For several decades, wild species of Solanum L. section Petota Dumort. have been involved in potato cultivar breeding for robust resistance to pests and diseases. Potato late blight (LB) is caused by oomycete Phytophthora infestans (Mont.) de Bary, and the genes for race-specific resistance to P. infestans (Rpi genes) have been introgressed into cultivated potatoes by remote crosses and trans- or cigsaw, first from S. demissum Buk. and, more recently, from other wild species, such as S. bulbocastanum Dun., S. stoloniferum Schlecht. et Bch. et Bché, and S. venturii Hawkes et Hjerting (according to the nomenclature by Hawkes, 1990). Most wild species already involved in breeding for LB resistance came from North and Central Americas: series Bulbocastanum (Rydby.) Hawkes, Demissa Buk. and Longipedicellata Buk., and some Rpi genes of these species have already been characterized in much detail. Rpi genes of South American species, including the series Tuberosa (Rydby.) Hawkes, have not been sufficiently investigated. Among the latter, this study focuses on the Rpi genes of S. alandiae Card. and S. okadae Hawkes et Hjerting. Four accessions of S. alandiae, one accession of S. okadae and 11 clones of interspecific potato hybrids comprising S. alandiae germplasm from the VfR collection were PCR-screened using specific SCAR (Sequence Characterized Amplified Region) markers for eight Rpi genes. SCAR amplicons of five Rpi genes registered in this study were validated by comparing their sequences with those of prototype genes deposited in the NCBI Genbank. Among the structural homologues of Rpi genes found in S. alandiae and S. okadae, of special interest are homologues of CC-NB-LRR resistance genes with broad specificity towards P. infestans races, in particular R2=Rpi-blb3, R8, R9a, Rpi-vnt1 and Rpi-blb2 (94 99, 94 99, 86 89, 92 98 and 91% identity with the prototype genes, respectively). Our data may help to better understand the process of Rpi gene divergence along with the evolution of tuber-bearing Solanum species, particularly in the series Tuberosa.

**Key words:** tuber-bearing Solanum species, Phytophthora infestans, CC-NB-LRR genes, structural homologues, SCAR markers.

South American species Solanum alandiae Card. and S. okadae Hawkes et Hjerting as potential sources of genes for potato late blight resistance

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Дикорастущие виды Solanum L. секции Petota Dumort., включая S. demissum Buk., а также S. bulbocastanum Dun., S. stoloniferum Schlecht. et Bch. et Bché, S. venturii Hawkes et Hjerting (здесь и далее по системе Hawkes, 1990) и другие – служат источниками генов устойчивости к Phytophthora infestans (Mont.) de Bary (генов для резистентности к P. infestans, Rpi-генов) в селекции картофеля на устойчивость к фитофторозу путем отдаленной гибридизации и транс-гиссенеза. В отличие от Rpi-генов, детально охарактеризованных у видов Solanum из Северной и Центральной Америки, прежде всего серий Bulbocastanum (Rydby.) Hawkes, Demissa Buk. и Longipedicellata Buk., Rpi-генов южноамериканских видов, включая серию Tuberosa (Rydby.) Hawkes, изучены очень слабо. К числу последних относятся S. alandiae Card. и S. okadae Hawkes et Hjerting. Четыре образца S. alandiae, образец S. okadae и 11 клонов межвидовых гибридов S. alandiae с культурным картофелем из коллекции ВИР исследовали методом ПЦР с помощью специфичных SCAR-маркеров (Sequence Characterized Amplified Region) восьми Rpi-генов. Последовательности SCAR-маркеров пяти обнаруженных Rpi-генов валидировали, сравнивая их с генами-прототипами из Генбанка NCBI. Среди структурных гомологов Rpi-генов, найденных у S. alandiae и S. okadae, особый интерес представляют гомологи Rpi-генов с широкой расовым специфичностью: R2=Rpi-blb3, R8, R9a, Rpi-vnt1 и Rpi-blb2 (соответственно 94–99, 94–99, 86–89, 92–98 и 91% сходства с генами-прототипами). Полученные данные будут способствовать лучшему пониманию дивергенции Rpi-генов в процессе эволюции клубненосных видов Solanum, особенно в серии Tuberosa.

**Key words:** клубненосные виды Solanum, Phytophthora infestans, CC-NB-LRR гены, структурные гомологи, SCAR-маркеры.
Introduction

Late blight (LB) caused by oomycete *Phytophthora infestans* (Mont.) de Bary is among the major obstacles on the road to sustainable potato production. To limit this disease, breeders deploy the technologies of remote crosses and trans- and cisgenesis to transfer the genes of resistance to *P. infestans* (*Rpi* genes) from wild *Solanum* species into susceptible potato cultivars, which are demanded by the market. However, due to rapid genome evolution and migration of new strains, severe outbreaks of the pathogen have been relentlessly overcoming plant defense barriers built up by breeders and negating, literally within a few days, their many years of effort (Cooke et al., 2012; Fry, 2016). By combining (pyramiding) several *Rpi* genes in one potato plant, breeders provide for robust and durable resistance of new cultivars to a broad range of *P. infestans* pathotypes. Therefore, it is an urgent task of plant biologists to constantly seek for new sources of resistance, predominantly in wild *Solanum* genotypes, which have not been as yet introduced into potato breeding, and expand the scope of *Rpi* genes thoroughly characterized and documented with molecular methods (Vossen et al., 2014; Bethke et al., 2019).

All presently characterized *Rpi* genes belong to the CC-NB-LRR type: their protein products comprise coiled-coil, nucleotide-binding and leucine-rich region domains (Hein et al., 2009; Jupe et al., 2012; Rodewald, Trognitz, 2013). The mechanisms of immediate molecular interactions between the products of *P. infestans* avirulence genes and the products of *Solanum Rpi* genes, that is, effector recognition by receptor kinase, have been sufficiently researched only in few cases, such as Avr3a – R3a interaction. However, such lack of information on the *Rpi* genes does not hinder the genetic studies aimed at mining *Solanum* collections for new *Rpi* genes and introducing these genes into breeding for durable LB resistance. Following the marker-assisted breeding and cloning of *Rpi* genes, recent years have seen a considerable progress in this direction. New methodologies, such as effectomics, allele profiling and diagnostic resistance gene enrichment sequencing (dRenSeq), tremendously facilitated the search for new *Rpi* genes and new alleles of *Rpi* genes already characterized in other species of *Solanum L.* within the section Petota Dumort. (Vossen et al., 2014; van Weymers et al., 2016; Chen et al., 2018; Jiang et al., 2018; Armstrong et al., 2019). Currently some of these genes have been introgressed into commercial potato cultivars (Hae saert et al., 2015).

The potato genetic collection maintained at the N.I. Vavilov Institute of Plant Genetic Resources (VIR) in St. Petersburg is a major source of prospective genotypes for future breeding. It is facilitated the search for new *Rpi* genes and new alleles of *Rpi* genes already characterized in other species of *Solanum L.* (Mont.) de Bary (accepted by Spooner et al., 2014; as *S. brevicula* Bitter) and *S. okadai* Hawkes et Hjerting. We screened these species with specific SCAR (Sequence Characterized Amplified Region) markers of eight *Rpi* genes and found several structural homologues of *Rpi* genes with broad specificity toward *P. infestans* races: R2 / Rpi-blb3, R8, R9, Rpi-vnt1 and Rpi-blb2. Some data presented below have been reported at the XVII EuroBlight Workshop (Muratova [Fadina] et al., 2019).

Materials and methods

Plant material from the VIR potato genetic collection included the lines isolated from *S. alandiae* accessions k-18473, k-20408 and k-21240. The *S. okadai* line was isolated from the accession k-25397 derived from *P. infestans* resistant accession CGN 18279, which was kindly provided by Roel Hoekstra (Wageningen University & Research, Wageningen [WUR]). In addition, our study included potato interspecific hybrids containing *S. alandiae* germplasm and several potato cultivars and hybrids employed as references in LB assessments and as positive and negative controls in the marker analysis. These hybrids and cultivars are maintained in the collection of the All-Russian Research Institute of Phytopathology, Moscow Province, Russia (http://www.vnipfi.ru).

Resistance of wild species to *P. infestans* was evaluated in the laboratory tests with detached leaves according to Fadina et al. (2017) using a highly virulent and aggressive isolate of *P. infestans* 161 (races 1.2.3.4.5.6.7.8.9.10.11, mating type A1) collected in Moscow Province (the collection of the Institute of Phytopathology), and cv. ‘Santé’ as a reference. The LB resistance of potato hybrids and cultivars was assessed in the field trials at the Institute of Phytopathology and VIR under natural infestation by registering the area under the disease progress curve (AUDPC) against several cultivars used as references. All experimental data for LB resistance were converted to 1–9-point scores.

Plant DNA samples were PCR-screened for eight *Rpi* genes: R1, R2/Rpi-blb3, R3a, R3h, R8, Rpi-blb1 = Rpi-sto1, Rpi-blb2 and Rpi-vnt1 (Fadina et al., 2017). Specific SCAR markers are based on the sequences of *Rpi* prototype genes, that is, the genes extensively characterized by their structure and function. The markers were validated against *Solanum* genotypes comprising the functional alleles of the corresponding genes. Methods for DNA isolation, PCR primers (Fig. 1; Table 1) and protocols for PCR analysis, cloning and sequencing of DNA fragments as well as bioinformatic procedures were described previously (Fadina et al., 2017; Muratova [Fadina] et al., 2019). SCAR amplicons were verified by comparing their sequences to those of prototype genes deposited in the NCBI Genbank.

Results and discussion

Several accessions of *S. alandiae* were reported to manifest considerable resistance to *P. infestans* (Perez et al., 2001; Bhardwaj et al., 2018; Zoteyeva, 2019); however, the *Rpi* genes in *S. alandiae* have not been researched extensive- ly. By screening *S. alandiae* accessions and *S. alandiae* hybrids with potato cultivars we found the structural homologues of several *Rpi* genes of broad specificity toward *P. in-
Table 1. SCAR markers of Solanum Rpi genes (according to Muratova [Fadina] et al., 2019, modified)

| Gene | Prototype gene* | Marker and its size, bp | Position on the prototype genes, bp | Primer sequences | Anneal. temp, °C | Reference |
|------|-----------------|------------------------|-------------------------------------|-----------------|-----------------|-----------|
| R1   | AF447489        | R1-1205                | 5126-6331                           | F-cactctgacatatctcacta R-gtacacctctcttgcaagaat | 61             | Sokolova et al., 2011 |
| R2/Rpi-blb3 | FJS36325     | R2-686                 | 1370-2055                           | F-gcttctgatagattcagctat R-agggcctctcttgcaagaat | 54             | Kim et al., 2012 |
| R3a  | AY849382        | R3a-1380               | 1677-3056                           | F-gtacacctctctcttgcaagaat R-agggcctctctcttgcaagaat | 64             | Sokolova et al., 2011 |
| R3b  | JF900492        | R3b-378                | 94818-95195                         | F-gtacagtgaatctgtttcttgcaagaat R-acggcttcttgaatgaa | 64             | Rietman et al., 2012 |
| Rpi-blb1 = Rpi-sto1 | AY336128     | Rpi-blb1-820          | 2304-3124                           | F-aacctgtatggcagtggcatg R-gtcagaaaagggcactcgtg | 62             | Wang et al., 2008 |
|      | EU884421        | Rpi-sto1-890          | 241-1130                            | F-accagggcacaagattcct R-ctcgcggtctgccgtaataca | 65             | Haesaert et al., 2015 |
| Rpi-blb2 | DQ122125       | Rpi-blb2-976          | 3226-4202                           | F-gtcagggcacaagattcct R-ctcgcggtctgccgtaataca | 55             | Van der Vossen et al., 2005 |
| Rpi-vnt1 | FJ423046       | Rpi-vnt1.3-612        | 89-701                              | F-ccttctctctctctctctttagct R-gtacagtgaatctgtttcttgcaagaat | 58             | Pel, 2010 |
| R8   | KU53015         | R8-1276                | 73694-74970                         | F-aacaagagatgattaaatctcttggact RCC R-gtacagtgaatctgtttcttgcaagaat | 62.5           | Modified after Vossen et al., 2016 |

* Accession numbers in the NCBI Genbank (https://www.ncbi.nlm.nih.gov/)
* Номера последовательностей в Генбанке NCBI (https://www.ncbi.nlm.nih.gov/)

Fig. 1. SCAR markers of the Rpi genes are schematically positioned against the CC-NB-LRR domains (according to Muratova [Fadina] et al., 2019, modified)

Рис. 1. Схема положения SCAR-маркеров в последовательностях CC-NB-LRR доменов Rpi-генов [по Muratova [Fadina] et al., 2019, с изменениями]
festans races (Table 2, Table 3). Comparison of marker sequences to those of the prototype genes showed that the S. alandiae genome comprised the structural homologues of R2/Rpi-blb3, R8, R9a, Rpi-vnt1 and Rpi-blb2 (with 94 99, 94 99, 86 89, 92 98 and 91% identity with the prototype genes, respectively). The marker of the R1 gene, found only in potato cultivars and their hybrids, was apparently derived from that of S. demissum. The markers of the R3b and Rpi-blb1 = Rpi-sto1 genes were not found in the analyzed genotypes.

This is the first report on the structural homologue of the Rpi-vnt1 gene in S. alandiae. S. okadae amplicons sequenced in this study were 97–98% identical to the Rpi-vnt1.3 homologue from the S. alandiae genome (Table 3). The Rpi-vnt1.2 allele was reported in the accession CGN 18279 (Pel, 2010), corresponding to S. okadae k-25397; however, Pel and his associates (Pel, 2010) presumed that this accession belonged to the species S. venturii.

The structural homologues of the Rpi-vnt1 gene from S. alandiae and S. okadae differed from the prototype gene

Table 2. Presence or absence of the SCAR markers Rpi genes in accessions of Solanum okadae, S. alandiae and in S. alandiae hybrids with potato cultivars (according to Muratova [Fadina] et al., 2019, modified)

| Genotype | VIR accession number and pedigree* | R1-1250 | R2-686 | R3a-1380 | Rpi-blb2-976 | R8-1259 | Rpi-vnt1.3-612 | Resistance to P. infestans, points |
|----------|-----------------------------------|---------|--------|----------|-------------|---------|----------------|----------------------------------|
| S. alandiae | k-21240 D17-329 | 0 | 0 | 0 | 1 | 1 | 0 | 5 |
| S. alandiae | k-18473-1 | 0 | 1 | 0 | 1 | 1 | 1 | nd |
| S. alandiae | k-19443 | 0 | 0 | 0 | 1 | 0 | 0 | 5 |
| S. alandiae | k-20408 | 0 | 0 | 0 | 1 | 1 | 0 | 3 |
| S okadae | k-25397-1 | 0 | 1 | 0 | 1 | 1 | 1 | nd |
| cv. Atzimba P1 | nd | 0 | 0 | 0 | 1 | 1 | 0 | 5 |
| 39-1-2005 | Atzimba × S. alandiae | 0 | 0 | 0 | 0 | 1 | 1 | 6 |
| cv. Elizaveta P2 | acl, adg, dms, phu, sto, vrn | 1 | 0 | 1 | 0 | 0 | 0 | 4 |
| cv. Svitanok Kievskij P2 | dms | 0 | 1 | 1 | 0 | 1 | 0 | 5 |
| 24-1 | Atzimba × S. alandiae | 0 | 0 | 0 | 1 | 1 | 1 | 6 |
| 24-2 | 0 | 1 | 0 | 1 | 0 | 1 | 6 |
| 117-1 | 0 | 0 | 0 | 1 | 0 | 1 | 5 |
| 117-2 | 0 | 0 | 0 | 1 | 1 | 1 | 5 |
| 25-1-2007 | 24-1 × Elizaveta | 1 | 0 | 0 | 1 | 0 | 0 | 5 |
| 25-2-2007 | 1 | 0 | 0 | 1 | 0 | 1 | 4 |
| 134-6-2006 | 24-2 × Svitanok Kievskij | 0 | 0 | 1 | 0 | 1 | 1 | 5 |
| 134-2-2006 | 0 | 0 | 0 | 0 | 1 | 1 | 6 |
| 135-1-2006 | Svitanok Kievskij × 24-2 | 1 | 1 | 1 | 0 | 0 | 0 | 4 |
| 135-2-2006 | cv. Alouette | 0 | 0 | 1 | 0 | nd | 1 | 8 |

* acl, S. acaule Bitt.; adg, S. tuberosum subsp. andigena Hawkes; acln, S. alandiae Cár. dms, S. demissum Lindl.; phu, S. phureja Juz. et Buk.; sto, S. stoloniforum Schlechtd. et Bché; vrn, S. vernei Bitt. et Wittm.; nd, no data.

1 presence of the marker, 0 absence of the marker

Приложение
**Table 3.** Similarity levels (% identity) of nucleotide sequences in sequenced fragments of *Solanum alandiae* and *S. okadae* genomes as compared with the homologous regions of known Rpi genes

| Homologue | R2/Rpi-blb3 | R2/Rpi-blb3 |
|-----------|-------------|-------------|
| S. demissum | FJ536325** | S. bulbocastanum | FJ536346 |
| S. okadae* | 94 | 97 | 98-99 |

| Homologue | 86-89 | 92-93 | 92-93 | 92-93 | 97-98 |
|-----------|-------|-------|-------|-------|-------|
| Rpi-vnt1.1 | Rpi-vnt1.2 | Rpi-vnt1.3 | Rpi-vnt1 | 99 | 85 | 94 |
| S. venturii | FJ423044 | FJ423045 | FJ423046 | S. okadae | k-25397* |
| S. lycopersicum | Sw5-b | S. lycopersicum | Sw5-b | S. okadae | k-25397* |

* Cloned in our laboratory; ** compared to the sequence published by Armstrong et al. (2019) as Appendix S1 (https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.12997).

Rpi-vnt1.1 of *S. venturii* (FJ423044) by several single nucleotide polymorphisms (SNPs) and one three-nucleotide insertion. Rpi-vnt1.1 clones from *S. alandiae* (accession k-18473) revealed two diverse variants of sequences with 90% identity: the sequence Rpi-vnt1.1 *S. alandiae* 2 comprised a 24-nucleotide deletion, which was also characteristic of the Rpi-vnt1.1 sequences in *S. okadae*, the prototype gene from *S. venturii* (FJ423044) and the marker Rpi-vnt1.3-612 (MH297492) cloned from *S. alandiae* (Fig. 2). One of the SNPs in Rpi-vnt1.3 from *S. alandiae* 1 resulted in a stop codon and, as a consequence, a shortened protein (Fig. 3), which was 83% identical to Rpi-vnt1-like amino acid sequence from *S. okadae* (ADB85624) and only by 77% to the corresponding sequence from *S. venturii* (ACJ66596). The amino acid sequences were identical to the corresponding Rpi-vnt1 sequence (ACJ66596) encoded by the functionally active Rpi-vnt1.3 allele of *S. venturii* (FJ423046). Thus, we can assume that at least some regions of Rpi-vnt1 in *S. alandiae* and *S. okadae* are translatable. In addition, *S. alandiae* comprised sequences resembling the Rpi-vnt1-like, which were different from the functional gene Rpi-vnt1.

The presence of the Rpi-vnt1.3-612 marker in *S. alandiae* and potato hybrids was significantly related to elevated LB resistance (the Spearman’s correlation coefficient 0.54 at 5% confidence level).

The Rpi-vnt1.3-612 sequences cloned from cv. Atzimba × *S. alandiae* hybrids resembled the functional gene R9a/Avr-vnt1 recognition in transient expression assays (van Weymers et al., 2016). In addition to *S. venturii*, M. A. Pel (2010) cloned Rpi-vnt1 homologues sharing 80 to 97% identity with the prototype gene from 13 wild South American species belonging to the series *Tuberosa*; the gene was represented by three alleles of monophyletic origin, with Rpi-vnt1.1 and Rpi-vnt1.3 alleles apparently evolved from Rpi-vnt1.2. We compared these sequences to Rpi-vnt1 homologues from
**Рис. 2.** Множественное выравнивание нуклеотидных последовательностей фрагментов структурных гомологов генов Rpi-vnt1 Solanum alandiae и S. okadae, а также участка функционального Rpi-vnt1-гена.

Rpi-vnt1 S. alandiae 1 и Rpi-vnt1 S. alandiae 2, маркер Rpi-vnt1.3-612 клонирован из S. alandiae k-18473; Rpi-vnt1 S. okadae, маркер Rpi-vnt1.3-612 клонирован из S. okadae k-25397; Rpi-vnt1 S. venturii, прототип гена Rpi-vnt1.1 (FJ423044); Rpi-vnt1 Alouette, маркер Rpi-vnt1.3-612 клонирован от сорта Alouette (MH297492). Идентичные нуклеотиды обозначены звездочками. Области различия выделены серой заливкой.

**Рис. 3.** Множественное выравнивание участков последовательностей белков Rpi-vnt1 из межвидовых гибридов Atzimba × S. alandiae (24-1, 24-2, 117-1, 117-2 и 39-1-2005), сорта Alouette и соответствующих последовательностей белков, кодируемых известными функциональными генами.

RPI-EDN2 соответствует Rpi-edn2 от S. edinense (US Patent 2014/0041072 A1), Rpi-vnt1.1, Rpi-vnt1.2 и Rpi-vnt1.3 – фрагменты соответствующих белков S. venturii ACJ66594, ACJ66595 и ACJ66596, RPP13-like – белок S. tuberosum гена устойчивости S. tuberosum к болезни XP, 015170549. Области различия выделены серой заливкой; KGA – аминокислотные остатки, характерные для белков Rpi-vnt-like и RPI-EDN2.
S. alandiae and S. okadae characterized in this study. To eliminate polymorphisms due to synonymous substitutions, we aligned amino acid sequences corresponding to the Rpi-vnt1 1.3-612 marker found in S. alandiae and S. okadae with the sequences corresponding to Rpi-vnt1 proteins in other South American species (Fig. 4).

These South American species comprise an insertion absent in other sequences, including S. okadae GU338334, and a deletion shared with S. bukasovii GU338315 and S. microdontum GU338312. It is of special significance that we failed to discern the marker Rpi-vnt1 1.3-612 in S. verrucosum Schltdl. accessions k-24995, k-24991, k-23760, k-24313, k-23015, k-23760, and PI195171, the only Tuberosa species in North and Central Americas (Spooner et al., 2014). The absence of Rpi-vnt1 in S. verrucosum was also reported using dRenSeq technology (Chen et al., 2018).

In addition to Rpi-vnt1 genes, SCAR marker analysis of S. alandiae and S. okadae revealed structural homologues of R2/Rpi-blb3, Rpi-blb2 and R8 characteristic of North and Central American species (Table 3). The S. alandiae fragment was 91% homologous to Rpi-blb2 in S. bulbocastanum (DQ122125) and differed from the prototype by several SNPs and deletions (Fig. 5). The S. alandiae and S. okadae fragments were both 99% identical to R8 from S. demissum (KU530153) and differed in several SNPs and one nucleotide deletion. The R8 homologue from hybrid 24-1 (Atzimba × S. alandiae k-21240) completely matched the R8-1276 sequence from S. alandiae k-20408 (Fig. 6). R8 homologues from S. alandiae and S. okadae differed by three deletions from the corresponding fragment of Sw5b (Fig. 6), which was only 83% identical to R8 in S. demissum (Jiang et al., 2018).

R2 homologues from S. alandiae and S. okadae were 94–97% identical to the prototype gene from S. demissum (FJ536325) and its orthologue Rpi-blb3 from S. bulbocastanum (FJ536346). Notably, the latter, together with R2 homologues from S. alandiae and S. okadae, differed from the R2 of S. demissum by several SNPs and a six-nucleotide insertion (Fig. 7).

Fig. 4. Multiple alignment of nucleotide sequences of the fragments of Rpi-vnt1 structural homologues from Solanum alandiae and S. okadae, and regions of the known Rpi-vnt1 genes from South American species of Tuberosa series (Pel, 2010).

Рис. 4. Множественное выравнивание нуклеотидных последовательностей фрагментов структурных гомологов генов Rpi-vnt1 Solanum alandiae и S. okadae, а также участков гена Rpi-vnt1 из южноамериканских видов серии Tuberosa (Pel, 2010).
Fig. 5. Structural homology between the cloned marker fragment Rpi-blb2-976 from Solanum alandiae accession k-19443, the Rpi-blb2 gene from S. bulbocastanum (DQ122125), and its analogue Mi-1 from S. lycopersicum L. (AF091048).

For other notations see Fig. 2

Рис. 5. Сравнение нуклеотидных последовательностей клонированного маркерного фрагмента Rpi-blb2-976 из образца k-19443 Solanum alandiae, последовательностей гена Rpi-blb2 из S. bulbocastanum (DQ122125) и его аналога Mi-1 из S. lycopersicum L. (AF091048).

Другие обозначения как на рис. 2

Fig. 6. Structural homology between the cloned marker fragment R8-1276 from Solanum alandiae accession k-20408, hybrid 24-1 comprising S. alandiae germplasm, and the prototype gene R8 from S. demissum (KU530153); Sw5b, R8 analogue from S. lycopersicum (AY007366).

For other notations see Fig. 2

Рис. 6. Сравнение нуклеотидных последовательностей клонированного маркерного фрагмента R8-1276 из образца k-20408 Solanum alandiae, гибрида 24-1, содержащего генетический материал S. alandiae, и гена-прототипа R8 из S. demissum (KU530153); Sw5b - аналог R8 из S. lycopersicum (AY007366).

Другие обозначения как на рис. 2
Fig. 7. Structural homology between the cloned marker fragment R2-686 from Solanum alandiae accession k-18437 and S. okadae accession k-25397, the prototype gene R2 from S. demissum (FJ536325), and its orthologue Rpi-blb3 from S. bulbocastanum (FJ536346). For other notations see Fig. 2

Rис. 7. Сравнение нуклеотидных последовательностей клонированного маркерного фрагмента R2-686, из образцов k-18437 Solanum alandiae и k-25397 S. okadae, гена-прототипа R2 из S. demissum (FJ536325) и его ортолога Rpi-blb3 из S. bulbocastanum (FJ536346). Другие обозначения как на рис. 2

Conclusion

The comparative SCAR marker analysis of S. alandiae and S. okadae accessions in the VIR collection discovered several structural homologues of already known Rpi genes. When analyzing these data, two caveats are appropriate. First, we dealt with rather short DNA fragments, which never covered more than one third of the complete gene sequence. Secondly, even when these fragments were translatable, the proof of functionality of Rpi homologues in S. alandiae and S. okadae must await further studies by independent methods. Our data match the evidence that the Rpi-vnt1 gene is specific to South American species of series Tuberosa (Pel, 2010) and is absent in Mexican S. verrucosum (Chen et al., 2018), despite the fact that the latter species is often grouped with South American S. verrucosum species of series Solanum. Other Rpi homologues found in S. alandiae and S. okadae resemble the R2, R8, R9 and Rpi-blb2 genes of Mexican species S. demissum and S. bulbocastanum. An Rpi-mcd1 gene orthologous to R2 but distinct in its chromosomal localization was reported in South American species S. microdontum (Hein et al., 2009; Pel, 2010; Vossen et al., 2014; van Weymers et al., 2016; Aguilera-Galvez et al., 2018); however, the sequence of Rpi-mcd1 has not yet been published. South American homologues of Rpi genes reported here for the first time are notably distinct from their Mexican prototypes. Among the Rpi homologues found in S. alandiae and S. okadae, especially promising for potato breeders are those resembling the genes of broad specificity toward P. infestans races, such as R2 / Rpi-blb3, R8, R9, Rpi-vnt1 and Rpi-blb2. In this context, it seems proper to mention our evidence that the presence of an Rpi-vnt1 marker significantly correlated with superior LB resistance.

Our search for new sources of Rpi genes among wild Solanum species takes us to two entwined issues of evolutionary genomics of the tuber-bearing Solanum: genome and species evolution in the section Petota and the origin of Rpi genes associated with the evolution of P. infestans – potato interactions often resulting in LB outbursts. In this context, the genes for defense against pests and diseases in S. alandiae and S. okadae are especially interesting as regards the evolution of the tuber-bearing Solanum. J. G. Hawkes (1990) emphasized close relations of these two species with S. microdontum and resemblance of S. okadae to the Argentinian endemic S. venturi. The areas of distribution of the two latter species are located in neighboring highlands of Bolivia and northwestern Argentina. S. alandiae is a Bolivian endemic from the bordering Departments of Cochabamba, Santa Cruz and Chuquisaca, with its plants growing in contrasting climates of cold and hot dry grasslands and warm and wet territories (Fuentes, 2014). The area of S. okadae is disjunct. In Bolivia, it is an endangered species, with few tiny islands in the Departments of Cochabamba and La Paz. Here, its natural habitat is alpine wet meadows and forests, where plants are affected by LB (Coca Morante, Castillo Plata, 2007). The Argentinian part of the S. okadae area of distribution is in the Provinces of Jujuy and Salta. On the basis of molecular evidence, the Argentinian S. okadae accessions have been recently identified as S. venturi (PBI Solanum Project, 2020). These data presume independent evolution of S. alandiae and S. okadae from S. venturi.

All presently characterized Rpi genes belong to the CCR-NB-RR structures, with their evolution widely researched in tuber-bearing Solanum species (Hein et al., 2009; Pel, 2010; Jupe et al., 2012; Rodewald, Trognitz, 2013; Aguilera-Galvez et al., 2018). These species are primarily found in the Mexican and Andean centers of Petota diversity (Spooner et al., 2014; Hardigan et al., 2017), where they successfully cohabit with local P. infestans races (Grünwald, Flier, 2005; Fry, 2016). Many aspects of Petota evolution and Rpi gene geography are hotly debated (Vossen et al., 2014; Spooner et al., 2014; Hardigan et al., 2015; Hardigan et al., 2017), and our data may supplement the research into divergence of Rpi genes as related to the evolution of Solanum species, emergence of tuber-bearing forms and their distribution between two Americas. An impressive illustration of the latter issue is the presumably reciprocal segregation of the Rpi-blb1 and Rpi-vnt1 genes between the Mexican and Andean species, respectively.

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Hardigan M.A., Laimbeer F.P.E., Newton L., Crisovan E., Hamilton J.P. Vaillancourt B. et al. Genome diversity of tuber-bearing Solanum uncovers complex evolutionary history and targets of domestication in the cultivated potato. *Proceedings of the National Academy of Sciences of the USA.* 2017;114(46):E9999-E10008. DOI: 10.1073/ pnas.1714380114

Hawkes J.G. The potato: evolution, biodiversity and genetic resources. London: Belhaven Press; 1990.

Hein I., Birch P.R.J., Danan S., Lefebvre V., Odeny D.A., Gebhardt C. et al. Progress in mapping and cloning qualitative and quantitative resistance against *Phytophthora infestans* in potato and its wild relatives. *Potato Research.* 2009;52(3):215-227. DOI: 10.1007/s11540-009-9129-2

Jiang R., Li J., Tian Z., Du J., Armstrong M., Baker K. et al. Potato late blight field resistance from QTL dPl90c is conferred by the NB-LRR gene *R8*. *Journal of Experimental Botany.* 2018;69(7):1545-1555. DOI: 10.1093/jxb/ery021

Jo K., Visser R.G.F., Jacobsen E., Vossen J.H. Characterisation of the late blight resistance in potato differential Ma99 reveals a qualitative resistance gene, *R9a*, residing in a cluster of *Tm-2* (2) homologs on chromosome IX. *Theoretical and Applied Genetics.* 2015;128(5):931-941. DOI: 10.1007/s00122-015-2480-6

Jupe F., Fritchard L., Etherington G.J., Mackenzie K., Cock P.J.A., Wright F. et al. Identification and localisation of the NB-LRR gene family within the potato genome. *BMC Genomics.* 2012;13:75. DOI: 10.1186/1471-2164-13-75

Kim H.J., Lee H.R., Jo K.R., Mortazaviv.S.M.M., Huigen D.J., Evenhuis B. et al. Broad-spectrum late blight resistance in potato differential set plants *MaR8* and *Ma99* is conferred by multiple stacked *R* genes. *Theoretical and Applied Genetics.* 2012;124(5):923-935. DOI: 10.1007/s00122-011-1757-7

Muratova (Fadina) O.A., Beketova M.P., Kuznetsova M.A., Rogozina E.V., Khavkin E.E. *Solanum alandiae* as a potential source of late blight resistance genes. In: H.T.A.M. Schepers (Ed.). *Proceedings of the Seventeenth EuroBlight Workshop. WUR Special Report No. 19.* Wageningen: Wageningen University & Research; 2019. p. 217-226. Available from: https://agro.au.dk/fileadmin/eurobliht/Workshops/Proceedings/Special_Report_19_Totaal_LR.pdf [accessed Feb. 25, 2020].

PBI *Solanum* Project. *Solanaceaee Source. Solanum okadae.* 2013. Available from: http://solanaceaesource.org/content/solanum-okadae [accessed Jan. 08, 2020].

Pel M.A. Mapping, isolation and characterization of genes responsible for late blight resistance in potato [dissertation]. Wageningen: Wageningen University & Research; 2010. Available from: https://library.wur.nl/WebQuery/wurpubs/392076 [accessed Jan. 08, 2020].

Pérez W.A.S., Salas A., Raymundo R., Huanan Z., Nelson R., Bonierbale M. Evaluation of wild potato species for resistance to late blight. In: *Scientist and Farmer: Partners in Research for the 21st Century: Program Report 1999–2000*. Lima, Peru: CIP; 2001. p.49-62. Available from: https://books.google.ru/books?id=GVBeX1l_hWIC&pg=PA49&hl=ru&rsrc=gsb_toc_r&cad=3#v=onepage&q&f=false [accessed Jan. 08, 2020].

Rietman H., Bijsterbosch G., Cano L.M., Lee H.R., Vossen J.H., Jacobsen E. et al. Qualitative and quantitative late blight resistance in the potato cultivar Sarpo Mira is determined by the perception of five distinct RXLR effectors.
Molecular Plant–Microbe Interaction. 2012;25(7):910-919. DOI: 10.1094/MPMI-01-12-0010-R

Rodewald J., Trognitz B. Solanum resistance genes against Phytophthora infestans and their corresponding avirulence genes. Molecular Plant Pathology. 2013;14(7):740-757. DOI: 10.1111/mpp.12036

Rogozina E.V., Chalaya N.A., Kuznetsova M.A., Demidova V.N., Rogozhin A.N., Smetanina T.I. et al. Late blight resistant potato hybrid clones in the VIR collection of plant genetic resources. Proceedings on Applied Botany, Genetics and Breeding. 2018;179(3):278-292. [in Russian] (Rogozina E.V., Chalaya N.A., Kuznetsova M.A., Demidova V.N., Rogozhin A.N., Smetanina T.I. and dr. Устойчивые к фитофторозу гибридные клоны картофеля в коллекции генетических ресурсов ранетей ВИР. Труды по прикладной ботанике, генетике и селекции. 2018;179(3):278-292). DOI: 10.30901/2227-8834-2018-3-278-292

Sokolova E., Pankin A., Beketova M., Khavkin E., Yashina I., Kuznetsova M. et al. SCAR markers of the R-genes and germplasm of wild Solanum species for breeding late blight-resistant potato cultivars. Plant Genetic Resources: Characterisation and Utilisation. 2011;9(2):309-312. DOI: 10.1017/S1479262111003438

Spooner D.M., Ghislain M., Simon R., Jansky S.H., Gavrilenko T. Systematics, diversity, genetics, and evolution of wild and cultivated potatoes. The Botanical Review. 2014;80(4):283-383. DOI: 10.1007/s12229-014-9146-y

Van der Vossen E.A.G., Van der Vossen E.A.G., Vosman, B. Allele mining in Solanum: conserved homologs of Rpi-bib1 are identified in Solanum stoloniferum. Theoretical and Applied Genetics. 2008;116(7):933-943. DOI: 10.1007/s00122-008-0725-3

Zoteyeva N.M. Late blight resistance of wild potato species under field conditions in the Northwest of Russia. Proceedings on Applied Botany, Genetics and Breeding. 2019;180(4):159-169. [in Russian] (Zoteeva N.M. Устойчивость лиственных видов картофеля к фитофторозу в полевых условиях Северо-Запада РФ. Труды по прикладной ботанике, генетике и селекции. 2019;180(4):159-169). DOI: 10.30901/2227-8834-2019-4-159-169

Van Weymers P.S.M., Baker K., Chen X., Harrower B., Cooke D.E.L., Gilroy E.M. et al. Utilizing “Omic” technologies to identify and prioritize novel sources of resistance to the oomycete pathogen Phytophthora infestans in potato germplasm collections. Frontiers in Plant Science. 2016;7:672. DOI: 10.3389/fpls.2016.00672

Vossen J.H., Jo K.R., Vosman B. Mining the genus Solanum for increasing disease resistance. In: R. Tuberosa (ed.). Genomics of Plant Genetic Resources. Dordrecht: Springer; 2014. p. 27-46. DOI: 10.1007/978-94-007-7575-6_2

Wang M., Alfels S., van den Berg R.G., Veleshovshwers V.G.A., van der Vossen E.A.G., Vosman, B. Allele mining in Solanum: conserved homologs of Rpi-bib1 are identified in Solanum stoloniferum. Theoretical and Applied Genetics. 2008;116(7):933-943. DOI: 10.1007/s00122-008-0725-3

Zoteyeva N., Chhranowska M., Flis B., Zimnoch-Guzowska E. Resistance to pathogens of the potato accessions from the collection of N.I. Vavilov Institute of Plant Industry (VIR). American Journal of Potato Research. 2012;89(4):277-293. DOI: 10.1017/s122230-012-9252-5

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