PAGV – SPECIAL REPORT NO 18 – 2017, 159-166

Stability of late blight resistance of potato hybrids with diverse genetic background

ELENA V. ROGOZINA[1], MARIYA A. KUZNETSOVA[2], OKSANA A. FADINA[3], MARIYA P. BEKETOVA[3], EKATERINA A. SOKOLOVA[3], LYUBOV YU. NOVIKOVA[1], EMIL E. KHAVKIN[3]

[1] N.I. Vavilov Institute of Plant Genetic Resources (VIR), St. Petersburg, Russia
e-mail: rogozinaelena@gmail.com
[2] Institute of Phytopathology (IP), Bol'shiye Vyazemy, Moscow region, Russia
[3] Institute of Agricultural Biotechnology (ARRIAB), Moscow, Russia

SUMMARY

Nine clones of interspecies potato hybrids and cv. Nayada recommended for cultivating on the territory of the northwestern Russia were tested for nine years under different infection loads in the field and following twice repeated artificial infection with a highly aggressive isolate of *Phytophthora infestans*. Stable resistance to late blight (LB) in the field through the entire period of observations was registered only in clone 99-4-1, meanwhile the responses of other genotypes varied depending on the year of the test. The results of the tests under natural and artificial infection when compared by the average degree of consistency (Kendall's W = 0.43) did not concur perfectly indicating that a comprehensive assessment is necessary. In the best characterized clones 171-3 and 99-4-1 comprising the genetic material of *S. anigenum, S. demissum* and *S. stoloniferum*, only two race-specific resistance genes were found: *R1* and *R3b* presuming that further study of the genetic nature of LB resistance in the interspecific hybrids is on the agenda.

KEYWORDS

Phytophthora infestans, Solanum section *Petota*, interspecific hybrids, durable resistance.

INTRODUCTION

The potato collection maintained in the N.I. Vavilov Institute of Plant Genetic Resources (VIR) comprises more than 120 potato hybrid clones developed and recommended to breeders as valuable sources of agronomic traits. Most of these hybrids were bred in the 1990s and have been propagated clonally till nowadays. These hybrids are maintained in the Pushkin experimental plots (VIR) located in the North West of Russia, where potato late blight (LB) outbreaks occur nearly every year. Complexity and high diversity of the races in the Pushkin population of *Phytophthora infestans* has been established both by phenotypic and molecular analysis of its structure (Kuznetsova et al., 2016; Sokolova et al., 2017; Zoteyeva, Patrikeeva, 2010). We selected nine hybrid clones with diverse genetic background and compared their phenotype and genetic profiles in order to examine the efficacy of LB resistance in the hybrid clones from the VIR collection and to pinpoint the combinations of the genes for race-specific resistance to *P. infestans* (*Rpi* genes) apparently providing for durable LB resistance.

MATERIALS AND METHODS

Potato genotypes tested for LB resistance

Nine clones of interspecific potato hybrids and cv. Nayada as a resistant standard were assessed for LB resistance in the field under conditions of natural infestation and in the laboratory assays. These clones originate from different parental lines and carry the genetic material from two to five tuber-bearing *Solanum* species, such as *S. alandiae, S. andigenum, S. bulbocastanum, S. demissum, S. polytrichon* (syn. *S. stoloniferum*)*, S. simplicifolium* (syn. *S. microdontum*) or *S. stoloniferum*, which were used as donors of LB resistance (Table 1). We initially selected the clones with haulm and tubers that resembled in the largest the cultivated potatoes and manifested high LB resistance scores (7-8). All tested genotypes belong to the middle maturity group.

LB resistance test

Field trials were carried out at the Pushkin Experimental Station of VIR (St. Petersburg) through cropping seasons 2008-2016. Ten plants of each clone per plot in two repetitions were planted in a randomized design into a common field of the potato collection. Observations started when first LB symptoms appeared on cv. Bintje used as a susceptible standard. The area of leaves affected by LB was scored 4-5 times during each cropping season and used for calculating the area under the disease progress curve (AUDPC). AUDPC values from each single experiment were transformed into the relative AUDPC (rAUDPC) score as described by Fry (1978). Maximum disease induced damage in potato foliage and tuber yields were assessed at the end of each cropping season.

The laboratory test with detached leaves was carried out in IP according to EuroBlight protocol Version 1.2 (euroblight.net). Three leaves per clone in two repetitions and cultivar Santé as a susceptible control have been infected with the complex race N161 (the IP collection) combining the virulence genes 1 to 11, A1 mating type and a high aggressiveness (Kuznetsova et al., 2016). The experimental data for LB resistance were transformed into 1-9-point scores.

Screening for of Rpi genes

Clones of hybrid clones and cv. Nayada were screened in ARRIAB with sequence characterized amplification region (SCAR) markers for seven genes: *R1*, *R2/Rpi-blb3*, *R3a*, *R3b*, *RB/Rpi-blb1=Rpi-sto1*, *Rpi-blb2* and *Rpi-vnt1.3* using the PCR protocols described elsewhere (Fadina et al., 2017).

RESULTS

Climatic conditions and LB emergency during nine growth seasons

Weather conditions for the growing seasons 2008-2016 were not equally favorable for potato plants and LB development. Through the whole period, the summer air temperatures exceeded the average long-term values, sometimes by 5-6.6°C (June 2013, July 2010, 2011, 2014, August 2015). The amount of precipitation in the summer months varied significantly. For example, in June 2009 and 2010, July 2015 and 2016 and in August 2008, 2009, 2012 and 2016, precipitation increased by 1.5-2 times as compared to the average monthly rate. Nevertheless, there were periods of lack of moisture - June 2011 and 2015, July 2008 and 2014, August 2015. Obviously, such instability of weather conditions strongly affected the patterns of both plant growth and development and LB emergence and spread. Upon the combined results of evaluating the defeat by LB of the entire field collection of potato hybrids comprising 120 clones, we established that the epiphytotic LB development took place in 2008 and 2016. Less sweeping but nevertheless as serious plant damage was noted in 2013. In 2009, 2011, 2012 and 2015, LB developed more slowly, and many cultivars and potato hybrids were in time to form a high yield. In 2010 and 2014, LB was registered on susceptible cultivars and some hybrids only at the very end of the growth season.

LB resistance of interspecific potato hybrids in the field trials

In both years of epiphytotic LB development (2008 and 2016), the progress of disease commenced as soon as the first damage symptoms were observed on cv. Bintje: 78 and 67 days after planting, respectively. The weather conditions favorable for the rapid LB development contributed to disease spread, in 5-6 days (apparently corresponding to the first cycle of the pathogen development), lesions appeared on cv. Nayada and eight hybrid clones. Clone 40-2000 was vividly affected much later: in 18-20 days from the beginning of the observations. In both epiphytotic years, LB progressed at different rate on the leaves of hybrid clones: most rapidly in clones 117-2 and much slower in clones 171-3 and 40-2000. In the years of moderate disease development, the disease progress and the extent of damage of nine hybrid clones and cv. Nayada considerably differed from the indices registered during epiphytoty. The values of rAUDPC for three-year study ranged from 0.05 to 0.53 (Table 1). The Friedman's ANOVA rank test indicates significant year-by-year variation in rAUDPC values in field tests ($\chi 2$Friedman = 6.95> $\chi 2$ (o.o5) = 5.99). In the years of epiphytotic LB manifestation, the minimal rAUDPC values were noted in clones 40-2000 and 171-3. In the years of moderate development, six clones:

40-2000, 91-19-3, 117-2, 171-3, 99-4-1 and 160-17 - produced similar rAUDPC values (Table 1). Variations in the rAUDPC indices in different years of testing are confirmed by statistical analysis. Kendall's coefficient of concordance W of 0.24 indicated weak coherence in year-by-year potato response to LB infection.

Table 1. *LB resistance and average productivity of potatoes with diverse genetic background*

Clone	Background	*Solanum* spp. in hybrid pedigree	*Rpi* assessed with SCAR markers	r AUDPC			DL	Yield, g/plant
				2008	2015	2016		
117-2	F1	*tbr, dms, aln*	*R2/Rpi-blb3, R3b, Rpi-blb2, Rpi-vnt1.3*	0.47	0.13	0.53	5 MS	550-1160
25-1-2007	BC1	*tbr, dms, aln*	*R1, R3b, Rpi-blb2*	0.33	0.23	0.33	5 MS	480-1000
99-4-1	BC2	*tbr, dms, adg, sto*	*R1, R3b*	0.23	0.16	0.28	5.5MS	475-860
171-3	F2 BC1	*tbr, adg, ryb, dms, sto*	*R3b*	0.12	0.16	0.19	6 MR	400-800
160-17	F2 BC1	same	*Rpi-blb2*	0.23	0.16	0.43	nd	660-1150
194-4т	BC2 (Fn)	*tbr, adg, phu, dms, sto*	*R3b, RB/Rpi-blb1*	0.27	0.34	0.45	5 MS	550-1400
34-5-2003	Fn	*tbr, adg, ryb, phu, dms, sto*	*Rpi-blb2*	0.36	0.21	0.30	5 MS	400-930
91-19-3	Fn	*tbr, adg, ryb, acl, blb, sto*	nd	0.23	0.13	0.41	nd	370-1160
40-2000	BC2F2 × BC2F1	*tbr, adg, dms, sto, plt, sml*	*R1, Rpi-vnt1.3*	0.05	0.13	0.25	4 MS	520-1200
cv. Nayada		*tbr, adg, phu, dms, vrn, sto*	*R1, R2/Rpi-blb3, Rpi-blb2*	0.27	0.23	0.45	nd	620-1100

**acl — S. acaule, adg — S. andigenum, aln — S. alandiae, dms — S. demissum, phu — S. phureja, plt — S. polytrichon, ryb — S. rybinii, sml- S. simplicifolium, sto — S. stoloniferum, tbr – S. tuberosum, vrn — S. vernei. DL – detached leaflet test, nd — no data.*

The extent of maximum damage by LB in the tested set of potato hybrid clones was assessed at the end of the growth period using the 1-9 scale, where 9 is the absence of lesions. The value of this index varied from 1 to 9 in clones 117-2, 25-1-2007, 194-4t and cv. Nayada (Figure 1). By the time of harvesting, in other six hybrid clones under study 30% or more of the leaf surface stayed unaffected by the disease. As far as this trait was concerned, clone 99-4-1 was the most durable: through nine years of observations, over 50% of the leaf surface remained unaffected (Figure 1). In clone 171-3 for 8 consecutive years of observations no more than by 25% of leaf surface was affected; however, by the end of the 2016 growth period, it was affected much stronger. Clone 40-2000 manifested lesser LB lesions by the end of growth during epiphytotic periods in 2008 and 2015 than in 2012 and 2013 (Figure 1). ANOVA proved a significant effect of the “year of tests” factor on the LB damage severity in the tested clones ($F = 15.3 > 3.4$).

The results of our long-term field experiments under the conditions of the North-West Russia concur with the data from the earlier tests of the same potato genotypes under other soil and climatic conditions. Thus, in the tests in 1990s run on the island of Sakhalin, which is very similar in its weather conditions to the valley of Toluca (Mexico), clones 91-19-3 was not affected by LB by the harvesting time, while the standard cultivars completely perished. Clone 99-4-1 was highly resistant to LB under high infection conditions in the tests performed in Belarus (Kozlov, Rogozina, 2014).

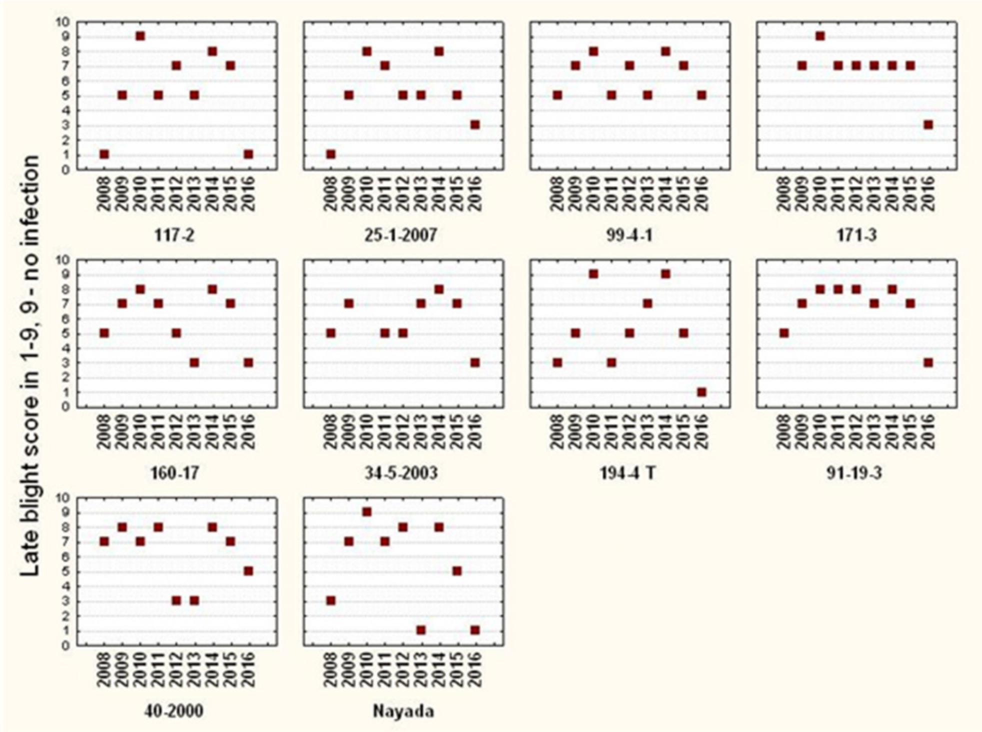

Figure 1. *Maximum damage caused by LB in nine hybrid clones and cv. Nayada in the 2008-2016 trials*

Laboratory tests for LB resistance

Based on the results of a two-year assessment of LB resistance of hybrid clones under the artificial infection, all investigated genotypes, except for clone 171-3, were classified as moderately susceptible. Clone 171-3 and cv. Sarpo Mira (currently a reference for high LB resistance) were found to be moderately resistant (7 MR). It is noteworthy that in contrast to Sarpo Mira, which is the late-season cultivar in the northwestern Russia, clone 171-3 belongs to the middle maturity group cultivars. These estimates of LB resistance under artificial infestation did not perfectly match the results of field trials: the Kendall's W value was equal to 0.43, probably due to a higher infection load in the laboratory tests and lack of competition between different *P. infestans* isolates colonizing potato plants in the field.

On the average, the consistency of LB resistance indices obtained in the field trials and laboratory tests with detached leaves was not high confirming the previous reports for wild species (Rogozina et al., 2010; Sharma et al., 2013). It follows that assessments for two to three years only in the field or only in the laboratory are not sufficient for evaluating the actual LB resistance.

Productivity

The average productivity of potato plants varied depending on the potato genotype and the year of testing. The most noticeable variation was observed in clones 194-4 and 91-19-3, wherein the weight of tubers collected from one plant varied by 2-3 times. Instability of productivity was also noted in cv. Nayada recommended for the northwestern Russia (Table 1). The link between LB damage and productivity was not strong: the final score of disease severity and tuber yield did not correlate (the Spearman's coefficient $r = 0.26$, $p < 0.001$). Plant productivity is a complex trait, and its manifestation depends on many factors. Two-way ANOVA indicated that the factor "year of trial" significantly affected the tuber yield. Obviously, in addition to LB, both other diseases and weather conditions significantly influenced the final tuber yield as confirmed by evaluating the productivity of clones 25-1-2007, 91-19-3 and 171-3: here the yield was higher in 2015, under heavy LB, than in the absence of disease in 2010.

Resistance genes

According to the SCAR marker analysis, nine potato clones and cv. Nayada comprised various patterns of one to four *Rpi* genes (Table 1). The relationship between LB resistance and *Rpi* gene profile of these potato clones was not evident and must to be studied further. Durable resistance of clone 99-4-1 to LB apparently involves the genes other than the *Rpi* genes registered in this study. This clone is one of the most probable sources of race non-specific LB resistance. Clone 171-3 is another promising target for gene mining: while this genotype considerably exceeds other hybrid clones by LB resistance, the marker analysis comes out with a single gene *R3b.*

DISCUSSION

Under the current changes in *P. infestans* populations, most clones of interspecies hybrids became more susceptible to the disease: of nine clones under study, durable LB resistance was found only in clone 99-4-1. Clones of different pedigrees probably differ in their defense mechanisms, such as resistance to infection penetration in clone 40-2000, slow pathogen development in the tissues of clone 99-4-1, or resistance to initial infection and its further spread in clone 171-3. Plant defense reaction in response to pathogen infection involves two defense systems (Jones, Dangl, 2006). The *Rpi* genes play a crucial role in defense as their products, e.g., receptor kinases, recognize pathogen effectors and respond to the changes in biochemical processes of plants affected by the invading pathogen. In the 20th century, the priorities of breeding potatoes for LB resistance changed dramatically: first, breeders employed the initial material comprising the *Rpi* genes of *S. demissum* and few other *Solanum* species, next, they turned to the *Rpi*-free genetic material providing for so-called horizontal LB resistance and finally, in the last decade, breeders focused again on the *Rpi* genes in a wider taxonomic context (Govers, Struik, 2009; Sliwka and Zimnoch-Guzowska, 2013). Discovery of *Rpi* genes with a broad resistance spectrum, such as *RB/Rpi-blb1, Rpi-blb2 and Rpi-vnt1* became a spectacular prerequisite for breeding potatoes resistant to numerous pathogen races due to pyramiding the effective *Rpi* genes. Using the methods of conventional breeding, several cultivars and advanced breeding lines have been developed, which carry four to seven *Rpi* genes (Khavkin et al., 2014; Kim et al., 2012; Rietman et al., 2012). The transcriptome analysis when employed for an in-depth study of the genetic nature of LB resistance of potato cultivars and non-tuber-forming *Solanum* species, revealed over 400 expressed *Rpi* genes in resistant forms of cultivated potatoes (Frades et al., 2015). In this regard, the detection of few *Rpi* genes in clones 171-3 and 99-4-1 presumes that LB resistance of these genotypes involves other genes than *R1* and *R3b* most probably transferred from *S. demissum*.

CONCLUSION

Clones of interspecific potato hybrids highly LB resistant in the 1990s tests became today more susceptible to the disease. Of nine clones under study, durable LB resistance was found only in clone 99-4-1. In clones 99-4-1 and 171-3 bred on the basis of potato cultivars and *S. andigenum*, *S. demissum* and *S. stoloniferum* accessions from the VIR collection, the already known genes *R1* and *R3b* are obviously complemented by other as yet unknown *Rpi* genes

ACKNOWLEDMENTS

The study was supported by the Russian Foundation for Basic Research (project 16-04-0098a).

REFERENCES

Fadina, O.A., M.P. Beketova, M.A. Kuznetsova, E.V. Rogozina and E.E. Khavkin. Revisiting late blight resistance genes in complex interspecific potato hybrids. In: Schepers H.T.A.M. (ed.). PAGV-Special Report, 2017, no. 18, Wageningen, DLO Foundation, pp. 245-256.

Frades, I., K.B. Abreha, E. Proux-Wéra, Å. Lankinen, E. Andreasson and E. Alexandersson (2015). A novel workflow correlating RNA-seq data to *Phytophthora infestans* resistance levels in wild Solanum species and potato clones. Front. Plant Sci.6:718. doi: 10.3389/fpls.2015.00718.

Fry, W.E. (1978). Quantification of general resistance of potato cultivars and fungicide effects for integrated control of potato late blight. Phytopathology 68:1650–1655.

Govers, A. and P.C. Struik (2009). Debate on the Exploitation of Natural Plant Diversity to Create Late Blight Resistance in Potato. Potato Research. 52:265–271 DOI 10.1007/s11540-009-9135-4

Jones, J.D.G. and J.L. Dangl (2006). The plant immune system. Nature 444, 323-329. doi:10.1038/nature05286.

Khavkin, E.E., O.A. Fadina, E.A. Sokolova, M.P. Beketova, P.E. Drobyazin, E.V. Rogozina, M.A. Kuznetsova, I.M. Yashina, R.W. Jones and K.L. Deahl (2014). Pyramiding R genes: genomic and genetic profiles of late-blight resistant interspecific potato hybrids. In: Schepers H.T.A.M. (ed.) PPO-Special Report no. 16, Wageningen, DLO Foundation, pp. 215-220.

Kim, H.-J., H.-R. Lee, K.-R. Jo, S.M.M. Mortazavian, D.J. Huigen, B. Evenhuis, G. Kessel, R.G.F. Visser, E. Jacobsen and J.H. Vossen, 2012. Broad-spectrum late blight resistance in potato differential set plants Ma*R8* and Ma*R9* is conferred by multiple stacked R genes. Theoretical and Applied Genetics 124, 923–935.

Kozlov, Rogozina (2014). Interspecific potato hybrid clones developed at VIR as sources of valuable traits for potato breeding in Belarus. Proceedings on Applied Botany, Genetics and Breeding. 175 (2):61-72 (In Russ.).

Kuznetsova, M.A., B.E. Kozlovsky, M.P. Beketova, E.A. Sokolova., O.P. Malyuchenko, Y.I. Alekseev, E.V. Rogozina and E.E. Khavkin (2016). Phytopathological and molecular characteristics of *Phytophthora infestans* isolates collected on resistant and susceptible potato genotypes. Mikologiya i fitopatologiya. 50, 175-184 (In Russ.).

Rietman, H., G. Bijsterbosch, L.M. Cano, H.R. Lee, J.H. Vossen, E. Jacobsen, R.G. Visser, S. Kamoun and V.G. Vleeshouwers (2012). 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 Interactions 25, 910-919.

Rogozina, E.V., M.A. Kuznetsova, M.V. Patrikeyeva, S.Y. Spiglazova, T.I. Smetanina, N.N. Semenova and Kenneth L. Deahl (2010). Late blight-resistant tuber-bearing Solanum species in field and laboratory trials. In: Schepers H.T.A.M. (ed.) PPO-Special Report no. 14, Wageningen, DLO Foundation, pp. 239-245.

Sharma, B.P., G.A. Forbes, H.K. Manandhar, S.M. Shrestha and R.B. Thapa (2013). Determination of Resistance to *Phytophthora infestans* on Potato Plants in Field, Laboratory and Greenhouse Conditions. Journal of Agricultural Science, 5 (5): 148-158.

Sliwka, J. and E. Zimnoch-Guzowska (2013). Resistance to Late Blight in Potato. Chapter 12. Translational Genomics for Crop Breeding, Volume I: Biotic Stress, First Edition. Edited by Rajeev K. Varshney and Roberto Tuberosa. John Wiley & Sons, Inc. Published 2013 by John Wiley & Sons, Inc. 221-240.

Sokolova, E.A., M.A. Kuznetsova, T.I. Ulaniva, A.N. Rogozhin, T.I. Smetanina, V.N. Demidova, M.P. Beketova, O.P. Malyuchenko, Ya. I. Alekseev, E.V. Rogozina and E.E. Khavkin. Pathogenicity of East European strains of *Phytophthora infestans* vs. resistance of colonized potato plants: the profiles of *AVR* genes vs. *R* gene pyramids. In: Schepers H.T.A.M. (ed.). PAGV-Special Report, 2017, no. 18, Wageningen, DLO Foundation, pp. 259-268.

Zoteyeva, N.M. and M.V. Patrikeeva. Phenotypic characteristics of North-West Russian populations of *Phytophthora infestans* (2003-2008). In: Schepers H.T.A.M. (ed.). PPO-Special Report, 2010, no. 14, Wageningen, DLO Foundation, pp.213-216.

http://euroblight.net/fileadmin/euroblight/Protocols/Detached_Leaf_Test.pdf