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Stacking Resistance Genes in Multiparental Interspecific Potato Hybrids to Anticipate Late Blight Outbreaks

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Abstract: Stacking (pyramiding) several resistance genes of diverse race specificity in one and the same plant by hybridization provides for high and durable resistance to major diseases, such as potato late blight (LB), especially when breeders combine highly efficient genes for broad-spectrum resistance that are novel to the intruding pathogens. Our collection of potato hybrids manifesting long-lasting LB resistance comprises, as a whole, the germplasm of 26 or 22 *Solanum* species (as treated by Bukasov and Hawkes, respectively), with up to 8–9 species listed in the pedigree of an individual hybrid. This collection was screened with the markers of ten genes for race-specific resistance to *Phytophthora infestans* (*Rpi* genes) initially identified in *S. demissum* (*R1, R2, R3a, R3b, and R8*), *S. bulbocastanum/S. stoloniferum* (*Rpi-blb1/ Rpi-sto1, Rpi-blb2, Rpi-blb3*) and *S. venturii* (*Rpi-vnt1*). The hybrids comprised the markers for up to four-six *Rpi* genes per plant, and the number of markers was significantly related to LB resistance. Nevertheless, a considerable portion of resistance apparently depended on presently insufficiently characterized resistance genes. Bred from these multiparental hybrids, the advanced lines with the stacks of broad-specificity *Rpi* genes will help anticipate LB outbreaks caused by rapid pathogen evolution and the arrival of new pathogen strains.

Keywords: *Phytophthora infestans*; *Solanum* species; genetic diversity; potato hybrids; late blight; durable resistance; genes for resistance to *P. infestans*; anticipatory breeding

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1. Introduction

Persistent and unrelenting, late blight (LB) of potato (*Solanum tuberosum* L.) caused by the oomycete *Phytophthora infestans* (Mont.) de Bary levies a permanent tax on potato growers: up to $10 billion is lost annually as direct crop losses and costs of chemical protection; the losses rise dramatically in the years of epidemic disease development [1–3]. The most economical and environment-friendly way to effectively contest and contain LB is to breed new cultivars with durable resistance. Durable resistance is empirically defined as resistance efficient over long periods of widespread crop cultivation under conditions favorable to disease, a compromise between plant defense capacity and the evolutionary potential of the pathogen [4]. Such resistance is reached by transferring the genes for resistance to *P. infestans* (*Rpi* genes) into cultivated potato [5]. Wild potatoes readily supply the necessary germplasm, and multiple *Rpi* genes have already been introgressed into marketable cultivars by the marker-assisted sexual and somatic hybridization or with the technologies of genetic engineering [3,5–11]. This resistance is gained slowly and with hard labor—and can be disappointedly lost, sometimes within few years, due to the rapid evolution of *P. infestans* genome and the arrival of new pathogen strains with novel repertoire of (a)virulence genes (*Avr* genes) [1–3,6,10,11].

An efficient strategy to overcome or at least alleviate this problem, when aiming at long-lasting and durable LB resistance, is to combine in one plant the *Rpi* genes that recognize several *Avr* genes. This strategy is called gene stacking, or pyramiding. Such gene pyramids will remain sustainable and effective as long as at least one *Rpi* component of the pyramid can recognize the corresponding *Avr* gene of the pathogen and trigger the defense response. Theoretically, a pyramid of four resistance genes would withstand pathogen invasion—on condition that both the resistance gene pyramids and the colonizing pathogen population(s) would concurrently fulfill several criteria. First, the stacked resistance genes should be highly effective and not leaky. Second, the best resistance genes and their combinations are those truly novel to the infecting pathogen population. Third, the pathogen genome should only rarely recombine, a criterion easily met only in a primarily asexual population. Fourth, the resistance will stay durable at a low level of gene flow due to pathogen migration [1,6,12–16].

In the case of potato, the most evident way to achieve long-lasting resistance against *P. infestans* is to recruit new *Rpi* genes into breeding schemes and to stack as many *Rpi* genes as possible into a single cultivar. The genetic diversity of cultivated potatoes that may provide such resources has been substantially pauperized in the process of conventional breeding [6,9–11]. Therefore within the last two decades, combining multiple resistance genes into a single plant genotype has heavily relied on the identification and cloning of *Rpi* genes of interest from the vast resource offered by wild *Solanum* species. Particularly inviting sources of germplasm enhancement are insufficiently explored South American wild potatoes, which have not been conspicuously involved in practical breeding, and the species that were never before reported to resist LB [7,9–11,17–20].

In the past centuries preceding the informed breeding, many cultivated genotypes in Mexico and South America had already harbored a significant contribution of wild germplasm [9]. Current germplasm enrichment by identifying and introgressing new *Rpi* genes and new alleles of already known *Rpi* genes must also include careful study of the gene pools presently used by breeders. Among other things, such exploration would lower the chance to undermine the efforts of breeders if they deploy *Rpi* genes that have already been broken by local pathogen strains [3,6,9,10,12–16].

The search for new *Rpi* genes and new alleles of previously characterized *Rpi* genes (allele mining) brings us to the mission of a wider span: identification of the full complement of *Solanum* genes contributing to the resistance to *P. infestans* [7,10,17,19–21]. For more than three decades, this field was successfully searched using various DNA markers [5,7,9,21–23]. Later, over 20 *Rpi* genes were identified and cloned from wild *Solanum* species. Recent breakthrough technologies of resistance gene enrichment sequencing (RenSeq) and the diagnostic version of this technology (dRenSeq) have opened new vistas to comprehensive exploration of *Solanum Rpi* genes and their introduction to advanced breeding schemes [24,25]; in addition, these technologies facilitate the wide-ranging characterization of allelic diversity enabling the evolutionary analysis of *Rpi* genes and prediction of new sources of these genes in genetic collections.

The multiparental potato hybrids described in this paper were obtained by remote crosses and combine genetic material from 20 wild and two cultivated *Solanum* species as treated by Hawkes [26], with up to 8–9 species reported per single hybrid pedigree. For over a decade, many of these hybrids and derived advanced lines have manifested high LB resistance. They are prospective donors containing pyramids of broad specificity genes that nowadays are readily involved in breeding, such as *Rpi-blb1* = *Rpi-sto1*, *Rpi-blb2*, *Rpi-vnt1*, *R2* = *Rpi-blb3*, etc. An important advantage of these breeding donors is that the introgressed *Rpi* genes maintain the genetic environment inherited from parental forms, including race-nonspecific resistance genes [5,18]. Rather than single genes, the remote crosses would transfer whole clusters of genes combining the *Rpi* genes of diverse race specificity and even the genes for resistance concurrently to several pests. These hybrid characteristics would ensure the stability of future cultivars and slow down the onset of more adapted pathogen forms in potato stands [5,17,18]. Here we present the

evidence obtained with the markers of ten *Rpi* genes characterized in more detail. Some data presented below have been reported earlier [27,28] at the Euroblight workshops (https://agro.au.dk/forskning/internationale-platforme/euroblight/).

2. Materials & Methods

2.1. Plant Material

The plant material explored in this study is predominantly represented by multi-parental interspecific hybrids. The pedigrees of these hybrids combine from two to nine species of *Solanum* L., section *Petota* Dumort. (Table 1). The sample under study includes ten hybrids with high field resistance to LB bred by I.M. Yashina at the Russian Potato Research Center (Korenevo, Moscow region), hereinafter Yashina's hybrids [29], by crossing *demissoid* potato varieties and/or breeding lines, which were backcrosses comprising the genetic material of *S. andigenum* Juz. & Buk. (=*S. tuberosum* ssp. *andigena* Hawkes), *S. chacoense* Bitt. and *S. chilotanum* (Buk. & Lechn.) Hawkes (=*S. tuberosum* ssp. *tuberosum* L.). Hereinafter, the names of *Solanum* species in the pedigrees of hybrids (Table 1) are listed according to Hawkes [26] and those of cultivars follow the information provided by breeders. Ten hybrids originally obtained by V.A. Kolobaev at the Institute of Plant Protection (Pushkin, St. Petersburg), hereinafter Kolobaev's hybrids [30], were bred using the accessions of wild *Solanum* species from the VIR collection, which were previously recognized as the sources of high LB resistance: *S. berthaultii* Hawkes, *S. pinnatisectum* Dunal., *S. polytrichon* Rydb., *S. simplicifolium* Bitt., and *S. verrucosum* Schlechtd. Thirty seven hybrids produced by E.V. Rogozina at VIR, hereinafter Rogozina's hybrids [30,31], represent two-species hybrids and backcrosses with the participation of South American species *S. alandiae* Cárd. and *S. okadae* Hawkes & Hjert., which have not been previously involved in potato breeding. They also include the hybrids obtained by crossing potato cultivars and breeding lines comprising the genetic material of cultivated and wild potato species: *S. andigenum* (=*S. tuberosum* ssp *andigena*), *S. leptostigma* Juz. (=*S. tuberosum* ssp. *tuberosum* L.), *S. phureja* Juz. & Buk., *S. rybinii* Juz. & Buk. (=*S. phureja*), *S. demissum* Lindl., *S. stoloniferum* Schlechtd. & Bché, *S. vallis-mexici* Juz., and *S. vernei* Bitt. & Wittm. It is significant to emphasize that the development of these hybrids involved many South American species rarely used by the Russian breeders. Many of these hybrids bred over several decades are particularly important as they possibly preserved the *Rpi* alleles that could have been lost in the world collections of wild *Solanum* species due to genetic drift and loss of individual accessions.

Table 1. Wild *Solanum* species section *Petota* Dumort. listed in the pedigrees of interspecific hybrids explored in this study. Species are listed as treated by Bukasov, Hawkes and Spooner [26,32,33].

Series in the Section *Petota*	Species	Countries	Germplasm Codes
Acaulia Juz.	*S. acaule* Bitt.	Argentina, Bolivia, Peru	acl
Bulbocastana (Rydb.) Hawkes	*S. bulbocastanum* Dun.	Guatemala, Mexico	blb
Commersoniana Buk.	*S. commersonii* Dun.	Argentina, Brazil, Paraguay, Uruguay	cmm
Demissa Buk.	*S. demissum* Lindl.	Mexico, Guatemala	dms
	S. × *edinense* Berth.	Mexico	edn
	S. × *semidemissum* Juz.	Mexico	sem

Table 1. *Cont.*

Series in the Section *Petota*	Species	Countries	Germplasm Codes
Longipedicellata Buk.	*S. antipoviczii* Buk. = *S. stoloniferum*	Mexico	ant
	S. polytrichon Rydb. = *S. stoloniferum*	Mexico	plt
	S. stoloniferum Schlechtd. & Bché.	Mexico	sto
	S. ×*vallis-mexici* Juz.	Mexico	vll
Megistacroloba Cárdenas & Hawkes	*S. megistacrolobum* Bitt.	Peru, Bolivia, Argentina	mga
Pinnatisecta (Rydb.) Hawkes	*S. pinnatisectum* Dun.	Mexico	pnt
Tuberosa (Rydb.) Hawkes	*S. alandiae* Cárd.	Bolivia	aln
	S. andigenum Juz. & Buk. = *S. tuberosum* ssp. *andigena* Hawkes	Argentina, Bolivia, Guatemala, Colombia, Ecuador, Mexico, Peru, Venezuela	adg
	S. berthaultii Hawkes	Bolivia	ber
	S. brevicaule Bitt.	Bolivia	brc
	S. chilotanum (Buk. & Lechn.) Hawkes (= *S. tuberosum* ssp. *tuberosum* L.).	Chile	chi
	S. leptostigma Juz. (= *S. tuberosum* ssp. *tuberosum* L.).	Chile	lpt
	S. microdontum Bitt.	Argentina, Bolivia	mcd
	S. okadae Hawkes & Hjert.	Argentina, Bolivia	oka
	S. phureja Juz. & Buk.	Ecuador, Colombia, Venezuela, Bolivia, Peru	phu
	S. rybinii Juz. & Buk. (=*S. phureja* Juz. & Buk.)	Ecuador, Colombia, Venezuela, Bolivia, Peru	ryb
	S. simplicifolium Bitt. = *S. microdontum*	Argentina, Bolivia	sim
	S. spegazzinii Bitt.	Argentina	spg
	S. vernei Bitt. & Wittm.	Argentina	vrn
	S. verrucosum Schlechtd.	Mexico	ver
Yungasensa Corr.	*S. chacoense* Bitt.	Argentina, Bolivia, Brazil, Paraguay, Uruguay	chc

In addition to all these hybrids, our study also included several registered varieties, some of which come from complex interspecific hybrids: Alouette (https://varieties.ahdb.org.uk/varieties/view/Alouette; [25]), Sarpo Mira and Sarpo Axona (http://sarpo.co.

uk/portfolio/; https://pomidom.ru/sarpo-mira-potatoes/), Mastenbroeck-Black potato differential set plants R5, R8 and R9 [34] and others. These varieties were used to verify SCAR markers of the *Rpi* genes; besides, they also served as positive and negative controls for PCR screening. Other cultivars, some of which are also interspecific hybrids, were employed as controls when assessing plant LB resistance (Table 2).

As a whole, this collection (Tables 1 and 2) contains potato hybrids with their pedigrees representing nine series of *Solanum* L. section *Petota* Dumort. and listing 22 wild and four cultivated *Solanum* species as treated by Bukasov [32], which correspond to 20 wild and two cultivated *Solanum* species as treated by Hawkes [26] and 15 wild and one cultivated species as treated by Spooner [33]. The pedigrees of some individuals include as many as nine *Solanum* species. To verify SCAR markers of *Rpi* genes we also employed the accessions of wild *Solanum* species in the VIR collection.

2.2. Resistance to Pathogens

Late blight resistance of leaves was evaluated in long-term field trials under conditions of natural infestation in two European regions of the Russian Federation, i.e., the Northwest (VIR, Pushkin, St. Petersburg; 59.42′ E, 30.25′ N) and the Central (Institute of Phytopathology, Ramenskaya Gorka, Moscow region; 55.63′ E, 36.95′ N).

In the Northwest region, the growing seasons during the period of field trials were different: in 2016 and 2017, abundant precipitation and cool temperatures were favorable for the early manifestation and development of LB; in 2014, 2019 and 2020, moderate rainfall and unstable temperatures delayed the appearance of disease. In the Central region, dry weather early in the 2014 growing season delayed the LB progress; however, heavy rainfall and a drop in temperature early in August provided extremely favorable conditions for the LB development on potato haulms and later, damage to tubers. Through the following six years (2015–2020), the weather conditions were favorable for a fairly early (the middle of June) LB development, and later LB epiphytoty. Within this period, the air temperatures in June and the first half of July were below the long-term values. In addition, significant precipitation was recorded annually.

Pathogen population at two sites was represented by numerous diverse and highly aggressive complex races of *P. infestans* comprising seven to eleven virulence genes [35].

The field assessment of the partial LB resistance of potato plants was carried out every 10–12 days, and these data were used to calculate the area under the disease progress curve (AUDPC) in the course of the growth season and the corresponding yield losses caused by the early destruction of leaves (%). To evaluate the LB resistance level, the calculated yield losses were converted into 1–9-point scores, where 9 points correspond to the highest resistance level [35].

Resistance to LB in the laboratory tests was evaluated with detached leaves according to the Eucablight protocol (www.euroblight.net/). Detached leaves of plants grown in a greenhouse were infected with a highly virulent and aggressive isolate of *P. infestans* N161 (race 1.2.3.4.5.6.7.8.9.10.11, mating type A1) isolated in the Moscow region (the collection of the Institute of Phytopathology), using cv. Santé as a reference [35]. The aggressiveness of N161 in the Lapwood test [36] with Santé tubers exceeded the indices registered with all isolates collected in the potato stands under study. The experimental data for LB resistance were transformed to 1–9-point scores.

The experimental data were processed by the methods of nonparametric statistics (Kruskal-Wallis test of variance, Spearman's rank correlation and cluster analysis) using the STATISTICA Advanced package (StatSoft Russia; http://statsoft.ru/products/).

Table 2. Multiparental interspecific hybrids and potato cultivars included in this study.

Hybrid, Cultivar *	Bred from	Pedigree ****		LB Resistance *****	
		♀Female	♂Male	Field	Laboratory
Hybrids bred by I.M. Yashina					
2585-67	F1	Nikulinskij {Mavka × [Apta (Interspecific hybrid × Hindenburg)] × Karpatskij}	Peterburgskij [(Omega (adg, dms, chi) × E 109/11]×	7	6
2585-70	F1	Nikulinskij	Peterburgskij	5	4
2585-80	F1	Nikulinskij	Peterburgskij	7	6
97.12-18	F1	Nikulinskij	88.16/20 {[(*S.chacoense* × *S. tuberosum*) × Kameraz]} × Belorusskij 3}	5	4
2359-13	F1	Nikulinskij	88.16/20 {[(*S.chacoense* × *S. tuberosum)* × Kameraz]} × Belorusskij 3}	6	5
2584-7	F1	Nikulinskij	Ausonia (Wilja × Konst 63-655 adg)	6	4
97.13-9	F1	Nikulinskij	375.333.1 (cmm, dms, mga)	5	3
97.1.17	F1	Lugovskoj (164-1C/72 × 60C/73)	88.16/20 {[(*S.chacoense* × *S. tuberosum)* × Kameraz]} × Belorusskij 3}	7	4
2372-60	F1	1977-76	Zarevo (7692 C 68 × Bekra) adg, dms, plt	8	6
2522-173	F1	Utenok {Adretta × [(Saskia × Ora) × [(Apta × MPI 44335 1309 (adg,dms)) × Schwalbe] Lu.59.884/3 × Axilia] × 15-26 [Lyubimec × 172m-7 (*S.chacoense*×*S. tuberosum*)]}	90/2	6	3
Hybrids bred by V.A. Kolobaev					
10/5-09	F1	Zagadka Pitera (dms, phu, sto, tbr, vrn)	mixture of pollen ***	6–7	4
11/6-09	F2	Zagadka Pitera (dms, phu, sto, tbr, vrn)	mixture of pollen	6–7	4
12/1-09	F4	*S. pinnatisectum* k-17464	Fausta (Sommerstarke (dms) × W8102/214)	6–7	6
13/11-09	F1	F2 (*S. pinnatisectum* k-17464 × Gitte (adg))	mixture of pollen	7	5
14/8-09	F5	(*S. polytrichon* k-5345 × MPI 50-140\5 (ant = sto, dms))	MPI 50-140\5 (ant = sto, dms)	6	4
15/13-09	F1	(*S. pinnatisectum* k-17464 × Gitte (adg))	F2 [(*S. polytrichon* k-5345 × MPI 50-140/5 (ant=sto)) × MPI 50-140/5] × ׀F3[(*S. verrucosum* × MPI 50-140/5) × Licaria] × F2 {F2[(*S. polytrichon* k-5345 × MPI 50-140/5) × MPI 50-140/5] × {[(*S. simplicifolium* k-5400 × MPI 50-140/5) × Mariella (adg, dms)] × Desiree}}׀	6	6

Table 2. *Cont.*

Hybrid, Cultivar *	Bred from	Pedigree ****		LB Resistance *****	
		♀Female	♂Male	Field	Laboratory
Hybrids bred by I.M. Yashina					
16/27-09	F1	[(*S. berthaultii* k-8510 × Tajga (adg, dms)) × Omega (adg, chi, dms)] × F2[(*S. polytrichon* k-5345 × MPI 50-140/5 (ant=sto) × MPI 50-140/5] × F2{[(*S. simplicifolium* k-5400 × MPI 50-140/5) × Gitte (adg)] × Hera}}|	Nayada (adg, dms, phu, sto, tbr, vrn)	7	6
18/40-2000	F2	[(*S. polytrichon* k-5345 × MPI 50-140/5 (ant=sto)) × Umbra] × Fausta (dms)	[(*S. simplicifolium* k-5400 × MPI 50-140/5) × Gitte (adg)] × Hera	6	5
111 (38 KVA)	F1	Fermer	F4{F2[(*S. polytrichon* k-5345 × MPI 50-140/5 (ant=sto)) × MPI 50-140/5] × F2{[(*S. simplicifolium* k-5400 × MPI 50-140/5) × Gitte (adg)] × Hera}}	6.5–8	6
113 (50/1 KVA)	F1	Zagadka Pitera (dms, phu, sto, tbr, vrn) × mixture of pollen	Nayada (adg, dms, phu, sto, tbr, vrn) × mixture of pollen	6–7	6
Hybrids bred by E.V. Rogozina					
117-1	F1	Atzimba (adg, dms)	*S. alandiae* k-21240		
117-2	F1	Atzimba	*S. alandiae* k-21240	5–7	5
39-1-2005	F1	Atzimba	*S. alandiae* k-21240	6–7	6
24-1	F1	Atzimba	*S. alandiae* k-21240	6–8	7
24-2	F1	Atzimba	*S. alandiae* k-21240	6–8	7
25-1-2007	F1	Elizaveta	24-1 (Atzimba × *S. alandiae* k-21240)	5	5
25-2-2007	F1	Elizaveta	24-1 (Atzimba × *S. alandiae* k-21240)	4–5	4
134-2-2006	F1	24-2 (Atzimba × *S. alandiae* k-21240)	Svitanok kievskij	6–7	6
134-3-2006	F1	24-2 (Atzimba × *S. alandiae* k-21240)	Svitanok kievskij	2–3	3
134-6-2006	F1	24-2 (Atzimba × *S. alandiae* k-21240)	Svitanok kievskij	5–6	5
135-1-2006	F1	Svitanok kievskij	24-2 (Atzimba × *S. alandiae* k-21240)	5–7	5
135-2-2006	F1	Svitanok kievskij	24-2 (Atzimba × *S. alandiae* k-21240)	4.5–7	4
139 (4-1-2012)	F1	Atzimba × *S. alandiae* k-21240	F5 [(*S. polytrichon* k-5345 × MPI 50-140\5) × MPI 50-140\5]	7–9	6
97-155-1	F1	Bobr (adg, dms, sto)	91-21-4 (adg, dms, ryb)	7–8	6

Table 2. *Cont.*

Hybrid, Cultivar *	Bred from	Pedigree ****		LB Resistance *****	
		♀Female	♂Male	Field	Laboratory
Hybrids bred by I.M. Yashina					
128-05-03	F1	97-155-1 (adg, ryb, sto)	Nayada (adg, dms, phu, sto, tbr, vrn)	6–7	5
118 (118-5-2011)	F2	Bobr (adg, dms, sto)	91-21-4 (adg, dms, ryb)	5–8	6
120 (118-6-2011)	F2	Bobr (adg, dms, sto)	91-21-4 (adg, dms, ryb)	5–7	5
160-1	F2	Bobr (adg, dms, sto)	91-21-4 (adg, dms, ryb)	7–8	nd
160-17	F2	Bobr (adg, dms, sto)	91-21-4 (adg, dms, ryb)	6–7	5
106 (171-3)	F2	Bobr (adg, dms, sto)	91-21-4 (adg, dms, ryb)	6–7	6
123 (128-6)	F2	Bobr (adg, dms, sto)	91-21-4 (adg, dms, ryb)	6–8	6
90-6-2	F1	194-4 (adg, phu, sto)	CIP-1039 (adg)	7	nd
99-6-5	F1	90-6-2 (adg, phu, sto)	Hertha (adg, dms, ryb, tbr)	3–4	nd
99-6-6	F1	90-6-2 (adg, phu, sto)	Hertha (adg, dms, ryb, tbr)	5	nd
97-153-2	F1	90-6-2 (adg, phu, sto)	91-21-4 (adg, dms, ryb)	6	5
2 (194-4т)	F1	Zagadka Pitera (dms, sto, vrn, phu, tbr)	99-6-6 (adg, dms, phu, ryb, sto, tbr)	6–7	5
99-4-1	F1	180-1 (sto)	Hertha (adg, dms, ryb, tbr)	5–7	5
7 (93-5-30)	F1	41.85.6 (adg, phu, ryb)	91-19-2 (acl, blb, sto)	5–7	5
190-4	F1	Gibridnyj 14 (dms, vll)	194-4 (adg, phu, sto)	7–8	4
97-162-2	F1	91-15-2 (adg, ryb, sto)	90-21-1 (adg, mcd, ryb, spg, sto)	3	nd
34-6	F1	97-162-2 (adg, mcd, ryb, spg, sto)	190-4 (adg, dms, phu, sto, vll)	5	nd
53 (34-5-2003)	F1	97-162-2(adg, mcd, ryb, spg, sto)	190-4 (adg, dms, phu, sto, vll)	6	5
135-3-2005	F1	*S. okadae* k-20921	*S. chacoense* k-19759	5	nd
135-5-2005	F1	*S. okadae* k-20921	*S. chacoense* k-19759	5	nd
8-1-2004	F1	*S. okadae* k-20921	*S. chacoense* k-19759	5	nd
8-3-2004	F1	*S. okadae* k-20921	*S. chacoense* k-19759	3	nd
8-5-2004	F1	*S. okadae* k-20921	*S. chacoense* k-19759	5	nd
Other hybrids and cultivars employed as standards					
R5	nd **			nd	nd
R8	nd			nd	nd
R9	nd			nd	nd
Magellanes	nd	indigenous cultivar of Chile *S. tuberosum* ssp. *tuberosum* L.	-	nd	nd
Alouette	nd	AR 02-139-1	Laura	8–9	7

Table 2. *Cont.*

Hybrid, Cultivar *	Bred from	Pedigree ****		LB Resistance *****	
		♀Female	♂Male	Field	Laboratory
Hybrids bred by I.M. Yashina					
Atzimba	F1	US 133.3	52- AT-1 (adg)	5	4
Sarpo Axona	nd	nd	nd	8	7
Sarpo Mira		76 PO 12 14 268	D 187	8	7
Alpha	F1	Paul Kruger	Preferent	4	3
Bintje	F1	Munstersen	Fransen	3	3
Desiree	F1	Urgenta	Depesche	4	2
Early Rose	nd	Garnet Chili	-	4	nd
Eersteling	nd	Duke of York	-	4	3
Escort	F1	Rental	Cebeco 64 197 16 (dms)	6–7	6
Gloria	nd	Alpha?	Bato	5	3–4
Jubel	F1	Victoria Augusta	78 92	7	nd
Robijn	nd	Rode Star	Preferent	5	4
Elizaveta	F1	acl, adg, dms, phu, sto, tbr, vrn	nd	5	4
Nayada	nd	adg, dms, phu, sto, tbr, vrn	nd	6	5
Negr	nd	indigenous cultivar of Chile *S. tuberosum* ssp. *tuberosum* L.	-	4	3
Priekul'skij rannij	nd	Irish Cobbler	Jubel	5	3
Svitanok kievskij	nd	Adretta (adg, dms)	3774c 71	5	4
Zagadka Pitera	nd	dms, phu, sto, tbr, vrn	nd	6	5

* https://www.europotato.org/varieties/view; ** nd, no data; *** see Table 1 for germplasm codes; **** mixture of pollen from several interspecific hybrids of high LB resistance; ***** 1–9-point scores, from susceptible to resistant.

2.3. Molecular and Bioinformatics Methods

Genomic DNA from young plant leaves was isolated with the AxyPrep Multisource Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA) or DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). DNA concentration was measured with an UV/Vis NanoPhotometer P300 (IMPLEN, München, Germany). Oligonucleotide primers were designed using the programs BLAST 2.0 (http://blast.ncbi.nlm.nih.gov/Blast.cgi.), SeqMan, Lasergene 7.0 (http://www.dnastar.com), Oligonucleotide Properties Calculator (http://www.basic.northwestern.edu/biotools/oligocalc.html) and synthesized by Syntol, Moscow (www.syntol.ru). Primer melting temperatures were adjusted empirically. DNA amplification was run in a MJ PTC-200 thermocycler (Bio-Rad, Hercules, CA, USA). The PCR mix contained 1 µL of 10× PCR buffer Mg^{2+} Plus for *Taq* DNA polymerase (Fermentas, St. Leon-Rot, Germany), 1 µL of dNTP mix (2.5 mM of each), 1 µL each of forward and reverse primers (1 µM), 5 U of *Taq* DNA polymerase, 30–60 ng/µL of genomic DNA, and sterile deionized water to 10 ul. PCR products were separated by electrophoresis in 1% (*w*/*v*) agarose in 1× TAE buffer for 40 min at 6 V/cm and visualized under UV after staining with ethidium bromide using a Gel Logic 100 Imaging System (Eastman Kodak Company, Rochester, NY, USA). Following electrophoretic separation, PCR-amplified DNA fragments were purified using QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The fragments were cloned using pGEM-T Easy Vector System I (Promega, Madison, WI, USA) and sequenced with nucleic acid analyzers ABI PRISM 3130xl (Applied Biosystems,

Foster City, CA, USA) or Nanophor 05 (Institute for Analytical Instrumentation, St. Petersburg, Russia). Sequenced fragments were assembled using SeqMan package, Lasergene 7.0. BLAST 2.0. and SeqMan, Lasergene 7.0 programs were used to mine genomic databases for *Rpi* genes and their homologues, and their phylogenetic analysis was performed with the MEGA6 package [37].

2.4. SCAR Markers for Resistance Genes

All SCAR markers (Table 3, Figure 1) were derived from the sequences of already well-characterized *Rpi* prototype genes deposited in the NCBI Genbank (https://www.ncbi.nlm.nih. gov/nucleotide/). Most markers were already reported elsewhere, and some were designed or modified following multiple alignment of the prototype gene sequences, their structural homologues and anonymous genome fragments lifted from the NCBI Genbank using BLAST and Vector NTI Suite 8 package (Invitrogen, Carlsbad, CA, USA). In the case of *R2/Rpi-blb3* and *Rpi-blb1/Rpi-sto1,* more than one marker was used to recognize the particular gene. Wherever possible, marker specificity was verified against wild species that were the initial sources of the prototype genes in the NCBI Genbank, including amplification, cloning and sequencing the marker amplicons and phylogenetic analysis of the marker sequences. To this end, multiple alignments of nucleotide sequences assembled using a combination of the Martinez and Needleman-Wunsch algorithms were performed with SeqMan, Lasergene 7.0 Sequences. The phylogenetic analysis was performed with MEGA6 (https://www.megasoftware.net/).

Table 3. SCAR markers of *Solanum Rpi* genes (see also Figure 1).

Gene	Prototype Gene *	Marker, Size, bp.	Position on the Gene, bp	Primers Sequences	Anneal. Temp., °C	References
Rpi-R1	AF447489	Rpi-R1-1205	5126–6331	F-cactcgtgacatatcctcacta R-gtagtacctatcttatttctgcaagaat	61	[21]
Rpi-R2/Rpi-blb3	FJ536325	Rpi-R2-686	1370–2055	F-gctcctgatacgatccatg R-acggcttcttgaatgaa	54	[38]
		Rpi-R2-1137	1277–2413	F-aagatcaagtggtaaaggctgatg R-atctttctagcttccaaagatcacg	60	[39]
	FJ536346	Rpi-blb3-305	5551–5855	F–agctttttgagtgtgtaattgg R-gtaactacggactcgaggg	63.5	[8]
Rpi-R3a	AY849382	Rpi-R3a-1380	1677–3056	F-gtagtacctatcttatttctgcaagaat R-agccacttcagcttcttacagtagg	64	[21]
Rpi-R3b	JF900492	Rpi-R3b-378	94818–95195	F-gtcgatgaatgctatgtttctcgaga R-accagtttcttgcaattccagattg’	64	[40]
Rpi-R8	KU530153	Rpi-R8-1276	73694–74970	F-aacaagagatgaattaagtcggtagc R-gctgtaggtgcaatgttgaagga	62.5	[41] modif.
Rpi-blb1 = Rpi-sto1	AY336128	Rpi-blb1-821	2304–3124	F-aacctgtatggcagtggcatg R-gtcagaaaagggcactcgtg	62	[42]
	AY336128	Rpi-blb1-226	3143–3368	F–cacgaggccctttctgac R-ttcaattgtgttgcgcactag	50	[43]
	EU884421	Rpi-sto1-890	241–1130	F-accaaggccacaagattctc R-cctgcggttcggttaataca	65	[8]
Rpi-blb2	DQ122125	Rpi-blb2-976	3226–4202	F-ggactgggtaacgacaatcc R-atttatggctgcagaggacc	55	[44]
Rpi-vnt1	FJ423046	Rpi-vnt1.3-612	89–701	F-ccttcctcatcctcacatttag R-gcatgccaactattgaaacaac	58	[45]

* Accession numbers in the NCBI Genbank (https://www.ncbi.nlm.nih.gov/genbank/).

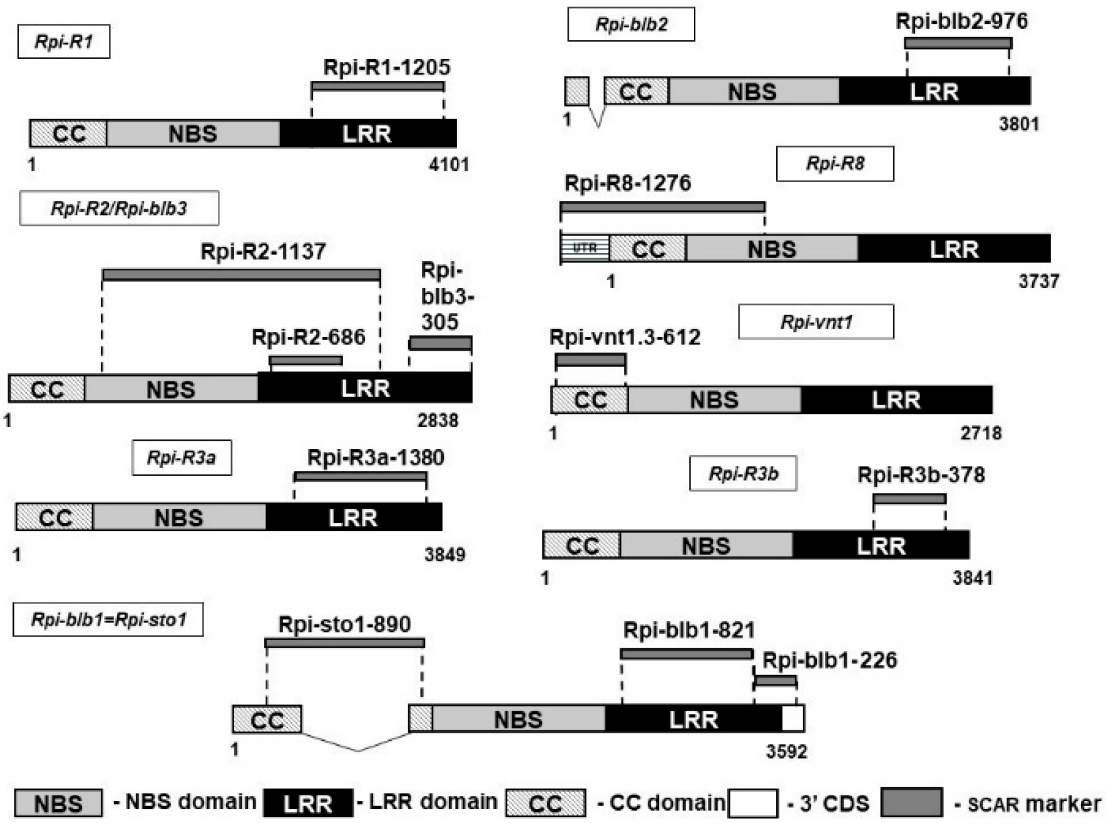

Figure 1. SCAR markers for the *Rpi* genes. The marker positions at the gene sequences are shown as regards the respective domains of CC-NBS-LRR kinases. For further details see Table 3.

3. Results & Discussion

3.1. LB Resistance of the Multiparental Potato Hybrids

In the field experiments, 50 hybrids and cultivars were assessed for their LB resistance in the span of seven years (2014–2020). Ten Yashina's hybrids, ten Kolobaev's hybrids and 23 Rogozina's hybrids were evaluated together with seven standard cultivars. For the sake of comparison, another seven cultivars (Alouette, Atzimba, Elizaveta, Nayada, Priekul'skij rannij, Svitanok kievskij and Zagadka Pitera) were tested in field trials for four years within the 2014–2020 period. The cvs. Alouette (8–9), Sarpo Mira (8) and Yashina's hybrid 2372-60 (8 points of resistance) were highly resistant to LB; Rogozina's hybrids 24-1, 24-2 and 123 (128-6) (6–8), 97-155-1, 160-1 and 190-4 (7–8), 139 (4-1- 2012) (7–9), Kolobaev's hybrid 111 (38 KVA) (6.5–8), Yashina's hybrids 2585-67 and 97.1.17 (both 7 points) were resistant (Table 2). Resistance indices of hybrids 2585-67, 2372-60, 97.1.17, 13 /11-09, 111 (38 KVA), 24-1, 24-2, 139 (4-1-2012) and cvs. Alouette and Sarpo Mira significantly differ from those of LB-susceptible cvs. Alpha and Bintje (3–4 points) by Kruskal-Wallis criterion ($H = 270.01$, $p = 0.001$). Resistance indices of cvs. Alouette and Sarpo Mira significantly differ from those of cvs. Priekul'skij rannij, Elizaveta, Eersteling, Gloria, Robijn and hybrids 2585-70, 97.12.18, 97.13-9, 25-1-2007, 25-2-2007, 97-162-2 and 134-3-2006 (3–5 points). In cvs. Escort, Atzimba, Nayada, Svitanok kievskij, Zagadka Pitera and 27 hybrids, the indices of field resistance varied from 5 to 7 points depending on the year of trial. These cultivars and hybrids manifested moderate LB resistance in the field trials as compared to resistant and susceptible potato genotypes.

Ten Yashina's hybrids, ten Kolobaev's hybrids, 24 Rogozina's hybrids and 16 cultivars were evaluated in laboratory tests with detached leaves. Resistant (7–8 points) were hybrids

24-1 and 24-2 and cvs. Alouette, Sarpo Axona and Sarpo Mira. Susceptible (2–3 points) were cvs. Alpha, Bintje, Desiree, Eersteling and hybrids 97.13-9, 2522-173, 134-3-2006 (Table 2). The data from field trials and laboratory assessments run in parallel for many years are in good agreement (Spearman's correlation coefficient r = 0.75 at $p < 0.05$).

Based on the evidence from long-term field trials and laboratory assessment, 55 hybrids and cultivars were grouped in the following way (Figures 2 and 3). Full coupling grouping using Euclidean distance and k-means clustering gave similar results. By the hierarchical classification, the sample of 55 hybrids and cultivars is separated into three groups with a similarity level >0.4. The k-means method also formed three disjoint subsets: each cluster consists of similar objects, and objects from different clusters differ significantly from each other. Cluster 1 comprises potato genotypes, which are moderately resistant to LB in the field trials and moderately susceptible in laboratory tests: cvs Nayada and Zagadka Pitera and hybrids 14/8-09; 18/40-2000; 10/5-09; 13/11-09; 16/27-09; 25-1-2007; 134-6-2006; 34-5-2003; 117-2; 128-05-03; 135-1-2006; 135-2-2006; 93-5-30; 99-4-1; 118-6-2011; 2584-7; 97.1.17. Potato cultivars and hybrids resistant to LB are pooled into cluster 2, which includes 16 genotypes: cvs. Alouette, Sarpo Mira and Escort, hybrids 11/06-09, 12/1-09, 113 (50/1 KVA), 111 (38 KVA), 134-2-2006, 118-5-2011, 128-6, 139 (4-1-2012), 24-1, 24-2, 2585-67, 2585-80, 2359-13. Susceptible genotypes are combined into cluster 3, which includes cultivars Alpha, Bintje, Eersteling, Elizaveta, Gloria, Priekul'skij rannij, Robijn, Svitanok kievskij, and hybrids 2585-70, 97.12.18, 97.13- 9, 2522-173, 25-2-2007 and 134-3-2006 (Table 2).

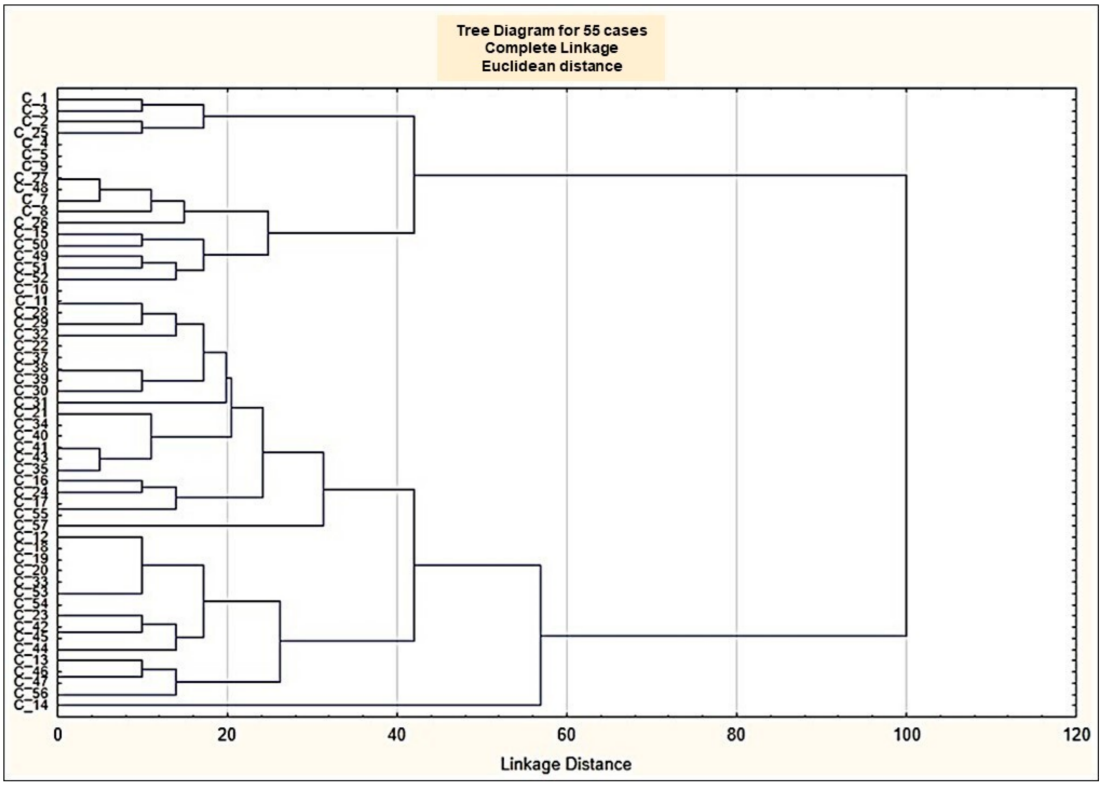

Figure 2. The hierarchical clustering dendrogram of potato genotypes. Potato genotypes: n = 55. C_1, Alpha; C_2, Bintje; C_3, Eersteling; C_4, Gloria; C_5, Robijn; C_7, Elizaveta; C_8, Priekul'skij rannij; C_9, Svitanok kievskij; C_10, Nayada; C_11, Zagadka Pitera; C_12, Escort; C_13, Sarpo Mira; C_14, Alouette; C_15, 14/8-09; C_16, 18/40-2000; C_17, 10/5-09; C_18, 11/6-09; C_19, 113 (50/1 KVA); C_20, 12/1-09; C_21, 13/11-09; C_22, 15/13-09; C_23, 111(38 KVA); C_24, 16/27-09; C_25, 134-3-2006; C_26, 25-1-2007; C_27, 25-2-2007; C_28, 134-6-2006; C_29, 34-5-2003; C_30, 97-153-2; C_31, 117-2; C_32, 128-05-03; C_33, 134-2-2006; C_34, 135-1-2006; C_35, 135-2-2006; C_37, 171-3; C_38, 194-4т; C_39, 39-1-2005; C_40, 93-5-30; C_41, 99-4-1; C_42, 118-5-2011; C_43, 118-6-2011; C_44, 128-6; C_45, 139(4-1-2012); C_46, 24-1; C_47, 24-2; C_48, 2585-70; C_49, 2522-173; C_50, 2584-7; C_51, 97.12-18; C_52, 97.13-9; C_53, 2585-67; C_54, 2585-80; C_55, 97.1.17; C_56, 2359-13; C_57, 2372-60.

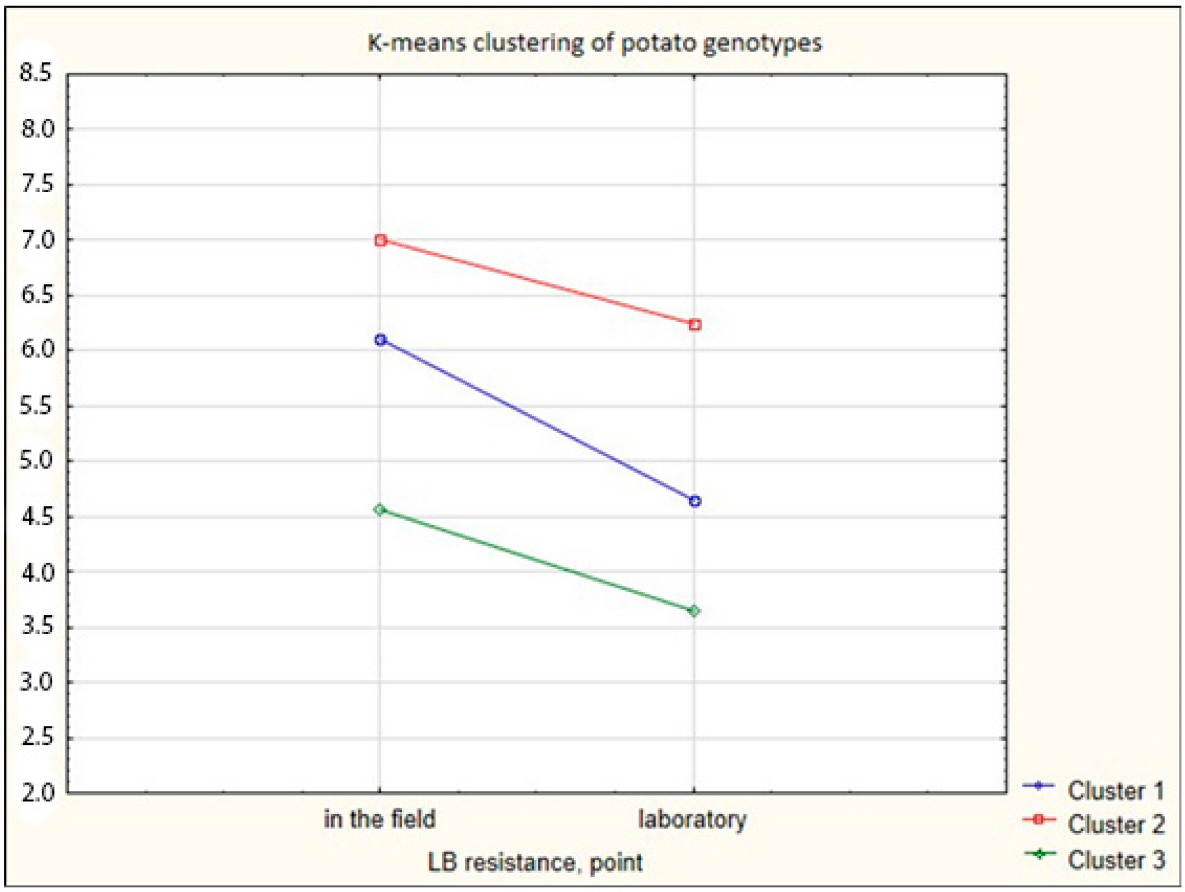

Figure 3. K-means clustering of potato genotypes.

3.2. Rpi Genes in the Multiparental Potato Hybrids

As expected, the hybrids and standard cultivars with *Demissa* species in their pedigrees, including cvs Atzimba [46] and both Sarpos (https://pomidom.ru/sarpo-mira-potatoes/) contain as many as three to five markers of genes *Rpi-R1—Rpi-R8* (Table 4). However, several *demissoid* hybrids, such as 2585-80, 2584-7, 97.1.17, 12/1-09, 97-153-2, and 99-4-1 seem to comprise only one or two of *Rpi-R1—Rpi-R8* genes. Potato differentials R5, R8 иR9 each harbored four to five markers of these genes. The *Rpi-R8* gene is expected in differentials R8 and R9 [38], but not in R5.

To recognize the *Rpi-R2/Rpi-blb3* genes, we used three SCAR markers corresponding to different regions of this gene (Figure 1, Table 3). Marker Rpi-R2-686 covers about half of the Rpi-R2-1137 sequence, and the evidence for these two markers matches in most cases (Table 4). The third marker Rpi-blb3-305 usually follows Rpi-R2-1137. The *Rpi-R2/Rpi-blb3* family of genes in the cluster on chromosome 4 has been reported in many Mexican species [20,40,47,48], and to distinguish the input of particular germplasms in the interspecific hybrids should become the goal of future studies. It is difficult to explain the presence of *Rpi-R2* markers in *S. chacoense* × *S. okadae* hybrid (135-3-2005)—especially when other segregants of this combination are free of these markers.

Table 4. Markers of *Rpi* genes in multiparental interspecific hybrids and reference potato cultivars (1/0—presence/absence of markers).

Geno-Types	*Solanum* Species in Hybrid Pedigrees *	Genes												The Number of Genes
		R1	*R2 = Rpi-blb3*			*R3a*	*R3b*	*R8*	*Rpi-blb 1 = Rpi-sto1*			*Rpi-blb2*	*Rpi-vnt1-3*	
		R1-1205	R2-1137	R2-686	Rpi-blb3-305	R3a-1380	R3b-378	R8-1276	RB-226	Rpi-blb1-821	Rpi-sto1-890	Rpi-blb2-976	Rpi-vnt1.3-612	
Hybrids bred by I.M. Yashina														
2585-67	adg, chi, dms, tbr	0	1	1	1	1	1	0	0	0	0	0	0	3
2585-70	adg, chi, dms, tbr	0	1	1	1	1	1	0	0	0	0	0	0	3
2585-80	adg, chi, dms, tbr	0	1	1	1	0	0	0	0	0	0	0	0	1
2359-13	chc, dms, tbr	1	1	1	1	1	1	0	0	0	0	0	0	4
2584-7	adg, chc, dms, edn, ryb, tbr	0	1	1	1	0	1	0	0	0	0	0	0	2
97.12-18	chc, dms, tbr	0	0	0	0	1	1	1	0	0	0	0	0	3
97.13-9	cmm, dms, mga, tbr	0	1	1	1	1	1	1	0	0	0	1	0	5
2372-60	adg, chc, dms, lpt, sto, tbr,	1	1	1	1	1	1	0	0	0	0	0	0	4
2522-173	adg, chc, dms, tbr	0	0	0	0	1	1	1	0	0	0	0	0	3
97.1.17	adg, chc, dms, sem, tbr	0	1	1	1	0	0	0	0	0	0	0	0	1
10/5-09	dms, phu, sto, tbr, vrn	0	1	1	1	1	1	1	0	0	0	1	0	5

Table 4. *Cont.*

Geno-Types	*Solanum* Species in Hybrid Pedigrees *	Genes												The Number of Genes
		R1	*R2 = Rpi-blb3*			*R3a*	*R3b*	*R8*	*Rpi-blb 1 = Rpi-sto1*			*Rpi-blb2*	*Rpi-vnt1-3*	
		R1-1205	R2-1137	R2-686	Rpi-blb3-305	R3a-1380	R3b-378	R8-1276	RB-226	Rpi-blb1-821	Rpi-sto1-890	Rpi-blb2-976	Rpi-vnt1.3-612	
11/6-09	dms, phu, sto, tbr, vrn	0	1	1	1	1	1	1	0	0	0	1	0	5
12/1-09	dms, pnt, tbr	0	1	1	1	0	0	0	1	1	1	0	0	2
13/11-09	adg, pnt, tbr	0	0	0	0	0	1	1	1	1	1	0	1	4
14/8-09	Ant = sto, dms, plt = sto, tbr	0	1	0	0	1	1	1	0	0	0	0	1	5
15/13-09	adg, ant = sto, dms, plt = sto, pnt, sim = mcd, tbr, ver	0	1	1	0	0	1	1	1	1	1	1	0	5
16/27-09	adg, ant = sto, ber, chi, dms, phu, plt = sto, sim = mcd, tbr, vrn	1	0	0	0	0	0	1	1	1	1	1	0	4
18/40-2000	adg, dms, mcd, plt = sto, sto, tbr,	1	0	0	0	0	0	1	0	0	0	0	1	3

Table 4. *Cont.*

Geno-Types	*Solanum* Species in Hybrid Pedigrees *	Genes												The Number of Genes
		R1	*R2 = Rpi-blb3*			*R3a*	*R3b*	*R8*	*Rpi-blb 1 = Rpi-sto1*			*Rpi-blb2*	*Rpi-vnt1-3*	
		R1-1205	**R2-1137**	**R2-686**	**Rpi-blb3-305**	**R3a-1380**	**R3b-378**	**R8-1276**	**RB-226**	**Rpi-blb1-821**	**Rpi-sto1-890**	**Rpi-blb2-976**	**Rpi-vnt1.3-612**	
111 (38 KVA)	adg, ant = sto, dms, plt = sto, sim = mcd, tbr	0	1	1	1	1	1	0	0	1	1	1	0	5
113 (50/1 KVA)	adg, dms, phu, sto, tbr, vrn	1	0	0	0	0	0	1	1	0	0	1	0	4
Hybrids bred by E.V. Rogozina														
117-1	adg, aln = brc, dms, tbr	0	0	0	0	0	1	0	0	0	0	1	0	2
117-2	adg, aln = brc, dms, tbr	0	1	0	0	0	1	1	0	0	0	1	1	5
39-1-2005	adg, aln = brc, dms, tbr	0	0	0	0	0	1	0	1	1	1	0	1	3
24-1	adg, aln = brc, dms, tbr	0	0	0	0	0	1	1	0	0	0	1	1	4
24-2	adg, aln = brc, dms, tbr	0	1	1	1	0	1	0	0	0	0	1	1	4
25-1-2007	acl, adg, aln = brc, dms, phu, sto, tbr, vrn	1	0	0	0	0	1	0	0	0	0	1	0	3

Table 4. *Cont.*

Geno-Types	*Solanum* Species in Hybrid Pedigrees *	Genes												The Number of Genes
		R1	*R2 = Rpi-blb3*			*R3a*	*R3b*	*R8*	*Rpi-blb 1 = Rpi-sto1*			*Rpi-blb2*	*Rpi-vnt1-3*	
		R1-1205	R2-1137	R2-686	Rpi-blb3-305	R3a-1380	R3b-378	R8-1276	RB-226	Rpi-blb1-821	Rpi-sto1-890	Rpi-blb2-976	Rpi-vnt1.3-612	
25-2-2007	acl, adg, aln = brc, dms, phu, sto, tbr, vrn	1	0	0	0	0	1	1	0	0	0	1	1	5
134-2-2006	adg, aln = brc, dms, tbr	1	1	0	0	0	0	1	1	0	0	0	1	5
134-3-2006	adg, aln = brc, dms, tbr	0	0	0	0	0	0	1	0	0	0	0	0	1
134-6-2006	adg, aln = brc, dms, tbr	0	0	0	0	1	1	1	0	0	0	0	1	4
135-1-2006	adg, aln = brc, dms, tbr	0	1	1	1	1	1	0	0	0	0	0	1	4
135-2-2006	adg, aln = brc, dms, tbr	1	1	1	1	1	1	0	1	0	0	0	0	5
139 (4-1-2012)	adg, aln = brc, ant = sto, dms, plt = sto, tbr	1	0	0	0	0	0	0	0	1	1	1	0	3
97-155-1	adg, dms, ryb, sto, tbr	0	1	1	1	1	1	1	0	0	0	0	1	5

Table 4. *Cont.*

Geno-Types	*Solanum* Species in Hybrid Pedigrees *	Genes												The Number of Genes
		R1	*R2 = Rpi-blb3*			*R3a*	*R3b*	*R8*	*Rpi-blb 1 = Rpi-sto1*			*Rpi-blb2*	*Rpi-vnt1-3*	
		R1-1205	R2-1137	R2-686	Rpi-blb3-305	R3a-1380	R3b-378	R8-1276	RB-226	Rpi-blb1-821	Rpi-sto1-890	Rpi-blb2-976	Rpi-vnt1.3-612	
128-05-03	adg, dms, phu, ryb, sto, tbr, vrn	0	1	1	1	1	1	1	0	0	0	0	0	4
118 (118-5-2011)	adg, dms, ryb, sto, tbr	0	1	0	0	1	1	0	1	0	0	0	0	4
120 (118-6-2011)	adg, dms, ryb, sto, tbr	0	1	0	0	1	1	1	0	0	0	0	0	4
160-1	adg, dms, ryb, sto, tbr	0	0	0	0	0	0	1	0	0	0	1	0	2
160-17	adg, dms, ryb, sto, tbr	0	0	0	0	0	0	1	0	0	0	1	0	2
106 (171-3)	adg, dms, ryb, sto, tbr	0	0	0	0	0	1	1	0	0	0	0	0	2
123 (128-6)	adg, dms, ryb, sto, tbr	1	0	0	0	1	1	1	0	0	0	1	0	5
90-6-2	adg, phu, sto, tbr	1	1	0	0	0	0	1	1	0	0	0	1	5
99-6-5	adg, dms, phu, sto, tbr	0	1	0	0	0	1	1	1	0	0	0	1	5
99-6-6	adg, dms, phu, sto, tbr	1	1	0	0	1	1	0	1	0	0	0	1	6
97-153-2	adg, dms, phu, sto, tbr	0	0	0	0	1	0	1	0	0	0	0	0	2

Table 4. *Cont.*

Geno-Types	*Solanum* Species in Hybrid Pedigrees *	Genes												The Number of Genes
		R1	*R2 = Rpi-blb3*			*R3a*	*R3b*	*R8*	*Rpi-blb 1 = Rpi-sto1*			*Rpi-blb2*	*Rpi-vnt1-3*	
		R1-1205	**R2-1137**	**R2-686**	**Rpi-blb3-305**	**R3a-1380**	**R3b-378**	**R8-1276**	**RB-226**	**Rpi-blb1-821**	**Rpi-sto1-890**	**Rpi-blb2-976**	**Rpi-vnt1.3-612**	
2 (194-4т)	adg, dms, phu, ryb, sto, tbr, vrn	0	0	0	0	0	1	1	0	1	0	0	0	3
99-4-1	adg, dms, ryb, sto, tbr	1	0	0	0	0	1	0	0	0	0	0	0	2
7 (93-5-30)	acl, adg, blb, dms, phu, ryb, sto, tbr	0	0	0	0	0	1	1	0	0	0	1	0	3
190-4	adg, dms, phu, sto, tbr, vll	1	1	1	0	0	0	1	1	0	0	0	1	5
97-162-2	adg, mcd, ryb, spg=brc, sto, tbr	0	0	0	0	0	0	1	0	0	0	1	0	2
34-6	adg, mcd, ryb, spg=brc, sto, phu, tbr, vll	1	0	0	0	0	0	1	0	0	0	1	0	3
53 (34-5-2003)	adg, mcd, ryb, spg=brc, sto, phu, tbr, vll	0	0	0	0	0	0	1	0	0	0	1	0	2
135-3-2005	chc, oka	0	1	1	1	0	0	1	0	0	0	1	0	3

Table 4. *Cont.*

Geno-Types	*Solanum* Species in Hybrid Pedigrees *	Genes												The Number of Genes
		R1	*R2 = Rpi-blb3*			*R3a*	*R3b*	*R8*	*Rpi-blb 1 = Rpi-sto1*			*Rpi-blb2*	*Rpi-vnt1-3*	
		R1-1205	**R2-1137**	**R2-686**	**Rpi-blb3-305**	**R3a-1380**	**R3b-378**	**R8-1276**	**RB-226**	**Rpi-blb1-821**	**Rpi-sto1-890**	**Rpi-blb2-976**	**Rpi-vnt1.3-612**	
135-5-2005	chc, oka	0	0	0	0	0	0	1	0	0	0	1	0	2
8-1-2004	chc, oka	0	0	0	0	0	0	0	0	0	0	1	0	1
8-3-2004	chc, oka	0	0	0	0	0	0	1	0	0	0	1	0	2
8-5-2004	chc, oka	0	0	0	0	0	0	1	0	0	0	1	0	2
Reference genotypes														
R5	dms, tbr	1	1	1	1	0	1	1	0	0	0	1	0	5
R8	dms, tbr	0	0	0	0	1	1	1	0	0	0	1	0	4
R9	dms, tbr	1	1	1	1	1	1	0	0	0	0	1	0	5
Magel-lanes	*S. tuberosum* ssp. *tuberosum* L.	1	0	0	0	0	0	0	0	0	0	1	0	2
Alouette	vnt	0	0	0	0	1	1	0	0	0	0	0	1	3
Atzimba	adg, dms, tbr	0	0	0	0	0	0	1	1	0	0	1	0	3
Sapro Axona	dms, tbr	0	0	0	0	0	1	1	0	0	0	0	0	2
Sapro Mira	dms, tbr	0	0	0	0	1	1	1	0	0	0	0	0	3
Alpha	tbr	0	0	0	0	0	0	1	0	0	0	0	0	1
Bintje	tbr	0	0	0	0	0	0	0	0	0	0	0	1	1
Desiree	tbr	0	0	0	0	0	0	1	0	0	0	0	0	1

Table 4. *Cont.*

Geno-Types	*Solanum* Species in Hybrid Pedigrees *	Genes												The Number of Genes
		R1	*R2 = Rpi-blb3*			*R3a*	*R3b*	*R8*	*Rpi-blb 1 = Rpi-sto1*			*Rpi-blb2*	*Rpi-vnt1-3*	
		R1-1205	**R2-1137**	**R2-686**	**Rpi-blb3-305**	**R3a-1380**	**R3b-378**	**R8-1276**	**RB-226**	**Rpi-blb1-821**	**Rpi-sto1-890**	**Rpi-blb2-976**	**Rpi-vnt1.3-612**	
Early Rose	tbr	0	0	0	0	0	0	0	0	0	0	1	1	2
Eersteling	tbr	0	0	0	0	0	0	1	0	0	0	0	0	1
Escort	dms, tbr	1	1	1	0	1	1	0	0	0	0	0	0	4
Gloria	adg, dms, tbr	0	1	0	0	1	1	0	0	0	0	0	0	3
Jubel	dms?, tbr	1	1	0	0	0	0	1	0	0	0	1	1	5
Robijn	tbr	0	1	0	0	0	0	0	0	0	0	0	0	1
Elizaveta	acl, adg, dms, phu, sto, tbr, vrn	1	0	0	0	1	1	0	1	0	0	0	0	4
Nayada	adg, dms, phu, sto, tbr, vrn	1	1	1	1	0	0	1	0	0	0	1	0	4
Negr	*S. tuberosum* ssp. *tuberosum* L.	0	1	0	0	0	0	0	0	0	0	0	0	1
Priekul'skij rannij	tbr	0	0	0	0	0	0	1	0	1	0	0	0	2
Svitanok kievskij	dms, tbr	0	1	1	1	1	1	1	0	1	0	0	0	5
Zagadka Pitera	dms, phu, sto, tbr, vrn	0	0	0	0	1	1	1	0	0	0	0	1	4

* For germplasm codes see Table 1.

The marker Rpi-R3a was previously reported in several *Demissa* and *Longipedicellata* species and also in *S. microdontum* [21]. The functional *Rpi-R3a* analogues were found in several series of *Petota* species with the effectoromics technology [47], whereas the complete *Rpi-R3a* cdc was cloned from *S. stoloniferum* (Genbank accession HQ731037). Two genes, *Rpi-R3a* and *Rpi-R3b*, are located in one cluster on chromosome 11, and their markers go together in most though not all hybrids (Table 4).

Using the effectoromics technology to mine cv. Sarpo Mira, Rietman et al. [40] reported five *Rpi* genes: *Rpi-3a, Rpi-3b, Rpi-R4, Rpi-Smira1* (*Rpi-R9*) and *Rpi-Smira2* (*Rpi-R8*). Our marker analysis of this cultivar confirmed the presence of genes *Rpi-3a, Rpi-3b,* and *Rpi-R8.* All these genes were most probably transferred from *S. demissum* and *S. stoloniferum* (https://pomidom.ru/sarpo-mira-potatoes/).

Now, let us turn to one more gene assayed with several SCAR markers: *Rpi-blb1/Rpi-sto1*. Two markers, Rpi-blb1-821 иRpi-sto1-890, which cover different regions of the gene sequence (Figure 1), perfectly concurred in a range of *Bulbocastana* and *Longipedicellata* accessions [49] and now in most hybrids containing the genetic material of these species (Table 4). In addition to the predictable presence of the markers Rpi-blb1-821 and Rpi-sto1-890 in such hybrids, these markers were unexpectedly found in the Atzimba × *S. alandiae* hybrid 39-1-2005. Only single marker Rpi-blb1-821 was found in cvs Priekulskiy rannij and Svitanok kievskij. Previously this marker was also reported in a highly resistant accession VIR5399 of *S. microdontum* [49].The short marker Rpi-blb1-226 usually accompanied two longer markers of the gene; however, Rpi-blb1-226 alone was found in four genotypes that contained *Longipedicellata* genetic material (113 (50/1 KVA), 118(118-5-2001), 190-4 and cv. Elizaveta), whereas the hybrids 134-2-2006, 135-2-2006, 90-6-2, 90-6-5, 99-6-6 and Atzimba also containing this marker are free from the *stoloniferum* germplasm as the most probable source of this gene.

Our collection lacks the hybrids with the genetic material of *S. venturii*. However, the *Rpi-vnt1* analogues and pseudogenes are widely distributed in South American *Tuberosa* species, including *S. microdontum* and *S. okadae* [45]. Indeed, we registered one allele of this gene, *Rpi-vnt1*-3, in two thirds of hybrids containing the germplasm of *S. alandiae* and *S. microdontum:* the comparison of this allele sequence to that of the prototype *Rpi-vnt1* gene indicated 92–98% identity [50]. In addition, the *S. alandiae* genome comprised the structural homologues of *R2/Rpi-blb3*, *R8*, *R9a*, *Rpi-vnt1* and *Rpi-blb2*; respective homologues were 94–99, 94–99, 86–89, and 91% identical with the prototype genes [50]. It is also relevant to mention that the complete *Rpi-vnt1*-like sequence was cloned from *S. microdontum* ssp. *gigantophyllum* (Genbank accession GU338312). We failed to find the marker Rpi-vnt1.3-612 in all hybrids comprising *S. okadae* genetic material (Table. 4), whereas this marker was found in the *S. okadae* accession k-25397-1 different from the accession к-20921 used as the male parent of the hybrids [50].

In each group of hybrids of similar descent created by Yashina and Kolobaev and hybrids with the participation of *S. alandiae* bred by Rogozina, we find a highly consistent inheritance of markers. Thus, the Yashina's hybrids 2585-67, 2585-70, 2585-80, 2359-13, 2584-7 and 97.13-9 (descended from cv. Nikulinsky as a female parent), seem to comprise the *Rpi-R2/Rpi-blb3*, *Rpi-R3a* and *Rpi-R3b* genes. The Kolobaev's hybrids 10/5-09 and 11/6-09 (descended from cv. Zagadka Peter as a female parent) inherited the *Rpi-R2/Rpi-blb3*, *Rpi-R3a*, *Rpi-R3b*, *Rpi-R8* and *Rpi-blb2* genes. In Rogozina's hybrids 25-1-2007 and 25-2-2007, the *Rpi-R1* and *Rpi-R3b* genes were inherited from the female parent cv. Elizaveta, whereas the *Rpi-blb2* gene was transferred from the paternal form—hybrid 24-1. In most hybrids based on *S. alandiae*, the first generation from crosses and backcrosses inherited the marker of *Rpi-vnt1.*

Of special interest are resistant and moderately resistant hybrids (6 and more points) that nonetheless contain only one or two markers of *Rpi* genes. Such discrepancy is especially surprising as many of these hybrids seem to include *demissum* and/or *stoloniferum* germplasm: 2585-80, 2584-7, 97.1.17, 12/1-09, 160-17, 106 (171-3), 97-153-2, 99-4-1, and 53 (34-5-2003) (Tables 2 and 4). Presumably, these hybrids comprise as yet unidentified

Rpi genes or new alleles of already known *Rpi* genes [7,9] that are not recognized with our markers. Two *Rpi* genes (*Rpi1* and *Rpi2*) on chromosome 7 of *S. pinnatisectum* [20,51] may exemplify such case in hybrid 12/1-09. Three hybrids with low numbers of markers: 97-162-2, 34-6 and 53 (34-5-2003) reportedly include genetic material of *S. microdontum* insufficiently researched by molecular methods. SCAR marker analysis of the South American species *S. alandiae* and *S. okadae* accessions in the VIR collection also revealed several structural homologues of already known *Rpi-R2*, *Rpi-R8* and *Rpi-blb2* genes of the Mexican species *S. demissum* and *S. bulbocastanum* [50].

3.3. LB Resistance is Enhanced by Pyramiding Rpi Genes

The numbers of *Rpi* genes combined in particular potato hybrids are clearly in line with plant LB resistance in the field experiments. We compared LB resistance in field trials in cultivars and hybrids in two contrasting subsets of potato genotypes: those containing only one *Rpi* gene and those with five genes. The former subset of nine genotypes comprises six cultivars (Desiree, Bintje, Alpha, Negr, Eersteling, and Robijn) and three hybrids (134-3-2006, 2585-80, and 97.1.17), wherein only one *Rpi* gene, either *Rpi-R2/Rpi-blb3* or *Rpi-R8*, was found (Table 4). In the latter subset of 18 genotypes five-six genes were recognized (Tables 4 and 5). Two subsets significantly differ in their LB resistance in field trials by the Mann-Whitney criterion: $U_{observed} = 33 < U_{critical} = 42$ at $p < 0.05$. The Spearman' correlation coefficient ($R_{observed} = 0.514 > R_{critical} = 0.382$ at $p < 0.05$) is another proof of statistically significant relationship between the number of *Rpi* genes and LB resistance in these subsets of potato cultivars and hybrids.

Mundt [14] demonstrated that under optimal conditions, a stack of four efficient resistance genes would provide a durable protection against the pathogen. We therefore focused on the genotypes that comprised four and more *Rpi* genes per plant (Table 5). Over 80% of these hybrids, together with the cultivars derived from multiparental hybrids, manifest significant and long-lasting field resistance to LB (6 points and higher). The predominant resistance genes of these genotypes are *demissoid Rpi-R3b* (with the frequency of 0.79), *Rpi-R2/Rpi-blb3* (0.74), *Rpi-R8* (0.66), and *Rpi-R3a* (0.59); the frequencies of other genes are 0.41–0.44 (Table 5).

Table 5. Potato hybrids with 4+ *Rpi* genes.

Genotype	Pedigree	*Rpi-R1*	*Rpi-R2/Rpi-blb3*	*Rpi-R3a*	*Rpi-R3b*	*Rpi-R8*	*Rpi-blb1/Rpi-sto1*	*Rpi-blb2*	*Rpi-vnt1*	Total Gene Number	Field Resistance
2359-13	chc, dms, tbr	1	1	1	1	0	0	0	0	4	6
97.13-9	cmm, dms, mga, tbr	0	1	1	1	1	0	1	0	5	5
2372-60	adg, chc, dms, lpt, sto, tbr	1	1	1	1	0	0	0	0	4	8
10/5-09	dms, phu, sto, tbr, vrn	0	1	1	1	1	0	1	0	5	7
11/6-09	dms, phu, sto, tbr, vrn	0	1	1	1	1	0	1	0	5	7
13/11-09	adg, pnt, tbr	0	0	0	1	1	1	0	1	4	7
14/8-09	Ant = sto, dms, plt = sto, tbr	0	1	1	1	1	0	0	1	5	6
15/13-09	adg, ant = sto, dms, plt = sto, pnt, sim = mcd, tbr, ver	0	1	0	1	1	1	1	0	5	6
16/27-09	adg, ant = sto, ber, chi, dms, phu, plt = sto, sim = mcd, tbr, vrn	1	0	0	0	1	1	1	0	4	7
111 (38 KVA)	adg, ant = sto, dms, plt = sto, sim = mcd, tbr	0	1	1	1	0	1	1	0	5	8

Table 5. *Cont.*

Genotype	Pedigree	*Rpi-R1*	*Rpi-R2/Rpi-blb3*	*Rpi-R3a*	*Rpi-R3b*	*Rpi-R8*	*Rpi-blb1/Rpi-sto1*	*Rpi-blb2*	*Rpi-vnt1*	Total Gene Number	Field Resistance
113 (50/1 KVA)	adg, dms, phu, sto, tbr, vrn	1	0	0	0	1	1	1	0	4	7
117-2	adg, aln = brc, dms, tbr	0	1	0	1	0	0	1	1	4	7
24-1	adg, aln = brc, dms, tbr	0	0	0	1	1	0	1	1	4	8
24-2	adg, aln = brc, dms, tbr	0	1	0	1	0	0	1	1	4	8
25-2-2007	acl, adg, aln = brc, dms, phu, sto, tbr, vrn	1	0	0	1	1	0	1	1	5	5
134-2-2006	adg, aln = brc, dms, tbr	1	1	0	0	1	1	0	1	5	7
134-6-2006	adg, aln = brc, dms, tbr	0	0	1	1	1	0	0	1	4	6
135-1-2006	adg, aln = brc, dms, tbr	0	1	1	1	0	0	0	1	4	7
135-2-2006	adg, aln = brc, dms, tbr	1	1	1	1	0	1	0	0	4	7
97-155-1	adg, dms, ryb, sto, tbr	0	1	1	1	1	0	0	1	5	8
128-05-03	adg, dms, phu, ryb, sto, tbr, vrn	0	1	1	1	1	0	0	0	4	7
118 (118-5-2011)	adg, dms, ryb, sto, tbr	0	1	1	1	0	1	0	0	4	8

Table 5. *Cont.*

Genotype	Pedigree	*Rpi-R1*	*Rpi-R2/Rpi-blb3*	*Rpi-R3a*	*Rpi-R3b*	*Rpi-R8*	*Rpi-blb1/Rpi-sto1*	*Rpi-blb2*	*Rpi-vnt1*	Total Gene Number	Field Resistance
120 (118-6-2011)	adg, dms, ryb, sto, tbr	0	1	1	1	1	0	0	0	4	7
123 (128-6)	adg, dms, ryb, sto, tbr	1	0	1	1	1	0	1	0	5	8
90-6-2	adg, phu, sto, tbr	1	1	0	0	1	1	0	1	5	7
99-6-5	adg, phu, sto, tbr	0	1	0	1	1	1	1	0	5	4
99-6-6	adg, phu, sto, tbr	1	1	1	1	0	1	0	1	6	5
190-4	adg, dms, phu, sto, tbr, vll	1	1	0	0	1	1	0	1	5	8
Escort	dms, tbr	1	1	1	1	0	0	0	0	4	7
Jubel	dms?, tbr	1	1	0	0	1	0	1	1	5	7
Elizaveta	acl, adg, dms, phu, sto, tbr, vrn	1	0	1	1	0	1	0	0	4	5
Nayada	adg, dms, phu, sto, tbr, vrn	1	1	0	0	1	0	1	0	4	6
Svitanok kievskij	dms, tbr	0	1	1	1	1	1	0	0	5	5
Zagadka Pitera	dms, phu, sto, tbr, vrn	0	0	1	1	1	0	0	1	4	6
Frequency		0.44	0.74	0.59	0.79	0.66	0.41	0.44	0.44		

* For germplasm codes see Table 1.

4. Conclusions

High and long-lasting LB resistance is a major prerequisite for sustainable potato production. In this project, a considerable collection of potato interspecific hybrids and standard cultivars was assayed with SCAR markers for ten *Rpi* genes, and plant LB resistance was evaluated in the field trials and laboratory tests with detached leaves. These hybrids combine several *Rpi* genes that are currently in high demand with potato breeders, such as *Rpi-R2*/*Rpi-blb3*, *Rpi-blb1*/*Rpi-sto1*, *Rpi-blb2*, and *Rpi-vnt1*. The level of LB resistance manifested by these hybrids is significantly related to the number of *Rpi* genes stacked in a single hybrid. This evidence seems to support the concept of pyramiding *Rpi* genes for durable LB resistance. However, when the patterns of gene stacking are examined with SCAR markers, it seems proper to focus on several caveats.

First, a considerable portion of resistance manifested by the investigated hybrids was not associated with the markers used in this study, and we believe that such resistance depended on some new or insufficiently characterized *Rpi* genes, which are not recognized by the markers employed to screen the hybrids. To exemplify such possibility, *S. chacoense* germplasm is found in many hybrids examined in the present study (Tables 2 and 4), and some of their LB resistance could be related to the *Rpi-chc1* gene [7]. Indeed, screening such hybrids with the marker for this gene developed in our laboratory produced the positive signal in hybrids 2372-60, 2522-173 and 2584-7 but not in 2359-13. Among five *S. okadae* k-20921 × *S. chacoense* k-19759 hybrids, only 135-3-2005 was positive, other four segregants of this hybrid and the accession *S. chacoense* k-19759 itself responded negatively (M. Beketova, personal communication). Another possibility would link such resistance to other defense pathways, including non-specific tolerance.

Second, in such a complex assortment of genetic material, the gene stacks may comprise several alleles of one and the same gene introgressed from different *Solanum* species, e.g., *S. chacoense*, *S. demissum*, *S. pinnatisectum*, *S. phureja*, *S. stoloniferum*, etc. [7,20,47,51,52]. It is not always possible to distinguish such alleles. At least, in this study, by using the markers that reliably discriminate between *demissum* and *stoloniferum* alleles of *Rpi-R1* [53], we demonstrated that nine hybrids combining *demissum* and *stoloniferum* germplasms comprised only the former allele of *Rpi-R1* and were devoid of the latter.

Third, the SCAR markers employed in this study do not stretch over the full-size sequences of candidate genes, especially in the case of short markers Rpi-R3b-378 and Rpi-blb3-305. The changes in the candidate gene under study beyond the region covered by the particular marker would render this gene inactive. Perhaps, the presence of pseudogenes would explain the occurence of markers of *Rpi* genes in the standard cultivars believed to be devoid of such genes: *Rpi-R1* in cv. Magellanes, *Rpi-R2* in cv. Robijn, *Rpi-R8* in cvs Alpha, Desiree, and Eersteling, *Rpi-blb2* in cvs Magellanes and Early Rose, and *Rpi-vnt1* in cvs Bintje and Early Rose (Table 4). Similarly, when the presence of markers in the hybrids is not supported by their pedigrees, such discrepancy can be explained by the presence of inactive homologues. In support of these suggestions, the BLAST search recognized the homologues of all these genes except *Rpi-vnt1* in a true *S. tuberosum* cv. Solyntus [54] (the corresponding Genbank accessions CP055238, CP055237, CP055242, CP055241, and CP055239).

Fourth, even when the complete sequences of candidate genes are assessed (e.g., with the dRenSeq technology [25]), the proof for their functionality must be obtained by independent methods, such as effectoromics [40,47,55].

There are two ways to combine a sufficient number of *Rpi* genes of broad specificity towards diverse pathogen races and in this way to develop the basis of long-lasting and durable LB resistance: to stack several efficient genes in a single potato genotype or to produce a mosaic of *Rpi* genes in a potato stand combining several cultivars. When bred from the multiparental hybrids, the advanced lines with the stacks of broad-specificity *Rpi* genes will become prospective breeding donors immediately at hand when new pathogen strains arrive with *Avr* genes virulent to existing potato cultivars [1,13,14]. These breeding strategies usually aim at supporting and expanding the genetic diversity in potato stands.

Developing such sources of resistance to combat future pathotypes is called pre-emptive, or anticipatory breeding [56,57]. In the case of *P. infestans*, with its extremely plastic genome [58] and rapid changes in the repertoire of *Avr* genes [1,59], the advanced lines bred from multiparental hybrids would help withstand LB outbreaks caused by rapid pathogen evolution and invasion of new pathotypes.

By their productivity (0.89–1.25 kg of tubers per plant), most tested hybrids were comparable to cv. Sarpo Mira, the international standard of LB resistance, and considerably overtook the susceptible standard cv. Bintje. However, within the selection of highly resistant genotypes with 4+ markers of *Rpi* genes per plant (Table 5), it is difficult to relate tuber yield immediately to plant resistance and the number of resistance genes.

In many aspects, the success of pyramiding *Rpi* genes depends on the breeder's appraisal of the agricultural ecosystem as a whole [60] and the knowledge of potato *Rpi* genes and *Avr* genes of *P. infestans* in the particular potato stands. In the latter case, rapid and efficient assessment of *Rpi* and *Avr* gene profiles with dRenSeq and PenSeq technologies [25,59] seems most hopeful as regards the prediction of crop losses and evaluation of breeders' efforts.

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