CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

New homologues of the *Rpi-chc1* gene in wild and cultivated *Solanum* species

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Abstract: *The Rpi-chc1 gene confers resistance to late blight (LB) in the wild South American species Solanum chacoense. The goal of this study was to enhance our insight into polymorphism of this gene in the genus Solanum, which is the source of valuable donors of resistance to LB. To this end, we analyzed 122 accessions of the working collection, consisting of potato cultivars, complex interspecific hybrids, and representatives of 11 wild Solanum species. We studied the polymorphism of the region of this gene that encodes the most polymorphic LRR domain. As a result, in the species S. chacoense, S. berthaultii, S. tuberosum, S. microdontum, and S. maglia we found previously unknown variants of the Rpi-chc1 gene, which differ in their amino acid sequence from both the functional and non-functional variant of the Rpi-chc1 gene. Therefore, the function of these homologues cannot be unambiguously predicted, but must be further studied.*

Keywords: *Resistance genes, DNA markers, potato,* Phytophthora infestans*, late blight*

INTRODUCTION

Potato (*Solanum tuberosum*) is the third most important food crop after rice and wheat. Late blight (LB) caused by the oomycete *Phytophthora infestans* is one of the most devastating potato diseases. The global economic cost of LB is approximately €9.4 billion per year (Haverkort et al. 2016). One strategy to control LB is the introgression of LB resistance genes (*R* genes) from potato wild relatives. Most of these resistance genes have been introduced into commercial potato varieties from the wild species *S. demissum*. In particular, in *S. demissum* the *R1*, *R2*, *R3a*, *R3b*, *R8*, and *R9a* genes were mapped, characterized, and then bred to cultivated potato varieties (Ballvora et al. 2002, Huang et al. 2005, Lokossou et al. 2009, Li et al. 2011, Jo et al. 2015, Vossen et al. 2016). However, the resistance conferred by these genes is being overcome by new virulent strains of *P. infestans* (Jo et al. 2014). Therefore, it is important to search for new *R* genes that provide broad-spectrum resistance to a wide range of pathogen races at once. The main sources of these new LB resistance genes (*Rpi* genes) are wild *Solanum* species. To date, over 70 *Rpi* genes have been identified and mapped in 32 *Solanum* species (Paluchowska et al. 2022). One of these genes is the *Rpi-chc1* gene discovered in the wild species *S. chacoense* (Vossen et al. 2011).

S. chacoense is a diploid South American wild potato species native to Bolivia. The locus associated with resistance to LB has been mapped in *S. chacoense* on chromosome 10, and the gene conferring this resistance has been found at this locus. This gene was named *Rpi-chc1*. The *Rpi-chc1* gene encodes a protein

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from the NB-LRR family, consisting of 1302 amino acids and containing 29 leucine-rich repeats (LRR) (Vossen et al. 2011). Later it was found that the *Rpi-chc1* gene had two allelic variants, *Rpi-chc1.1* and *Rpi-chc1.2*, and it was shown that these alleles recognized different effectors from the PexRD12/31 superfamily of effector proteins of *P. infestans*. The *Rpi-chc1.1* allele recognizes several PexRD12 proteins, and the *Rpi-chc1.2* allele recognizes several PexRD31 proteins (Monino-Lopez et al. 2021). In addition, homologues of the *Rpi-chc1* gene were found in some *Solanum* species other than *S. chacoense*, in particular in *S. berthaultii*, *S. tarijense* and *S. tuberosum*, and among these homologues were both functional and non-functional variants (Monino-Lopez et al. 2021). At the same time, the functional variant *Rpi-chc1.1* was found to differ from its non-functional homologue from *S. tuberosum* by 21 amino acid substitutions, of which 19 are in the LRR domain (Monino-Lopez 2021). These data suggest that the *Rpi-chc1* gene is a member of a large *R* gene family that is still poorly understood in Solanaceae. Therefore, the search for new homologues of the *Rpi-chc1* gene in *S. chacoense* and other *Solanum* species makes sense, since in order to keep up with the rapid evolution of *Avr* genes, *Rpi* genes also rapidly evolve with the formation of new variants with different functional activities (Leister 2004, Mcdowell and Simon 2006). Besides, it should be noted that new data on the polymorphism of the primary structure of the *Rpi-chc1* gene and its homologues and the possible relationship of this polymorphism with the function will help in selecting targets for genome editing when breeding new LB-resistant potato varieties.

Thus, the goal of this research was to study the polymorphism of the *Rpi-chc1* gene in potato varieties and interspecific hybrids cultivated in Russia, as well as in accessions of wild potato species from the collection of the N.I. Vavilov Institute of Plant Genetic Resources (VIR). This collection is one of the largest collections of cultivated potato and wild tuber-bearing species in the world.

MATERIAL AND METHODS

Plant material

For a plant material we used 95 accessions of wild *Solanum* species from the collection of the N.I. Vavilov Institute of Plant Genetic Resources (VIR). In particular, six accessions of *S. andigenum*, five accessions of *S. bertaultii*, 13 accessions of *S. bulbocastanum*, ten accessions of *S. cardiophillum*, 19 accessions of *S. chacoense*, two accessions of *S. maglia*, eight accessions of *S. microdontum*, seven accessions of *S. phureja*, six accessions of *S. pinnatisectum*, eight accessions of *S. stoloniferum*, and 11 accessions of *S. verrucosum*. Also in our study we used plants of 17 registered potato cultivars, “Alpha”, “Atzimba”, “Desiree”, “Bintje”, “Early Rose”, “Eesterling”, “Escort”, “Gloria”, “Jubel”, “Robijn”, “Sarpo Mira”, “Sarpo Axona”, “Negr”, “Elizaveta”, “Zagadka Pitera”, “Nayada” and “Priekul’skij rannij”, as well as ten multiparental interspecific hybrids, 2372-60, 97.13-9, 97.1.17, 2522-173, 2584-7, 2359-13, 97.12-18, 25-85-70, 2585-80 and 2585-67, bred by I.M. Yashina at the Russian Potato Research Center by crossing potato varieties and/or breeding lines, which were backcrosses containing the genetic material of wild *Solanum* species (Yashina et al. 2010).

LB resistance assessment

LB resistance of varieties and hybrids was evaluated in the laboratory tests by the method of infection of detached leaves. Detached leaves of plants grown in a greenhouse were infected by applying to their surface 0.1 ml of a suspension of zoosporangia (approximately 3000 zoosporangia) of a highly virulent and aggressive isolate of *P. infestans* N161 from the collection of the Institute of Phytopathology (race 1.2.3.4.5.6.7.8.9.10.11, mating type A1), using the cultivar Santé as a reference control (Kuznetsova et al. 2014). Leaves were placed bottom side out in wet chambers. The lesion was evaluated in five days post infection. The resistance of the sample was scored on a 9-point scale, wherein 9 points correspond to the highest resistance level. The average score for each sample was calculated based on the results of damage of three leaves.

DNA isolation

Total DNA was isolated from young leaves of two-week-old plants using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer’s protocol. For each accession DNA was isolated from eight plants and then these DNA preparations were combined into one common sample.

Primer design and PCR conditions

PCR primers for specific amplification of the *Rpi-chc1* gene were designed based on the sequence of this gene from the International Patent Application WO2011/034433 (Vossen et al. 2011). In this application it was designated as 543-5_C10_C15_C24. We made a multiple alignment of this sequence with the sequences of other closely related homologues of the *Rpi-chc1* gene described in this patent application, as well as potato homologues available from the NCBI database. As a result, primers were selected that could discern the *Rpi-chc1* gene from its homologues due to the specific 3'-terminal sequence characteristic only of this gene. We designed primers that amplify the region of the *Rpi-chc1* gene encoding the LRR domain of the receptor protein, since this domain is responsible for pathogen recognition and it is the most polymorphic. The nucleotide sequences of the designed primers were as follows: 5¢-CTATTTGACTTCCCTCGAATTCT-3¢ for the forward primer and 5¢-CTTCTAACAATGGACAATCACGT-3¢ for the reverse primer. DNA amplification was performed in a thermal cycler GeneAmp PCR System 2700 (Applied Biosystems, Inc., USA) using the following cycling condition: one cycle of 94 °C for 5 min followed by 35 cycles of 94 °C for 35 sec, 60 °C for 35 sec and 72 °C for 1 min 20 sec; and final extension at 72 °C for 7 min. The volume of the reaction mixture was 25 µl. A sample of 50 ng of total DNA was taken per each reaction. PCR products were separated by electrophoresis in 1% agarose gel in 1X TAE buffer and visualized under UV after staining with 0.5 µg/ml ethidium bromide.

Cloning and sequencing

PCR products were cloned using pAL-TA vector (Evrogen, Moscow, Russia) in accordance with the manufacturer's protocol and then sequenced with a nucleic acid analyzer ABI PRIZM 3730 (Applied Biosystems, Inc., USA) using the Big Dye Terminator v.3.1 reagent kit (Applied Biosystems, Inc., USA) according to the manufacturer's instructions.

Bioinformatics analysis

Multiple alignment of nucleotide sequences was performed with the Clustal Omega software (http://www.ebi.ac.uk/Tools/msa/clustalo/), followed by analysis of this alignment with the GeneDoc software. Phylogenetic analysis was performed with the Treecon software (Van de Peer and de Wachter 1994). Deduced amino acid sequences were obtained with the EditSeq software.

RESULTS AND DISCUSSION

Occurrence of homologues of the *Rpi-chc1* gene in the working collection

We amplified the total DNA samples isolated from 122 accessions of the working collection, consisting of potato varieties, interspecific hybrids and wild *Solanum* species, with primers specific to the *Rpi-chc1* gene. As a result, the expected PCR product was obtained only in 30 samples. However, we probably failed to amplify the specific PCR product in some other samples due to the inherent disadvantage of the PCR method, which is that if mutations occur in the DNA at the primer binding sites, then PCR is not feasible, resulting in false negative results. PCR results for these 30 samples are shown in Figure 1. In any of the five studied North American species *S. bulbocastanum*, *S. cardiophillum*, *S. pinnatisectum*, *S. stoloniferum* and *S. verrucosum* the specific PCR product was not detected. This finding may support the assumption that the ancestral form of the *Rpi-chc1* gene arose after the separation of the North American and South American *Solanum* species, and the presence of functional alleles of *Rpi-chc1* gene in the latter may be the result of a recent cross of the species (Monino-Lopez et al. 2021). Among South American species we found the specific PCR product not only in *S. chacoense*, *S. berthaultii* and *S. tuberosum*, but also in two species, *S. microdontum* and *S. maglia*. In particular, in the case of *S. chacoense*, out of 19 analyzed samples, the expected PCR product was present in six, and out of eight analyzed samples of *S. microdontum* it was found in two samples. In recent decades, the species *S. microdontum* has already been used in programs for breeding LB resistant potato varieties (Sandbrink et al. 2000), and one LB resistance gene *Rpi-mcd1* was discovered in this species (Tan et al. 2008). In our study, for the first time, we found in *S. microdontum* a homologue of the *Rpi-chc1* gene, which was the most identical to the prototype gene (see Table 1 and discussion below). This finding makes *S. microdontum* an even more valuable donor of LB resistance and should serve as a starting point in an in-depth study of the structural and functional features of the homologue of the *Rpi-chc1* gene in this species. Also, the expected PCR product was found in *S. bertaultii* (in four samples out of five) and in both analyzed samples of *S. maglia*. For the species *S. maglia*, the homologue of the *Rpi-*

chc1 gene obtained in this study is the first reported homologue of the LB resistance gene. In varieties of cultivated potatoes, the specific PCR product was found in 12 of the 17 analyzed samples, and it was found in only four of ten screened hybrids.

Structural features of new homologues of the *Rpi-chc1* gene

In order to determine the nucleotide sequence of the PCR products obtained in all five abovementioned South American species, we cloned the amplified fragment from seven samples. These were samples of the wild species *S. chacoense* K19264, *S. microdontum* CGN 20640, *S. berthaultii* K19961 and *S. maglia* K240604 from the VIR collection and samples of cultivated potato *S. tuberosum*, represented by varieties "Sarpo Mira" and "Bintje" and a complex interspecific hybrid 2372-60. The cloned fragment corresponded to the region of the LRR domain in the C-terminal part of the *Rpi-chc1* protein. Five clones were sequenced for each sample. Hence, 35 nucleotide sequences of structural homologues of the *Rpi-chc1* gene were obtained in total. For each sample, the sequences of five clones were substantially similar to each other. A few single-nucleotide polymorphisms (from four to six) can be considered as artifactual, since each of them occurs only in one clone out of five. An exception was the "Sarpo Mira" sample. In this sample, four clones were almost identical (99.8% of sequence identity), and the fifth clone was significantly different from them (91.2% of sequence identity).

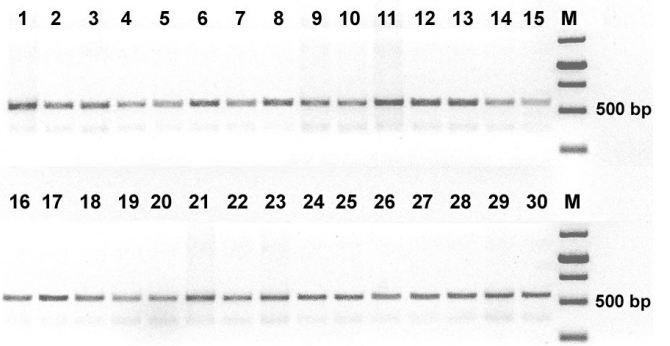

Figure 1. Results of PCR amplification of total DNA isolated from accessions of wild and cultivated *Solanum* species with primers specific to the *Rpi-chc1* gene. **1** – *S. berthaultii* K23047, **2** – *S. berthaultii* K23154, **3** – *S. berthaultii* K24267, **4** – *S. berthaultii* K19961, **5** – *S. chacoense* PI189219, **6** – *S. chacoense* GLKS 176, **7** – *S. chacoense* GLKS 1006, **8** – *S. chacoense* GLKS 135, **9** – *S. chacoense* CGN 22725, **10** – *S. chacoense* K19264, **11** – *S. maglia* K24604, **12** – *S. maglia* K2883, **13** – *S. microdontum* CGN 20640, **14** – *S. microdontum* CGN 23050, **15** – "Bintje", **16** – "Desiree", **17** – "Early Rose", **18** – "Eersteling", **19** – "Escort", **20** – "Gloria", **21** – "Jubel", **22** – "Nayada", **23** – "Priekul'skij rannij", **24** – "Sarpo Axona", **25** – "Sarpo Mira", **26** – "Zagadka Pitera", **27** – hybrid 2372-60, **28** – hybrid 2522-173, **29** – hybrid 2584-7, **30** – hybrid 2585-67, **M** – 1 kb DNA Ladder.

Thus, it suggests that the cultivar "Sarpo Mira" is polymorphic in the *Rpi-chc1* gene and has at least two variants of this gene in its genome. The highly resistant cultivar "Sarpo Mira" was known to have not only homologues of the *R3a*, *R3b* and *R4* genes from *S. demissum*, but also own resistance genes *Rpi-Smira1* and *Rpi-Smira2* (Rietman et al. 2012).

Table 1. Homology of the obtained sequences (%) with *Rpi-chc1.1* and *Rpi-chc1.2*

	Homology (%)							
	S. chacoense OQ411253	*S. tuberosum* cultivar "Bintje" OQ411252	*S. tuberosum* cultivar "Sarpo Mira" OQ414957	***S. tuberosum* cultivar "Sarpo Mira" OQ414958**	***S. microdontum* OQ411254**	*S. tuberosum* hybrid 2372-60 OQ411255	***S. berthaultii* OQ411256**	*S. maglia* OQ411257
Rpi-chc1.1	90.47	90.81	90.47	**95.48**	**97.55**	90.64	**94.26**	90.99
Rpi-chc1.2	90.85	91.19	90.85	**96.35**	**95.48**	91.02	**94.26**	91.36

The most homologous sequences are in bold.

In our study, we showed for the first time that, in addition to the known *Rpi* genes, the "Sarpo Mira" genome contains at least two variants of the *Rpi-chc1* gene homologues. This discovery sheds light on the pedigree of the cultivar "Sarpo Mira" and suggests that the genetic material of *S. chacoense* was used when this cultivar was being bred. The size of the cloned fragment for all samples was 575 nucleotides, except for the *S. microdontum* sample, for which the size of the resulting product was 572 nucleotides. Due to the high identity, we deposited one of the obtained sequences for each sample at the NCBI GenBank under the following accession numbers: OQ411253 for the *S. chacoense* K19264, OQ411254 for the *S. microdontum* 20640, OQ411256 for the *S. berthaultii* 19961, OQ411257 for the *S. maglia* K240604, OQ411252 for the *S. tuberosum* cultivar "Bintje" and OQ411255 for the *S. tuberosum* hybrid 2372-60. The exception was the "Sarpo Mira" sample, for which we deposited two sequences found in this sample. The accession numbers of these sequences are as follows: OQ414957 and OQ414958.

Thus, further we analyze and discuss these eight deposited sequences. The sequences from *S. chacoense*, *S. berthaultii*, *S. maglia*, the cultivar "Bintje" and the hybrid 2372-60, as well as the sequence OQ414958 from the cultivar "Sarpo Mira", are translated *in silico*, suggesting that they are the expressed genes. In contrast, the translation of the sequence from *S. microdontum* and the second sequence OQ414957 from the cultivar "Sarpo Mira" terminates at an early stop-codon, indicating that most probably these sequences are pseudogenes, or they are coding a truncated protein.

We compared the obtained sequences with the sequences of two allelic variants of the prototype gene *Rpi-chc1.1* and *Rpi-chc1.2*. The results are shown in Table 1. As can be seen from this table, sequences from *S. microdontum*, the cultivar "Sarpo Mira" (the sequence OQ414958) and *S. berthaultii* are the most similar to the prototype, and sequences from *S. chacoense*, *S. maglia*, the hybrid 2372-60 and the cultivar "Bintje", as well as from the cultivar "Sarpo Mira" (the sequence OQ414957) are significantly less similar to the prototype. At the same time, the level of homology of all obtained sequences with both *Rpi-chc1.1* and *Rpi-chc1.2* is approximately the same - on average 91% for less homologous sequences and 96% for more homologous sequences, while the homology between the variants *Rpi-chc1.1* and *Rpi-chc1.2* is 98.57%, and the homology between variants *Rpi-chc1.1*/*Rpi-ber1.1*/*Rpi-tar1.1* is 99%. Therefore, none of the obtained homologues is the known variant *Rpi-chc1.1* or *Rpi-chc1.2*. Then, we compared the obtained nucleotide sequences with known homologues of the *Rpi-chc1* gene in *S. berthaultii*, *S. tarijense* and *S. tuberosum* described in Monino-Lopez et al. (2021).

The results of the comparison are presented as a phylogenetic tree in Figure 2. On this dendrogram the sequences of known variants of the *Rpi-chc1* gene from *S. chacoense*, *S. berthaultii*, *S. tarijense* and *S. tuberosum* form a large common cluster (Cluster I), and within this cluster, homologues of the *Rpi-chc1* gene are not grouped according to species origin, but according to belonging to the variant of this gene. The sequences obtained in this study are clustered separately from the previously known sequences of the *Rpi-chc1* gene and its homologues and form their own distinct cluster (Cluster IV). The exceptions are the sequence from *S. berthaultii*, which clusters together with the sequence of the known homologue *Rpi-ber1.4* (Cluster III), and the sequences from "Sarpo Mira" OQ414958 and *S. microdontum*, which form their own separate cluster (Cluster II). It is worth mentioning that the new obtained

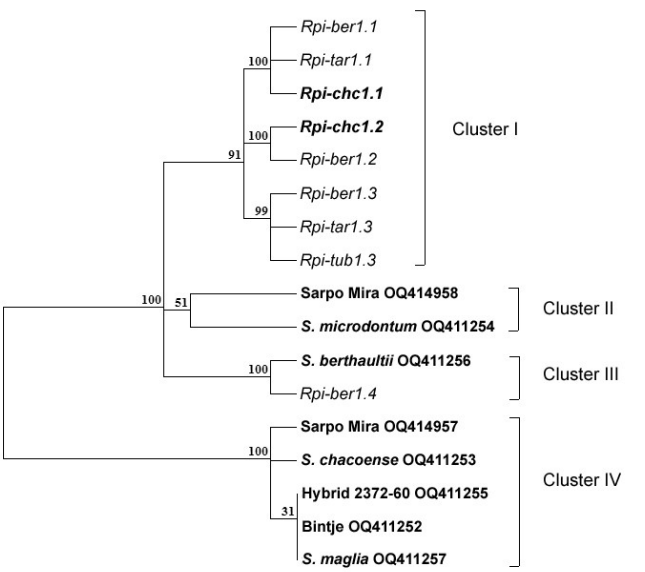

Figure 2. Phylogenetic tree of nucleotide sequences of the *Rpi-chc1* gene variants and homologues thereof in *Solanum* species. *Rpi-chc1.1*, *Rpi-chc1.2*, *Rpi-ber1.1*, *Rpi-ber1.2*, *Rpi-ber1.3*, *Rpi-ber1.4*, *Rpi-tar1.1*, *Rpi-tar1.3* and *Rpi-tub1.3* – previously known variants of the *Rpi-chc1* gene in *S. chacoense*, *S. berthaultii*, *S. tarijense* and *S. tuberosum* (NCBI GenBank accession nos. MW383255, MW410797, MW390806, MW410793, MW410798, MW410803, MW390807, MW410799 and MW410800, respectively). New homologues of the *Rpi-chc1* gene found in this study and their NCBI GenBank accession nos. are in bold. Also, variants of the prototype gene *Rpi-chc1.1* and *Rpi-chc1.2* are highlighted in bold. Bootstrap values are shown near the branches.

Table 2. Amino acid differences between functional *Rpi-chc1.1* and non-functional *Rpi-tub1.3*

Gene	Function-related amino acid positions (by Monino-Lopez 2021)						
	1035	1057	1096	1158	1161	1175	1188
Rpi-chc1.1	**C**	**V**	**K**	**D**	**E**	**T**	**V**
Rpi-tub1.3	*F*	*E*	*E*	*N*	*K*	*R*	*E*
S. chacoense OQ411253	Q	K	*E*	**D**	D	*R*	S
S. tuberosum cultivar "Bintje" OQ411252	Q	K	*E*	**D**	D	*R*	S
S. tuberosum cultivar "Sarpo Mira" OQ414957	Q	K	*E*	**D**	D	*R*	S
S. tuberosum cultivar "Sarpo Mira" OQ414958	*F*	*E*	**K**	**D**	**E**	*R*	**V**
S. microdontum OQ411254	*F*	**V**	**K**	**D**	*K*	**T**	**V**
S. tuberosum hybrid 2372-60 OQ411255	Q	K	*E*	**D**	D	*R*	S
S. berthaultii OQ411256	Q	*E*	*E*	**D**	**E**	*R*	**V**
S. maglia OQ411257	Q	K	*E*	**D**	D	*R*	S

Bold letters – amino acids of the functional variant *Rpi-chc1.1*; italic letters – amino acids of the non-functional variant *Rpi-tub1.3*.

homologue of the *Rpi-chc1* gene from *S. chacoense* is closer to the homologues of this gene from potato than to the homologues from *S. chacoense*, and together with the former forms a common cluster.

The functional variant *Rpi-chc1.1* in *S. chacoense* was shown to differ from its non-functional homologue *Rpi-tub1.3* in *S. tuberosum* by 21 amino acid substitutions (Monino-Lopez 2021). Seven of these substitutions are located in the region of the LRR domain that we amplified (Figure 3), and we compared the amino acids at these positions in the obtained homologues and the functional and non-functional variants of the *Rpi-chc1* gene. The comparison results are shown in Table 2. As can be seen from this table, none of the obtained homologues in terms of its amino acid composition at these positions corresponds to both the functional and non-functional variant of the *Rpi-chc1* gene. Homologues from the cultivar "Sarpo Mira" (OQ414958) and *S. microdontum* are represented by a combination of amino acids characteristic of both functional and non-functional variants. The homologue from *S. microdontum* has five amino acids characteristic of the functional variant, while the homologue from the cultivar "Sarpo Mira" contains four such amino acids. The homologue from *S. berthaultii* has three amino acids characteristic of the functional variant. The other obtained homologues have amino acid residues at positions 1035, 1057, 1161 and 1188 that have not been described for the functional and non-functional variants of the *Rpi-chc1* gene.

Summarizing the obtained results, we can conclude that the homologues obtained in this study cannot be unambiguously classified as any of the previously known variants of the *Rpi-chc1* gene, with the exception of the homologue from *S. berthaultii*, which most likely is the *Rpi-ber1.4*, since it is clustered with the *Rpi-ber1.4* on the dendrogram. All other

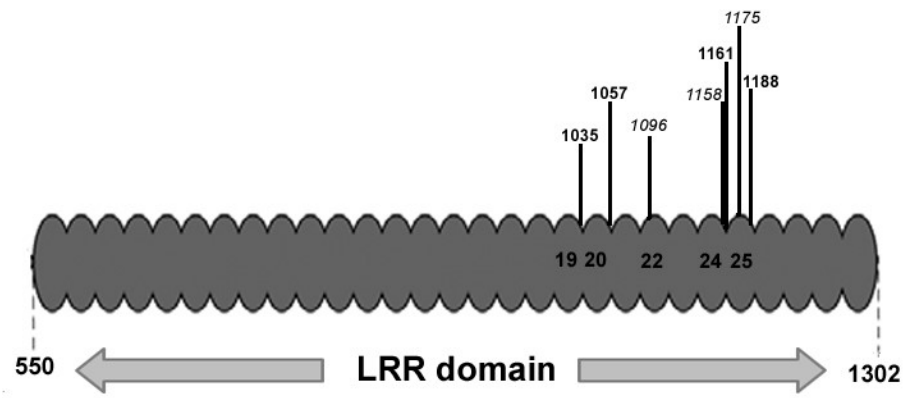

Figure 3. LRR domain of the *Rpi-chc1* protein. Arrows indicate the location of this domain (the numbers of the initial and final amino acids) relative to the full-length protein. Numbers above the vertical lines indicate the positions of the functionally important amino acids that are discussed in this article. Bold numbers indicate the positions in which previously unknown amino acids were found. Italicized numbers indicate the positions where the amino acids correspond to known variants of the *Rpi-chc1* protein. Numbers on the LRR domain itself indicate the LRR numbers in which the abovementioned substitutions are found.

homologues are the first-time reported variants of the *Rpi-chc1* gene in the corresponding species. These variants differ in their amino acid composition from both the functional and the non-functional variant of the *Rpi-chc1* gene, so their functional activity cannot be predicted, but must be further studied, for example, with using the effectoromics method.

Homologues of the *Rpi-chc1* gene as a potential marker of LB resistance

In order to study the possible contribution of the detected homologues of the *Rpi-chc1* gene to resistance to LB, we compared the results of molecular analysis with the data of laboratory resistance to LB of the studied cultivars and hybrids (Table 3). As a result, there was no unambiguous relationship between plant resistance to LB and the presence of the *Rpi-chc1* marker, since this marker was found both in highly resistant accessions and in accessions with low resistance. Among 12 cultivars in which this PCR product was found, only seven had relatively high resistance, and out of four hybrids with this PCR product two were highly resistant. However, all varieties and hybrids that lacked this marker had low resistance to LB. The only exception was the hybrid 2585-80, which had high resistance, but did not have the *Rpi-chc1* marker. The observed absence of relationship between the resistance of the studied accessions and the presence of the *Rpi-chc1* marker in them can be explained by the detection of non-functional homologues of *Rpi*-genes using PCR markers. For example, in the susceptible cultivar "Bintje", in which we found a homologue of the *Rpi-chc1* gene, a homologue of the *Rpi-vnt1* gene had been previously found (Rogozina et al. 2021). The discovery of the *Rpi-vnt1* gene in this cultivar is attributed by the authors of the abovementioned article to the use of insufficiently specific primers that amplify non-functional homologues of this gene (Rogozina et al. 2021). However, the discovery of even such homologues is valuable, as it has been shown that their activity can be restored by genome editing (Paluchowska et al. 2022), and this approach is a good alternative to transgenesis.

Table 3. Comparison of the results of molecular analysis with the data of laboratory resistance to LB of the studied cultivars and hybrids

Hybrid/ Cultivar	Presence of the *Rpi-chc1* marker	LB resistance score
2372-60	+	7
97.13-9	–	3
97.1.17	–	4
2522-173	+	3
2584-7	+	4
2359-13	–	4
97.12-18	–	4
25-85-70	–	3
2585-80	–	6
2585-67	+	6
"Alpha"	–	3
"Atzimba"	–	5
"Desiree"	+	3
"Bintje"	+	3
"Early Rose"	+	3
"Eesterling"	+	3
"Escort"	+	6
"Gloria"	+	3
"Jubel"	+	7
"Robijn"	–	4
"Sarpo Mira"	+	7
"Sarpo Axona"	+	7
"Negr"	–	3
"Elizaveta"	–	3
"Zagadka Pitera"	+	5
"Nayada"	+	5
"Priekul'skij rannij"	+	5

«+» - presence of the marker, «–» - absence of the marker

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