP-324	10	5.9	0.4	US-8	10	5.9	1.2
MP-324	11	5.1	1	US-8	11	3.7	1.4
MP-324	12	5.3	0.2		12	non tested	
MP-324	13	5.6	0.4	US-8	13	4.7	1
MP-324	14	7.1	0	US-8	14	6.5	0.6
MP-324	15	7.5	0	US-8	15	4.5	2.5
MP-324	16	4.6	0.8	US-8	16	4.7	1.4
MP-324	17	6.1	0.2		17	non tested	
MP-324	18	5.4	0.6	US-8	18	3.3	1.6
MP-324	19	6.0	0.4	US-8	19	5.5	1.6
MP-324	20	4.9	0.2	US-8	20	4.3	0.8
MP-324	21	3	1.6	US-8	21	2.8	2.2
MP-324	22	4.9	2	US-8	22	4.4	1.8
MP-324	23	5.7	0.2		23	non tested	
MP-324	24	5.6	0		24	non tested	
MP-324	25	5.3	1		25	non tested	
MP-324	26	4.1	3	US-8	26	4.1	3
	Controls:						
MP-324	Irys (S)	2.8	1.2	US-8	Irys	2.3	1.8
MP-324	Meduza(R)	6.5	0.6	US-8	Meduza	6.1	1.5

In tuber test performed at IHAR-Mlochow two isolates MP-324 (1.2.3.4.6.7.10.11.) and US-8 (1.2.3.4.5.6.7.10.11.) were applied. The difference in virulence between these two isolates was a lack of gene for virulence 5 in isolate MP-324 compared to isolate US-8. Results of the tests showed tuber resistance ranged from 3.0 to 7.5 grades after inoculation with isolate MP-324 and from 2.8 to 6.5 grades after inoculation with isolate US-8 (Table 2). To classify plants for tuber resistance the ones scored on average: from 1 to 4.9 grades were found as susceptible (S), from 5 to 6.4 grades as moderately resistant (MR) and up to 6.5 grades as resistant (R). The reaction of tubers of the standard cultivars inoculated with isolates MP-324 or US-8 showed sufficient infection pressure (Table 2). Tuber resistance of the susceptible cultivar Irys was scored on average 2.8 and 2.3 grades after inoculation with isolates MP-324 and US-8, respectively. The disease scores on tubers of resistant control (cultivar Meduza) were, respectively, 6.5 and 6.1 grades on average. The distribution of the resistance levels within 26 plants when tubers were inoculated with isolate MP-324 was 2R:16MR:8S. Twenty plants inoculated with isolate US-8 segregated for tuber resistance in proportion 1R:7MR:12S. The application of isolate US-8 resulted higher disease development on inoculated tubers regarding lesion sizes and mycelia growth: the lesion sizes score were 4.6 grades (US-8) and 5.3 grades (MP-324) grades on average and mycelia growth score were 0.8 grades (US-8) and 1.5 grades (MP-324) on average.

Statistical analyse using Minitab program showed that the resistance levels of hybrid progenies inoculated with different *P. infestans* isolates were differed significantly. Grouping Information using

Tukey Method showed that with mean values of MP-324 = 5,26 and Us-8 = 4,60 the resistance scores of lesion size were significantly different ($p < 0.0001$). A mean values of MP-324 = 0.84 and of US-8 = 1.45 showed significant differences for mycelium growth in tests with the use of isolates MP-324 or US-8 ($p < 0.0001$).

While leaf resistance of hybrid progenies was lower in test using the Swedish isolate SE 03058 compared to the test where the Polish isolate MP-324 was used, tuber resistance was higher in both tests performed at SLU-Alnarp. Resistance in 18 seedlings tested in 2008 was scored on average 7.6 grades and ranged from 6.2 to 9.0 grades (Table 1). Distribution by resistance levels in this test was 15R:3MR:0S.

Clones obtained from 20 seedlings in 2008 were tested in 2010. In this test resistance occurred to be lower and was scored on average 6.2 grades and ranged from 4.3 to 7.8 grades. Plant distribution within the population regarding resistance levels was 6R:11MR:3S. In 2010 resistance was scored with lower grade on average due to higher number of moderately resistant plants and lower number of resistant ones compared to 2008 when tuber susceptible plants were not found at all.

A comparison of tuber test results where the isolates of *P. infestans* containing 8 (MP-324), 9 (US-8) and 6 (SE 03058) genes for virulence showed higher resistance in the tests where isolate containing lower number of genes for virulence were used. The use in the same test of two highly virulent isolates differing by the presence/absence of only one virulence gene (out of the range R1 – R11) resulted in significantly different tuber resistance score values.

In all tests performed hybrid populations were found segregating for foliar and tuber resistance. In each test the genotypes with combined foliar and tuber resistance were found.

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