

ASSESSMENT OF GENES *R1* AND *R3* CONFERRING RESISTANCE TO LATE BLIGHT AND OF GENE *R_{YSto}* CONFERRING RESISTANCE TO POTATO VIRUS Y IN TWO WILD SPECIES ACCESSIONS AND THEIR HYBRID PROGENIES

Nadezhda Zoteyeva*[#], Ieva Mežaka**, Daiga Vilcāne**, Ulrika Carlson-Nilsson***, Ilze Skrabule**, and Nils Rostoks****

* N. I. Vavilov Institute of Plant Industry, B. Morskaya Str., 42, St. Petersburg, RUSSIA; nzoteyeva@gmail.com

** State Priekulji Plant Breeding Institute, Zinātnes iela 2, Priekulju pag., Priekulju nov., LV-4126, LATVIA

*** Swedish University of Agricultural Sciences, Alnarp, SE 23053, SWEDEN

**** University of Latvia, Kronvalda bulv. 4., Rīga, LV-1586, LATVIA

[#] Corresponding author

Communicated by Isaak Rashal

The oomycete Phytophthora infestans Mont. (de Bary), which causes potato late blight, and Potato Virus Y (PVY) are economically important potato pathogens. More virulent P. infestans strains have evolved and are able to overcome resistance genes introgressed earlier to cultivated potato from wild Solanum species, especially from S. demissum Lindl. Potato cultivars resistant to the previously common type of PVY^N may be susceptible to the more virulent isolates of PVY. In previous research, S. guerreroense Corr. (grr) and S. neoantipoviczii Buk. (nan) accessions were rated as resistant to P. infestans and to two (grr) and three (nan) strains of PVY. These parental accessions and their respective hybrid offspring were screened using polymerase chain reaction (PCR) tests to detect alleles conferring resistance to P. infestans (R1 and R3) and to PVY (R_{YSto}). Screenings were made in detached leaflet tests (P. infestans), and with the aid of ELISA tests (PVY). The resistance of two hybrids derived from grr was rated as high, mostly due to a hypersensitivity reaction. The allele R3 was detected in only one grr plant among 20 plant populations for each grr and nan accession. R1 and R3 alleles were more frequently detected in one hybrid of grr (grr × B.d.R5.). The resistance allele R_{YSto} was found in nan and provided host plant resistance to three strains of PVY, including PVY^{NTN}. Hybrids derived from this accession were characterised by a high frequency of plants bearing the resistant allele R_{YSto}. In the parental clone tbr × phu, as well as in grr and its hybrids, only the susceptible allele at R_{YSto} locus was detected.

Key words: host plant resistance, interspecific hybrids, potato, *Phytophthora infestans*, *Potato Virus Y*.

INTRODUCTION

Phytophthora infestans causes late blight, a disease that significantly reduces potato yield in susceptible cultivars worldwide. The estimated annual costs of potato plant protection against this disease are above €1 billion (Haverkort *et al.*, 2008). An integrated management approach can control late blight (Fry, 2008). Although breeding for host plant resistance offers one of the most promising and environmentally friendly strategies, plant breeding efforts are yet to reduce significantly yield loss due to late blight. The incorporation of resistance genes from crop wild relatives could also broaden the genetic base of the cultivated potatoes. Sources for horizontal and vertical resistance are available

in the potato gene pool (Wastie, 1991). Sustainable crop genetic enhancement consists of identifying useful characters, manipulating their genetic variation and putting genes into a usable form using DNA markers to monitor chromosomal changes (Ortiz, 2012). There are 11 resistance (R) genes used in potato breeding, which originate from the wild species *S. demissum* (Muller and Black, 1952): *R1*, *R2*, *R3*, and *R4* (Black *et al.*, 1953); *R5* and *R6* (Eide *et al.*, 1959); *R7*, *R8*, and *R9* (Malcolmson and Black, 1966); and *R10* and *R11* (Malcolmson, 1969).

The *R1* gene is located on chromosome 5 on which multiple other disease resistance genes had been mapped. In comparison to the susceptibility allele, the resistance allele at the

R1 locus represents a large insertion of a functional R gene (Ballvora *et al.*, 2002). Substantial structural variation exists among the three *R1* haplotypes from the allohexaploid *S. demissum* (Kuang *et al.*, 2005). The *R3a* locus is highly frequent in *S. demissum*. There are 30 to 45 *R3a* homologs per haplotype (Friedman and Baker, 2007). The results of study done on *R10* and *R11* genes using R-gene differentials of Black showed that *R10* and *R11* are allelic versions of genes at the *R3* locus on chromosome 11 (Bradshaw *et al.*, 2006). Using functional allele mining with *Avr3a*, gene *R3a* homologs characterised by high sequence conservation were detected in *Solanum stoloniferum* (Champouret, 2010). The R genes provoke hypersensitivity in potato plants when affected by avirulent *P. infestans* isolates, preventing therefore further growth of the pathogen because of rapid plant cell death. Most cultivars in Europe and some in North America bear R genes derived from the wild species *S. demissum* (Pavek and Corsini, 2001). The R genes confer race-specific hypersensitive resistance that does not, however, seem to last (Malcolmson and Black, 1966). New germplasm sources are therefore necessary to provide long lasting host plant resistance to late blight. They can be found in other potato wild relatives (Gebhardt and Valkonen, 2001; Villamon *et al.*, 2005). An effective breeding programme for host plant resistance to late blight needs to identify resistance alleles in the wild species, select the most promising sources for further use in crossing, and transfer the resistance to the cultigen pool to make these alleles suitable for cultivar development (Haverkort *et al.*, 2008). Multilines, cultivars mixtures and gene pyramiding have been proposed to increase the potential for durable resistance to late blight in potato (Niederhauser *et al.*, 1996; Smilde *et al.*, 2005). Combining R alleles with minor genes providing high levels of field resistance may contribute to enhance the host plant response to late blight resistance in potato cultivars (Stewart *et al.*, 2003).

Potato virus Y (PVY) is a severe potato pathogen throughout the world. It is one of the most important viruses in Europe and affects not only potato yield but also that of other species in the Solanaceae family, such as tomato, tobacco and pepper (De Bokx and Huttinga, 1981). There are three main PVY strain groups: PVY^O, PVY^N and PVY^C (Jones, 1990; Swiezynski, 1994). In 1984, a new isolate was detected in cv. Wilga, which was grown in northwest Poland. This strain was named PVY^{NWi} (Chrzanowska, 1987). A new very aggressive strain of virus named PVY^{NTN} causes formation of a large necrotic ring on tubers (Weidemann, 1993; Le Romancer *et al.*, 1994). There are three known genes of extreme resistance to PVY that are specific to *S. tuberosum* Gp. Andigena (*Ry_{adg}*), *S. chacoense* (*Ry_{chc}*), *S. hougasii* and to *S. stoloniferum* (*Ry_{sto}*) (Cockerham, 1943, 1970; Munoz *et al.*, 1975; Galvez and Brown, 1980). Resistant cultivars often reduce virus concentration in plant, restrict its systemic spread in the field, and develop a necrotic response (i.e., cell death). Extreme resistance to PVY has been introgressed into *S. tuberosum* by Ross (1952; 1958), and various European potato cultivars bear *Ry_{sto}* (Ross, 1986). The search for sources of resistance to

PVY^{NTN} is especially important, because most grown resistant cultivars lack host plant resistance to this strain. In this regard, Zoteyeva *et al.* (2012) found a promising source of resistance to PVY^{NTN} in an accession of *S. neoantipovichii*.

The aim of this research was to further characterise the resistance to both late blight and PVY in accessions of *S. guerreroense* and *S. neoantipovichii* and some of their hybrids with the cultigen potato pool. We used greenhouse and field screening as well as DNA markers for detecting resistance genes in the host plant.

MATERIAL AND METHODS

This research was conducted at the State Priekuļi Plant Breeding Institute (SPPBI, Latvia) and at the Swedish University of Agricultural Science (SLU, Alnarp, Sweden).

Plant material. The F₁ interspecific hybrids ensued by direct crossing of *S. guerreroense* (grr) k-18407 and *S. neoantipoviczii* (nan) k-8505 with a selection from the accession of *S. tuberosum* Gp. Andigena (adg) VIR k-8077 from N. Vavilov Institute of Plant Industry (VIR, St. Petersburg, Russia), *S. tuberosum* Gp. Phureja (phu) DB 254 from the former Scottish Crop Research Institute (now James Hutton Institute, Dundee, United Kingdom), line R5 from set *R1-R11* of Black's differentials and an original hybrid *S. tuberosum* (tbr) × Gp. Phureja (phu). Hybrids derived from grr and from nan: (nan × (tbr × phu)) were obtained in 2009 at SLU-Alnarp and nan × phu in 1999 at IHAR-Mlochow Research Center (Poland). Both grr and nan accessions were female parents.

Host plant resistance. In 2010–2011, the parental accessions and F₁ hybrids were evaluated for resistance to *P. infestans* in laboratory tests at SPPBI (grr × adg, grr × B.D.R5., nan × (tbr × phu) and SLU (parental accessions and hybrids grr × adg, nan × phu). Parental accessions tbr × phu, adg and hybrids grr × adg and nan × phu were analysed for PVY infection using ELISA tests at SLU.

Late blight leaflet tests. The inoculation of detached leaflets was conducted using *P. infestans* isolate SE 03058 (1.3.4.7.10.11.) maintained at SLU. The genotypes of Black's differential set each possessing a single R-gene (*R1-R11*) were used to define genes for virulence. The differential set was obtained from IHAR-Mlochow. Detached leaflets collected from 20 to 30 plants of each accession grown in a greenhouse (SPPBI) and in the net-bench (SLU-Alnarp) were inoculated in the mid June. For inoculation, the *P. infestans* mycelium was multiplied on tuber slices of susceptible cultivar Madara at SPPBI and on rye agar media (2008 and 2009) as well on leaflets of cv. Desirée (2010) at SLU. The mycelium was washed with distilled water and used for preparation of the inoculum at a concentration 15000 sporangia/ml in tests done in both locations (SPPBI and SLU-Alnarp). The inoculum was incubated at 4 °C for 1 hour before inoculation. About 20 to 30 plants of each hybrid were tested. Three leaflets detached from each plant in

two replications were drop-inoculated (20 µl). Incubation proceeded during 6 days. Leaflets of susceptible cultivars Madara and Bintje or Desirée were used as controls at SPPBI and SLU, respectively. Disease rating was recorded on the 6th day after inoculation using a 1–9 grade scale, where 9 was the most resistant and 1, the most susceptible. General score criteria was a combination of percentage of affected leaf area and mycelia development intensity (Zarzycka, 2001).

Potato virus Y infection detection. ELISA kits were acquired from BIOREBA AG (Switzerland). The absorbance values of healthy controls ranged from 0 to 0.001, and any values above 0.1 were regarded as positive for virus infection.

DNA marker analyses. Molecular diagnostics using DNA markers for detecting resistance alleles *R1* and *R3a* (both for late blight) and *Ry_{sto}* (for PVY) in F₁ hybrids and parents were performed at SPPBI.

Extraction of genomic DNA. Genomic DNA of parents and F₁ hybrids was extracted from leaf material following the protocol of Wulff *et al.* (2002). DNA was extracted from fresh leaves of pot-grown plants. Collected leaves were stored at –20 °C until DNA extraction. Approximately 80 mg of leaf tissue per genotype were used for DNA extraction.

PCR amplification. The resistance allele of the *R1* was detected by primers 76-2sf2 and 76-2sR according to protocol adapted from Ballvora *et al.* (2002). The sequence of forward primer 76-2sf2 was

5'- CACTCGTGACATATCCTCACTA-3' and the reverse primer 76-2sR was 5'-CAACCCTGGCATGCCACG-3'. Amplification was carried out in 20 µl of 2.5 mM MgCl₂, 0.2 mM dNTPs, primers at concentration 0.3 mM each and 0.5 U *Taq* polymerase. The PCR conditions were: 3 min at 93 °C, followed by 35 cycles of 30s at 93 °C, 45s at 55 °C, 90 s at 72 °C and finally 5 min at 72 °C. Amplification of an approximately 1399 bp DNA fragment was scored as presence of resistance allele. The resistance allele of the *R3a* was detected by marker RT-R3a (Huang *et al.*, 2005). Sequences of the primers were

5'- ATCGTTGTCATGCTATGAGATTGTT-3' and 5'-CTTCAAGGTAGTGGGCAGTATGCTT-3', respectively. The PCR conditions were: 94 °C for 180 s followed by 35 cycles 94 °C for 30 s, 64 °C for 30 s, 72 °C for 80 s and a final extension time of 5 min at 72 °C. The PCR amplification was performed in 20 µl of 2.5 mM MgCl₂, 0.2 mM dNTPs, primers at concentration 0.3 mM each and 0.5 U *Taq* polymerase. Amplification of an approximately 981 bp band was scored as presence of the resistance allele.

Two sequence-tagged sites (STS) are available for detecting *Ry_{sto}*: YES3-3A (341 bp) and YES3-3B (286 bp). Both were developed from the E+ACC/M+CTC-365 — an amplified fragment length polymorphism. In our research the resistance allele of the *Ry_{sto}* gene was detected by marker YES3-3A (Song and Schwarzfischer, 2008). Sequences of

the primers were 5'-TAACTCAAGCGGAATAACCC-3' (3F) and 5'-AATTCACCTGTTTACATGCTTCTTGTG-3' (3A). The PCR amplification was carried out as described by Song and Schwarzfischer (2008). The amplification of a 341 bp band was scored as presence of the resistance allele.

RESULTS

Evaluation of host plant resistance. In laboratory tests the reactions of resistant and susceptible controls were found authentic indicating sufficient infection pressure for reliable characterisation of resistance. Weighted disease scores on leaflets of susceptible controls Madara (in tests done at SPPBI) and Bintje (in tests done at SLU) were 3.4 and 4.4, respectively.

The populations of parental accessions of *grr* and *nan*, as well as of original hybrid *tbr* × *phu*, expressed high resistance in leaflet tests performed at SLU. In this evaluation the selection from the accession of *S. tuberosum* Gp. Andigena was found susceptible. The *grr* hybrids were completely resistant to *P. infestans* in leaflet tests done at SPPBI (*grr* × *adg*, *grr* × B.D.R5) and at SLU (*grr* × *adg*). Some plants of *grr* × *adg* and *grr* × B.D.R5 reacted to inoculation with *P. infestans* isolate (containing genes for virulence *v.1* and *v.3*) with hypersensitivity reaction (Fig. 2). Hypersensitivity was observed on the 5th day after inoculation on leaflets of *grr* × *adg* and *grr* × B.D.R5 (Figs. 1 and 2). Individuals of *grr* × B.D.R5 bearing both genes expressed a high hypersensitivity (Fig. 3). Resistance levels of plants in populations of *nan* × *phu* and *nan* × (*tbr* × *phu*) were scored, respectively, with average grades 6.9 and 6.3, ranging from 4 to 9 and from 4 to 7.

ELISA tests showed presence of PVY infection in all plants of *adg* and *tbr* × *phu*, and in 7 (out of 30) plants of *grr* × *adg*. Other plants were virus-free. After two years of ELISA

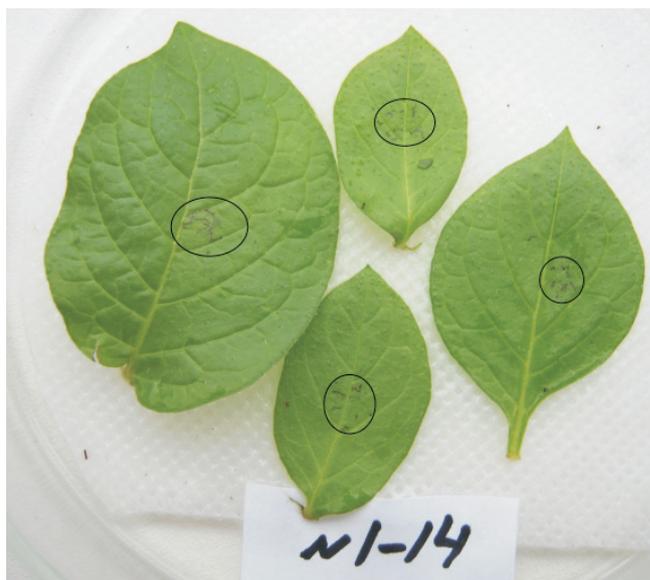


Fig. 1. Hypersensitivity reaction in plant 14 of *grr* × *adg* hybrid (with *R1* resistance allele detected) on the sixth day after inoculation with *Phytophthora infestans*.

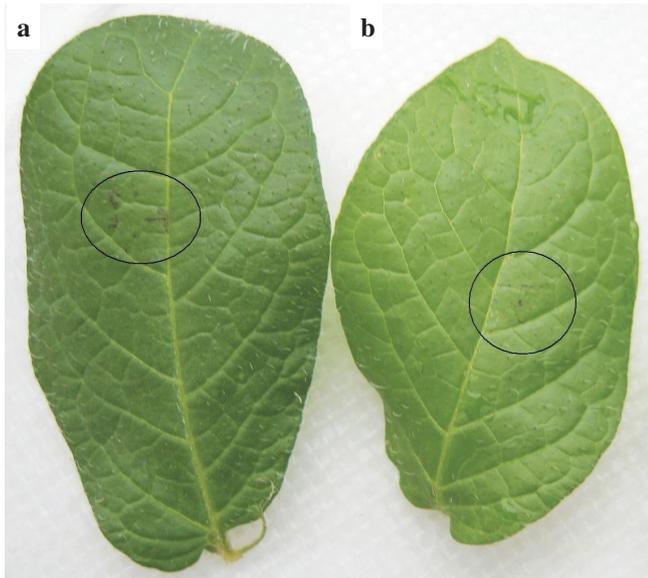


Fig. 2. Hypersensitive reaction in plant 23 of *grr* × B.D.R5 hybrid and plant 4 of *grr* × *adg* hybrid (without *R1* or *R3* resistance alleles detected) on sixth day after inoculation with *Phytophthora infestans*.

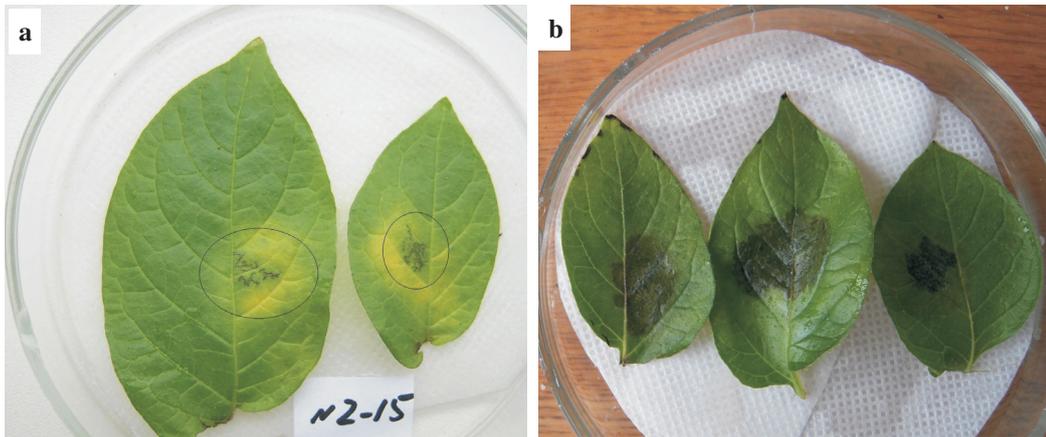


Fig. 3. **a.** Hypersensitivity reaction of plant 15 of *grr* × B.D.R5 hybrid (with resistance alleles of both *R1* and *R3a* genes detected). **b.** Infection on the leaflets of susceptible control.

Table 1

RESISTANT ALLELES *R1* AND *R3* TO *Phytophthora infestans* AND *Ry_{sto}* TO POTATO VIRUS Y DETECTED IN SEEDLINGS (*) AND CLONES (**) OF INTERSPECIFIC HYBRID POPULATIONS

| Accession | Tested plants | Number of plants with detected alleles | | | | |
|--|---------------|--|-----------|------------------------------------|--------------------|------------------|
| | | <i>R1</i> | <i>R3</i> | <i>Ry_{sto}</i> | | |
| | | | | Resistant allele (341 bp fragment) | Susceptible allele | No amplification |
| Parents | | | | | | |
| <i>S. guerreroense</i> (<i>grr</i>) * | 20 | 0 | 1 | 0 | 11 | 9 |
| <i>S. neoantipoviczii</i> (<i>nan</i>) * | 20 | 0 | 0 | 20 | 0 | 0 |
| <i>S. tuberosum</i> Gp. Andigena (<i>adg</i>) * | 20 | 0 | 0 | 0 | 0 | 20 |
| <i>S. tuberosum</i> (<i>tbr</i>) × <i>S. tuberosum</i> Gp. Phureja (<i>phu</i>) ** | 3 | 0 | 0 | 0 | 3 | 0 |
| Black differential line R5 (Bd.R5.)** | 6 | 6 | 0 | 0 | 0 | 6 |
| Hybrids | | | | | | |
| <i>grr</i> × <i>adg</i> | 23 | 0 | 0 | 0 | 14 | 9 |
| <i>grr</i> × Bd.R5. | 28 | 12 | 18 | 0 | 6 | 22 |
| <i>nan</i> × <i>phu</i> | 24 | n.t.*** | n.t. | 20 | 0 | 4 |
| <i>nan</i> × (<i>tbr</i> × <i>phu</i>) | 29 | n.t. | n.t. | 25 | 0 | 4 |

***n.t., none tested

tests (including 2011 season with a very strong aphid invasion) no virus infection in any of 26 plants from *nan* × *phu* was detected. The plants of cultivars King Edward and Magnum Bonum, which were grown in the same field, were strongly infected as shown by their high absorbance values.

Detection of resistant alleles with molecular markers.

Screening of parental accessions. DNA marker tags for R genes to *P. infestans* were found in only one (out of 20) plant of *grr* (*R3a*) and in all of 6 tested plants of B.d.R5 (*R1*). No *R1* or *R3* alleles were detected in *nan*, *adg* and *tbr* × *phu* parents (Table 1). The *Ry_{sto}*-specific PCR marker identified the resistant and susceptible potatoes by different size fragments. Marker YES-3A detects 341 bp, if resistant, but a larger fragment (size not known), if susceptible. Depending on genotype, two types of the target fragments were obtained in PCR tests: 341 bp fragment (lanes 3, 5–8, 10, 11, 14–17 in Fig. 4) was scored as resistant, while a larger fragment (lanes 2, 3 in Fig. 5 and lanes 18–22 in Fig. 7) was scored as susceptible. A 284 bp fragment was obtained with marker YES-3B. An apparent lack of target



Fig. 4. Detection of the *Ry_{sto}* gene conferring resistance to *Potato virus Y* (PVY) using *Ry_{sto}*-specific PCR marker in parental accessions *S. neoantipoviczii* (nan), *S. guerreroense* (grr) and their hybrids derived from crosses with *S. tuberosum* × *S. tuberosum* Gp. Phureja (tbr × phu) and Black differential line R5 (Bd.R5.). Lane 1, 23 Barbara (positive control); lanes 2, 24, Sante (negative control which amplifies neither resistant nor susceptible allele); lanes 3, 5–8, 10 and 11, PVY resistant nan × (tbr × phu); lanes 4, 9, 12, 13 nan × (tbr × phu), no amplification of either resistant or susceptible alleles; lanes 14–17, PVY resistant nan; lanes 18–20, PVY susceptible grr; lanes 21, 22, PVY susceptible grr × Bd.R5; lane 25 control without DNA.

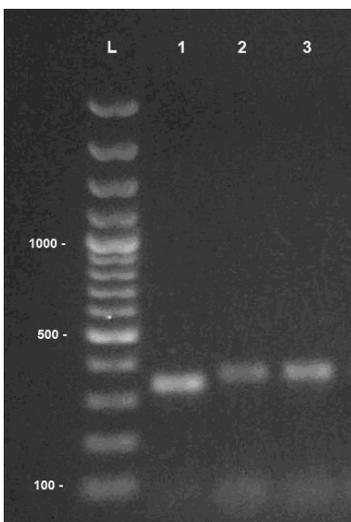


Fig. 5. Detection of the *Ry_{sto}* gene conferring resistance to *Potato virus Y* (PVY) using *Ry_{sto}*-specific PCR marker in plants of hybrid *S. tuberosum* × *S. tuberosum* Gp. Phureja. Lane 1, Barbara (positive control), lane 2 and 3, PVY sus-

ceptible plants. A 341 bp fragment was observed in several cases (lanes 4, 9, 12, 13 in Fig. 4), similarly to variety Sante, which showed amplification of neither resistant, nor susceptible alleles (Song and Schwarzfischer, 2008). The resistance allele of gene *Ry_{sto}* was detected in all 20 plants of nan (Table 1). The susceptible allele of *Ry_{sto}* gene was detected in 11 out of 20 plants of grr and 9 plants showed a lack of target fragment (Table 1).

Screening of F₁ hybrids. The amplification of specific fragments revealed the presence of resistance alleles *R1*, *R3a* and *Ry_{sto}* in some hybrids derived from the accessions mentioned above. *R1* and *R3a* were not detected in 23 plants of grr × adg. Molecular markers indicated presence of the *R1* gene, as well of the *R3a* gene, which were detected in 12 and 18 plants of the grr × B.D.R5. hybrid, respectively (Figs. 6, 7). The resistance allele *Ry_{sto}* was detected in 25 out of 29 tested plants of nan × (tbr × phu) hybrid (Fig. 8). Four plants showed no amplification of either resistant or susceptible marker alleles. Twenty out of 24 plants of the nan × phu amplified a 341 bp fragment characteristic of resistant plants.

DISCUSSION

In previous research we found high resistance to late blight and to three strains of PVY in *S. neoantipoviczii* and *S. guerreroense*. Neither spreading lesions, nor mycelia growth were observed on leaflets of a *S. guerreroense* accession using a standard, as well as 1.5 times higher inoculum concentrations in leaflet tests (Zoteyeva, 2000). In all tests some plants showed hypersensitivity. *S. neoantipoviczii* was mentioned by Hawkes (1990) as a synonym of *S. stoloniferum*

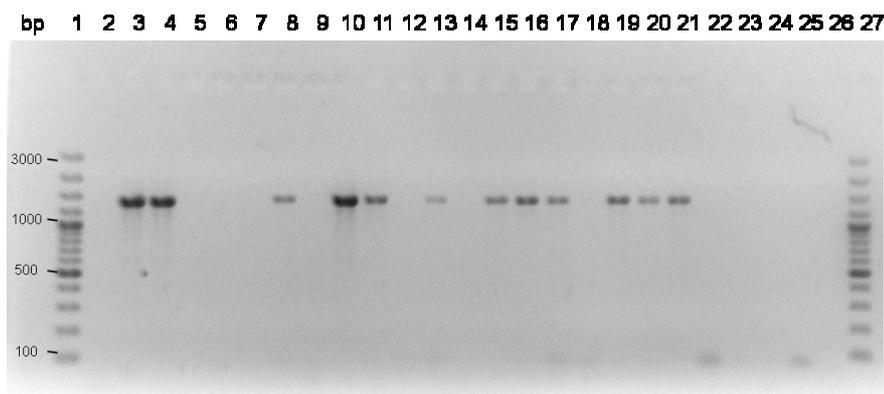


Fig. 6. Detection of the the *R1* gene in hybrid population *S. guerreroense* × Black differential line R5 using primers 76-2sf2 and 76-2sR. Lanes 2-16 and 18-25 depict PCR amplicons obtained from plants nr. 1 – nr. 24; lane 17 – positive control (cv. Vito), lane 26 – negative control; lanes 0 and 27 – Generuler™ 100bp Plus DNA Ladder (Fermentas, Lithuania). Lanes 2, 3, 8, 10, 11, 13, 15, 16, 19, 20, 21, late blight resistant plants; lanes 1, 4, 5, 6, 7, 9, 12, 14, 18, 22, 23, 24, 25, late blight susceptible plants.

bp 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27

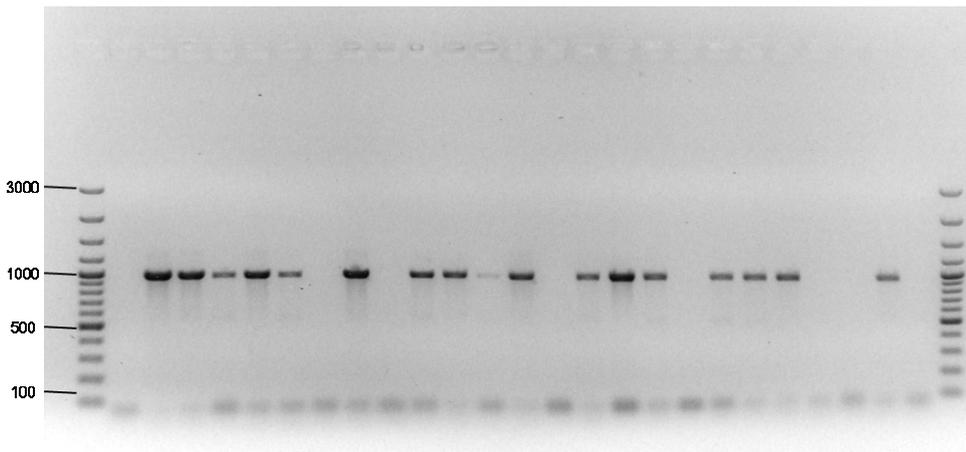


Fig. 7. Detection of the *R3a* gene in hybrid population *S. guerreroense* × Black differential line R5 using markers RT-R3a and RT-SH23-3. Lanes 2–25 amplicons obtained of plants from nr.1 to nr. 24. Lanes 1 and 27 – Generuler TM 100bp Plus DNA Ladder, Fermentas, Lithuania. Lanes 3, 4, 5, 6, 7, 9, 11, 12, 14, 16, 17, 19, 20, 21, 22, 25, late blight resistant plants; lanes 2, 8, 10, 13, 15, 19, 23, 24, late blight susceptible plants. Lane 26 – negative control.

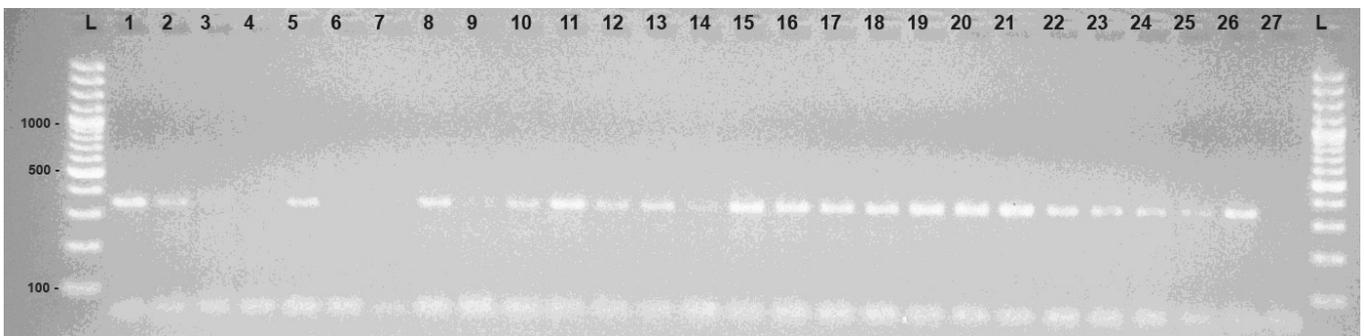


Fig. 8. Detection of the *Rysto* gene conferring resistance to *Potato virus Y* (PVY) using *Rysto*-specific PCR marker in plants *S. neoantipoviczii* × *S. tuberosum* Gp. Phureja. Lanes 1, 26, Ute (positive control); lanes 2, 25, Kuras, (positive control); lanes 3, 5, 8, 10 – 24, PVY resistant; lanes 4, 6, 7 and 9, no product amplified; lane 27 Sante (negative control which amplifies neither resistant, nor susceptible allele).

Schlecht. The host plant resistance in the accession of this species seems to be partial. In several tests the accession k-8505 segregated for resistance to *P. infestans* with a high predominance of resistant plants (from 80% to 95%) in 1999–2000 leaflet tests using standard inoculum concentration. Complete resistance to PVY⁰ and PVY^{N^{WI}} was noted in *S. guerreroense*, whereas *S. neoantipoviczii* had resistance to PVY⁰, PVYN^{WI} and PVY^{N^{NTN}} strains.

Systematic relationships within the group of tuber-bearing *Solanum* species are considered as criteria to select interesting materials for further use in genetic enhancement. Both *S. guerreroense* and *S. neoantipoviczii* are closely related to other well-studied wild species such as *S. demissum* (relative of *S. guerreroense*), which is highly resistant to late blight, and *S. stoloniferum* (relative of *S. neoantipoviczii*), which shows an extreme resistance to PVY. The total number of R genes for resistance to late blight in *Solanum* spp. is still unknown. Genes *R1–R11* were discovered in *S. demissum* in the middle of 20th century (Black *et al.*, 1953). Virulence genes 1 and 3 were often detected among European late blight populations (Andrивon *et al.*, 1994; Lehtinen *et al.*, 2003; Lebecka *et al.*, 2007; Runno-Paurson *et al.*, 2009). Potato genotypes containing *R1* and *R3* genes for resistance to late blight could be contributing to resistance of newly developed pre-breeding material. However, in our study no plants with *R1* gene and few plants with *R3* were

detected in *S. guerreroense*. No plants of *S. neoantipoviczii* showed resistance alleles of *R1* or *R3* genes.

The effect of *R1* and *R3a* resistance alleles to foliar resistance in wild parental accessions could not be determined due to lack of targeted alleles in *S. neoantipoviczii* and seldom occurrence of *R3* allele in *S. guerreroense* (Table 1). Both parental accessions were found highly resistant under inoculation with *P. infestans*. Furthermore, hybrids derived from grr also were completely resistant. It is obvious that complete (grr) and high partial (nan) resistance of both wild parental accessions is conditioned by gene(s) other than *R1* and *R3*. Besides the *R1–R11* genes from *S. demissum*, other genes for resistance to late blight have been identified in potato wild relatives, e.g. in *S. bertautilii* (Ewing *et al.*, 2000), *S. bulbocastanum* (Naess *et al.*, 2000; van der Vossen *et al.*, 2005), *S. venturii* (Foster *et al.*, 2009), *S. microdontum* (Tan *et al.*, 2008), *S. mochiquense* (Smilde *et al.*, 2005), *S. pinnitisectum* (Kuhl *et al.*, 2001), *S. paucissectum* (Villamon *et al.*, 2005), *S. phureja* and *S. ruiz-ceballosii* (Sliwka *et al.*, 2006; 2012). In leaflet tests, a necrotic reaction (i.e., hypersensitivity) was often observed in hybrids derived from *S. guerreroense* (Figs. 1 and 2). Hypersensitivity was observed on leaflets with or without detected alleles of *R1* or *R3* genes, thereby indicating the presence of other genes conferring resistance to *P. infestans*.

One of the sources for resistance to PVY is *S. stoloniferum* Schlecht. bearing *Ry_{sto}* (Cockerham, 1943). DNA marker genotyping supported presence of the *Ry_{sto}* gene in *S. neoantipovizii* and hybrids derived from crosses with *S. neoantipovizii* were all found to bear resistant allele *Ry_{sto}* (Figs. 6, 7). All plants of *S. guerreroense* and their hybrids derived from adg and B.D.R5. showed the susceptible allele in the *Ry_{sto}* locus or no amplification. Marker YES3-3A (Song and Schwarzfischer, 2008) successfully identified resistant and susceptible alleles in populations of parental accession of *S. neoantipovizii* and its hybrid progenies by amplifying different target sizes. Song and Schwarzfischer (2008) showed that the 341 bp fragment is present in resistant varieties and that a larger fragment (~370 bp) is present in susceptible varieties. DNA markers revealed the presence of *Ry_{sto}* resistance allele in the whole population of *S. neoantipovizii*. Within the populations of nan × phu and nan × (tbr × phu) hybrids the number of plants with resistance alleles of gene *Ry_{sto}* exceeded the number of plants without any PCR amplification.

The new sources of host plant resistance were also assessed for tuberisation under long day lengths. Hybrids F₁ obtained in crosses between *S. guerreroense* or *S. neoantipovizii* and cultivated potato species exceeded wild parents in tuber size (Zoteyeva *et al.*, 2011). Hybrid plants derived from both grr and nan accessions were involved in successful crossing with *S. tuberosum* when used as female parents. Thus, the presented results suggest that the analysed potato germplasm is a useful resource for breeding for late blight and PVY resistance. In order to recognise which genes are responsible for the late blight resistance in these accessions, further investigation is required.

ACKNOWLEDGMENTS

This study was co-supported by European Social Fund project: 2009/0218/1DP/1.1.1.2.0/09/APIA/VIAA/099 and E. and I. Nilssons foundation.

REFERENCES

Andrivon, D. (1994). Races of *Phytophthora infestans* in France, 1991–1993. *Potato Res.*, **37**, 279–286.

Ballvora, A., Ercolano, M. R., Weiã, J., Meksem, K., Bormann, C. A., Oberhagemann P., Salamini F., Gebhardt, C. (2002). The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. *Plant J.*, **30** (3), 361–371.

Black, W., Mastenbroek, C., Mills, W. R., Peterson, L. C. (1953). A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. *Euphytica*, **2**, 173–179.

Bradshaw, J. E., Bryan, G. J., Lees, A. K., McLean, K., Solomon, Blackburn, R. M. (2006). Mapping the *R10* and *R11* genes for resistance to late blight (*Phytophthora infestans*) present in the potato (*Solanum tuberosum*) R-gene differentials of black. *Theor. Appl. Genet.*, **112**, 744–751.

Champouret, N. (2010). Functional genomics of *Phytophthora infestans* effectors and *Solanum* resistance genes. PhD thesis. Wageningen University. 154 pp.

Cockerham, G. (1943). Potato breeding for virus resistance. *Ann. Appl. Biol.*, **30**, 105–108.

Cockerham, G. (1970). Genetic studies in resistance to potato virus X and Y. *Heredity*, **25**, 309–348.

Chrzanowska, M. (1987). Nowe izolaty wirusa Y zagrazajace ziemniakom w Polsce. *Hodowla Roslin i Nasiennictwo*, **5** (6), 8–11.

De Box, J. A., Huttinga, H. (1981). Potato virus Y. In: *Description of Plant Viruses*, No. 242 (No. 37 revised). CMI/AAB Ken, Surrey, United Kingdom. 6 pp.

Eide, C. J., Bonde, R., Gallegly, M. E., Graham, K., Mills, W. R., Niederhauser, J., Wallin, J. R. (1959). Report of the Late Blight Investigations Committee. *Amer. Potato J.*, **36**, 421–423.

Ewing, E. E., Šimko, I., Smart, C. D., Bonierbale, M. W., Mizubuti, E. S. G., May, G. D., Fry, W. E. (2000). Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. *Mol. Breed.*, **6**, 25–36.

Friedman, A. R., Baker, B. J. (2007). The evolution of resistance genes in multi-protein plant resistance systems. *Curr. Opin. Genet. Dev.*, **17**, 493–499.

Foster, S. J., Park, T. H., Pel, M., Brigneti, G., Śliwka, J., Jagger, L., van der Vossen, E., Jones, J. D. G. (2009). *Rpi-vnt1.1*, a *Tm-22* homolog from *Solanum venturii* confers resistance to potato late blight. *Mol. Plant Microbe Interact.*, **22**, 589–600.

Fry, W. E. (2008). *Phytophthora infestans*: The plant (and R-gene) destroyer. *Mol. Plant Pathol.*, **9**, 385–402.

Galvez, R., Brown, C. R. (1980). Inheritance of extreme resistance to PVY derived from *Solanum tuberosum* spp. *andigena*. *Amer. Potato J.*, **57**, 476–477.

Gebhardt, C., Valkonen, J. P. T. (2001). Organization of genes controlling disease resistance in the potato genome. *Ann. Rev. Phytopathol.*, **39**, 79–102.

Haverkort, A. J., Boonekamp, P. M., Hutten, R., Jacobsen, E., Lotz, L. A. P., Kessel, G. T., Visser, R. G. F., van der Vossen, E. A. G. (2008). Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. *Potato Res.*, **51**, 47–57.

Hawkes, J. G. (1990). *The Potato. Evolution, Biodiversity, Genetic Resources*. London: Belhaven Press. 259 pp.

Huang, S., van der Vossen, E. A. G., Kuang, H., Vleeshouwers, V. G. A. A., Zhang, N., Borm, T. J. A., van Eck, H. J., Baker, B., Jacobsen, E., Visser, R. G. F. (2005). Comparative genomics enabled the isolation of the R3a late blight resistance gene in potato. *Plant J.*, **42**, 251–261.

Jones, R. A. C. (1990). Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. *Ann. Appl. Biol.*, **117**, 93–105.

Kuang, H., Wei, F., Marano, M. R., Wirtz, U., Wang, X., Liu, J., Shum, W. P., Zaborsky, J., Tallon, L. J., Rensink, W., Lobst, S., Zhang, P., Tornqvist, C. E., Tek, A., Bamberg, J., Helgeson, J., Fry, W., You, F., Luo, M. C., Jiang, J., Robin Buell, C., Baker, B. (2005). The *R1* resistance gene cluster contains three groups of independently evolving, type I *R1* homologues and shows substantial structural variation among haplotypes of *Solanum demissum*. *Plant J.*, **44**, 37–51.

Kuhl, J. C., Hanneman, R. E., Havey, M. J. (2001). Characterization and mapping of *Rpi1*, a late blight resistant locus from diploid (1EBN) Mexican *Solanum pinnatisectum*. *Mol. Genet. Genomics*, **265**, 977–985.

Lebecka, R., Sliwka, J., Sobkowiak, S., Zimnoch-Guzowska, E. (2007). *Phytophthora infestans* population in Poland. In: *Proceedings of the 10th Workshop European Network for Development of an Integrated Control Strategy of Potato Late Blight, Bologna, 2nd–5th May* (pp. 155–159). Bologna.

- Lehtinen, A., Hannukkala, A., Andersson, B., Hermansen, A., Le, V. H., Nærstad, R., Brurberg, M. B., Nielsen, B. J., Hansen, J. G., Yuen, J. (2008). Phenotypic variation in Nordic populations of *Phytophthora infestans* in 2003. *Plant Pathol.*, **57**, 227–234.
- Le Romancer, M., Kerlan, M., Nedellec, M. (1994). Biological characterization of various geographical isolates of potato virus Y including superficial necrosis on potato tubers. *Plant Pathol.*, **43**, 138–144.
- Malcolmson, J. F. (1969). Races of *Phytophthora infestans* occurring in Great Britain. *Trans. Brit. Mycol. Soc.*, **3**, 417–423.
- Malcolmson, J. F., Black, W. (1966). New R genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica*, **15**, 199–203.
- Müller, K. O., Black, W. (1952). Potato breeding for resistance to blight and virus diseases during the last hundred years. *Z. Pflanzenzucht.*, **31**, 305–318.
- Munoz, F. J., Plaisted, R. L., Thurston, H. D. (1975). Resistance to potato virus Y in *Solanum tuberosum* spp. *andigena*. *Amer. Potato J.*, **52**, 107–115.
- Naess, S. K., Bradeen, J. M., Wielgus, S. M., Haberlach, G. T., McGrath, J. M., Helgeson, J. P. (2000). Resistance to late blight in *Solanum bulbocastanum* is mapped to chromosome 8. *Theor. Appl. Genet.*, **101**, 697–704.
- Niederhauser, J. S., Alvarez-Luna, E., Mackenzie, D. R. (1996). RETONA, a new strategy in the control of potato late blight. *Amer. Potato J.*, **73**, 225–229.
- Ortiz, R. (2012). Swimming in the breeding pool: Partnering for conservation of plant genetic resources through crop germplasm enhancement. *Proc. Latvian Acad. Sci.*, Section B, **66** (4/5), 143–147.
- Pavek, J. J., Corsini, D. L. (2001). Utilization of potato genetic resources in variety development. *Amer. J. Potato Res.*, **78**, 433–441.
- Ross, H. (1952). Studies on mosaic resistance in the potato. In: *Proc. 3rd Conf. Potato Virus Disease*, (40–47). Wageningen, The Netherlands.
- Ross, H. (1958). Inheritance of extreme resistance to virus Y in *Solanum stoloniferum* and its hybrids with *Solanum tuberosum*. In: *Proceedings of the 3rd Conference of Potato Virus Diseases, Lisse-Wageningen, 24–28 June, 1957* (pp. 204–211). Lisse-Wageningen, The Netherlands.
- Ross, H. (1986). Potato breeding-problems and perspectives. *Advances in Plant Breeding. J. Plant Breed.*, Suppl. **13**, Paul Parey, Berlin and Hamburg.
- Runno-Paurson, E., Fry, W. E., Myers, K. L., Koppel, M., Mand, M. (2009). Characterization of *Phytophthora infestans* isolates collected from potato in Estonia during 2002–2003. *Eur. J. Plant Pathol.*, **124**, 565–575.
- Sliwka, J., Jakuczun, H., Lebecka, R., Marczewski, W., Gebhardt, C., Zimnoch-Guzowska, E. (2006). The novel, major locus *Rpi-phu1* for late blight resistance maps to potato chromosome IX and is not correlated with long vegetation period. *Theor. Appl. Genet.*, **113**, 685–695.
- Sliwka, J., Jakuczun, H., Chmielarz, M., Hara-Skrzypiec, A., Tomczynska, I., Kilian, A., Zimnoch-Guzowska, E. (2012). Late blight resistance gene from *Solanum ruiz-ceballosii* is located on potato chromosome X and linked to violet flower colour. *BMC Genetics*, **13** (11). <http://www.biomedcentral.com/1471-2156/13/11>.
- Smilde, W. D., Brigneti, G., Jagger, L., Perkins, S., Jones, J. D. G. (2005). *Solanum mochiquense* chromosome IX carries a novel late blight resistance gene *Rpi-moc1*. *Theor. Appl. Genet.*, **110**, 252–258.
- Song, Y. S., Schwarzfischer, A. (2008). Development of STS markers for selection of extreme resistance (*R_{Ysto}*) to PVY and maternal pedigree analysis of extremely resistant cultivars. *Amer. J. Potato Res.*, **85**, 159–170.
- Stewart, H. E., Bradshaw, J. E., Pande, B. (2003). The effect of the presence of R-genes for resistance to late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*) on the underlying level of field resistance. *Plant Pathol.*, **52**, 193–198.
- Swiezynsky, K. (1994). Inheritance of resistance to viruses. In: Bradshaw, J. E., Mackay, G. (eds.). *Potato Genetics* (pp. 339–363). Wallingford, United Kingdom: CAB International.
- Tan, M., Adillah, Y., Hutten, R. C. B., Celis, C., Park, T.-H., Niks, R. E., Visser, R. G. F., van Eck, H. J. (2008). The *Rpi-mcd1* locus from *Solanum microdontum* involved in resistance to *Phytophthora infestans*, causing a delay in infection, maps on potato chromosome 4 in a cluster of NBS-LRR genes. *Mol. Plant. Microbe Interact.*, **21**, 909–918.
- Villamon, F. G., Spooner, D. M., Orrillo, M., Mihovilovich, E., Perez, W., Bonierbale, M. (2005). Late blight resistance linkages in a novel cross of the wild potato species *Solanum paucissectum* (series *Piurana*). *Theor. Appl. Genet.*, **111**, 1201–1214.
- Wastie, R. L. (1991). Breeding for resistance. *Adv. Plant Pathol.*, **7**, 193–224.
- Vossen van der, E. A. G. van der Gros, J., Sikkema, A., Muskens, M., Wouters, D., Wolters, P., Pereira, A., Allefs, S. (2005). The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato. *Plant J.*, **44**, 208–222.
- Villamon, F. G., Spooner, D. M., Orrillo, M., Mihovilovich, E., Pérez, W., Bonierbale, M. (2005). Late blight resistance linkages in a novel cross of the wild potato species *Solanum paucissectum* (series *Piurana*). *Theor. Appl. Genet.*, **111**, 1201–1214.
- Weidemann, H. L. (1993). Nekrotische Ringsymptome an Kartoffelknollen. Ein neuer Stamm des Kartoffelvirus Y als Ursache. *Kartoffelbau*, **44**, 308–309.
- Wulff, E., Torres, S., Vigil, E. (2002). Protocol for DNA extraction from potato tubers. *Plant Mol. Biol. Rep.*, **20**, 187–187.
- Zarzycka, H. (2001). Evaluation of resistance to *Phytophthora infestans* in detached leaflet assay. *Monografie i rozprawy naukowe 10b/2001*. IHAR, Radzikow, 75–77 pp.
- Zoteyeva, N. M. (2000). Wild potato species from the VIR collection as source of resistance to late blight. In: Khurana, S. M., Shekhewat, G. S., Singh, B. P., Pandey, S. K. (eds.). *Potato global research and developments*. Indian Potato Association, Shimla, India, **1**, 85–91.
- Zoteyeva, N., Skrabule, I., Carlson-Nilsson, U. (2011). Tuber formation in progenies derived from two short-day dependent Mexican potato species resistant to *Phytophthora infestans*. In: *Book of Abstracts. 18th Triennial Conference of the European Association of Potato Research, 24–29 July 2011, Oulu* (p. 24). Oulu, Finland.
- Zoteyeva, N., Chrzanowska, M., Flis, B., Zimnoch-Guzowska, E. (2012). Resistance to pathogens of the potato accessions from the collection of N. I. Vavilov Institute of Plant Industry (VIR). *Amer. J. Potato Res.*, **89**, 277–293.

Received 9 October 2014

IZTURĪBAS PRET LAKSTU PUVI GĒNU *R1* UN *R3* UN IZTURĪBAS PRET KARTUPEĻU VĪRUSU Y GĒNA *Ry_{sto}* NOVĒRTĒJUMS DIVU SAVVAĻAS SUGU LĪNIJĀS UN TO HIBRĪDOS

Oomicēte *Phytophthora infestans* Mont. (de Bary), kas izraisa kartupeļu lakstu puvi, un kartupeļu vīruss Y ir ekonomiski nozīmīgi kartupeļu patogēni. Evolūcijas gaitā ir izveidojušies virulentāki *P. infestans* patotipi, kuri spēj pārvarēt agrāk kultivētajos kartupeļu genotipos introgresētos savvaļas *Solanum* sugu, it īpaši *S. demissum* Lindl. rezistences gēnus. Pret līdz šim biežāk sastopamo PVY^N vīrusa celmu izturīgās kartupeļu šķirnes var būt jutīgas pret citiem virulentākiem PVY izolātiem. Iepriekšējie pētījumi norādīja, ka *S. guerreroense* Corr. (*grr*) un *S. neoanipoviczii* Buk. (*nan*) līnijas bija rezistentas pret *P. infestans* un pret diviem (*grr*) vai trijiem (*nan*) PVY celmiem. Šīs vecākaugu līnijas un to hibrīdie pēcnācēji tika pārbaudīti ar polimerāzes ķēdes reakcijas testiem, lai noteiktu slimību izturības gēnu alēles, kas nosaka izturību pret *P. infestans* (*R1* un *R3* gēni) un pret PVY (*Ry_{sto}*). Pārbaudes tika veiktas ar griezto lapu testu (*P. infestans*) un izmantojot ELISA testu (PVY). *Grr* izcelsmes hibrīdu izturība tika novērtēta kā augsta, galvenokārt hipersensitīvās atbildes dēļ. *R3* izturības alēle tika noteikta tikai vienā *grr* augā no 20 augus saturošām *grr* un *nan* populācijām. *R1* un *R3* izturības alēles tika noteiktas biežāk vienā no *grr* hibrīdu populācijas (*grr* x B.d.R5.). *Ry_{sto}* izturības alēle tika atrasta *nan* līnijā un nodrošināja izturību pret trijiem PVY celmiem ieskaitot PVY^{NTN}. No šīs līnijas veidotos hibrīdus raksturoja augsta *Ry_{sto}* izturības alēli saturošu augu frekvence. Vecāku klonā *tbr* x *phu*, kā arī *grr* līnijā un tās hibrīdos tika konstatēta tikai *Ry_{sto}* lokusa jutīgā alēle.