Plant Genetic Resources: Characterization and Utilization

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Insights into the genetic basis of the pre-breeding potato clones developed at the Julius Kühn Institute for high and durable late blight resistance

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Short Communication

Cite this article: Blossei J, Uptmoor R, Thieme R, Nachtigall M, Hammann T (2021). Insights into the genetic basis of the pre-breeding potato clones developed at the Julius Kühn Institute for high and durable late blight resistance. *Plant Genetic Resources: Characterization and Utilization* **19**, 461–464. https://doi.org/10.1017/S1479262121000447

Received: 12 March 2021
Revised: 11 May 2021
Accepted: 22 August 2021
First published online: 8 September 2021

Key words:
Crop wild relatives; gene pool; late blight (*Phytophthora infestans*); potato (*Solanum tuberosum* L.); resistance genes

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Abstract

Due to the high yield losses caused by late blight in potato cultivation, the development of resistant pre-breeding material is of great importance for cultivar breeding. The gene pool of the Julius Kühn Institute (JKI) includes a large collection of resistant clones whose resistance has not yet been analysed in detail with markers for relevant resistance genes. A panel of 52 pre-breeding potato clones developed at the JKI *via* interspecific crosses and highly resistant to late blight were tested for the presence of seven resistance genes (*Rpi-blb1/Rpi-sto1*, *Rpi-blb2*, *Rpi-blb3/R2/Rpi-abpt*, *R1*, *R3a*, *R3b*, *Rpi-phu1*) and one QTL allele (*QTL_phu-stn*) from *Solanum* species *S. bulbocastanum*, *S. demissum*, *S. phureja* and *S. stoloniferum*, respectively. Molecular marker assays based on sequence-specific primers revealed that 36 of the 52 pre-breeding clones carried either 1, 2, 3 or 4 resistance genes introgressed from these wild *Solanum* species. Results indicate that these resistance genes were retained over generations of breeding. Although highly resistant to late blight, 16 pre-breeding clones did not carry any of these resistance genes. Resistance in the gene pool may, thus, be based not only on individual resistance genes but also on QTL effects. Results help to better understand both inheritance and expression of late blight resistance of this unique gene pool and may be used for breeding programmes.

Introduction

Potato late blight, caused by *Phytophthora infestans* (Mont.) de Bary, leads to high yield losses worldwide (Dowley *et al.*, 2008; Wiik, 2014). At the former Institute of Potato Research (now Julius Kühn Institute (JKI), Groß Lüsewitz, Germany), a long-term pre-breeding programme for durable *P. infestans* resistance has been run since the 1950s. Initially, crosses were made with resistant wild *Solanum* species to introgress resistance into the cultivated gene pool. In particular, accessions of *S. demissum*, *S. okadae*, *S. phureja*, *S. sparsipilum*, *S. stoloniferum*, *S. tuberosum* ssp. *andigena*, *S. vernei* and *S. bulbocastanum* were used as resistant progenitors. Progenies were backcrossed several times with common cultivars to select clones combining resistance and acceptable agronomic and qualitative traits. Thus, a unique gene pool was developed over decades. By using gene-specific markers, tracing the transmission of resistance genes from wild species in the course of a breeding programme became possible. In a marker-assisted approach, we here report the presence of known late blight resistance genes in the JKI potato gene pool and draw conclusions on the genetic basis of late blight resistance in this gene pool.

Experimental

A total of 52 pre-breeding clones highly resistant to *P. infestans* were used for this study (online Supplementary Table S1). They originated from crosses carried out between 2001 and 2014 and represent higher backcross generations of BC5, BC6 or BC7. Additionally, eight common cultivars were included, five of which were described as susceptible ('Adretta', 'Belana', 'Gala', 'Krone', 'Princess') and three as moderately resistant ('Sarpo Mira', 'Alanis', 'Otolia'). Field resistance was evaluated in a randomized block design with two replications over 3 years at the JKI experimental station in Groß Lüsewitz. Plants were inoculated in early July with a *P. infestans* suspension containing races collected from the field over years. The field assessment was carried out twice a week until maturity. The relative

Table 1. Molecular markers used to detect corresponding late blight resistance genes

Gene/QTL	Origin	Marker	Forward/reverse primer	Chromosomal location, expected size (bp) of amplicon	Annealing temperature (° C)/touch down	Reference
Rpi-blb1/ Rpi-sto1	*S. bulbocastanum*	BLB 1/1′	F: CACGAGTGCCCTTTTCTGAC R: ACAATTGAATTTTTAGACTT	8, 213	60–50	Colton *et al.* (2006)
	S. stoloniferum	Sto1F/R	F: ACCAAGGCCACAAGATTCTC R: CCTGCGGTTCGGTTAATACA	8, 890	68–58	Zhu *et al.* (2012)
Rpi-blb2	*S. bulbocastanum*	BLB2F/R	F: GGACTGGGTAACGACAATCC R: AGCACGAGTTCCCCTAATGC	6, 773	60–50	Wang *et al.* (2008); Lokossou *et al.* (2010)
Rpi-blb3/R2/ Rpi-abpt	*S. bulbocastanum*	BLB3F/R	F: AGCTTTTTGAGTGTGTAATTGG R: GTAACTACGGACTCGAGGG	4, 305	60–50	Zhu *et al.* (2012)
	S. demissum	R2F/R	F: ATGGCTGATGCCTTTCTATCATTTGC R: TCACAACATATAATTCCGCTTC	4, 2500	60–50 (27 cycles)	Kim *et al.* (2012)
	S. demissum	R2-F1/R3	F: GCTCCTGATACGATCCATG R: ACGGCTTCTTGAATGAA	4, 686	50	Kim *et al.* (2012)
R1	*S. demissum*	76–2sf2/ 76-2SR	F: CACTCGTGACATATCCTCACTA R: CAACCCTGGCATGCCACG	5, 1400	60–50	Ballvora *et al.* (2002)
R3a	*S. demissum*	SHa-F/R	F: ATCGTTGTCATGCTATGAGATTGTT R: CTTCAAGGTAGTGGGCAGTATGCTT	11, 982	68–57	Huang *et al.* (2005)
R3b	*S. demissum*	R3b-F4/R5	F: GTCGATGAATGCTATGTTTCTCGAGA R: ACCAGTTTCTTGCAATTCCAGATTG	11, 378	55	Rietman (2011)
Rpi-phu1	*S. phureja*	GP94F/R	F: ATGTATCACAATCACATTCTTGCTC R: TGTAAAACCAACAAGTAGTGTTGC	9, 300	45–35	Śliwka *et al.* (2006)
QTL_phu-stn	*S. phureja*	GP198F-1/R	F: TTTGCTTACTCTTGTTGTATG R: TCACTTTGGTGCTTCTGTCG	3, 450	60–50 (25 cycles)	Wickramasinghe *et al.* (2009)

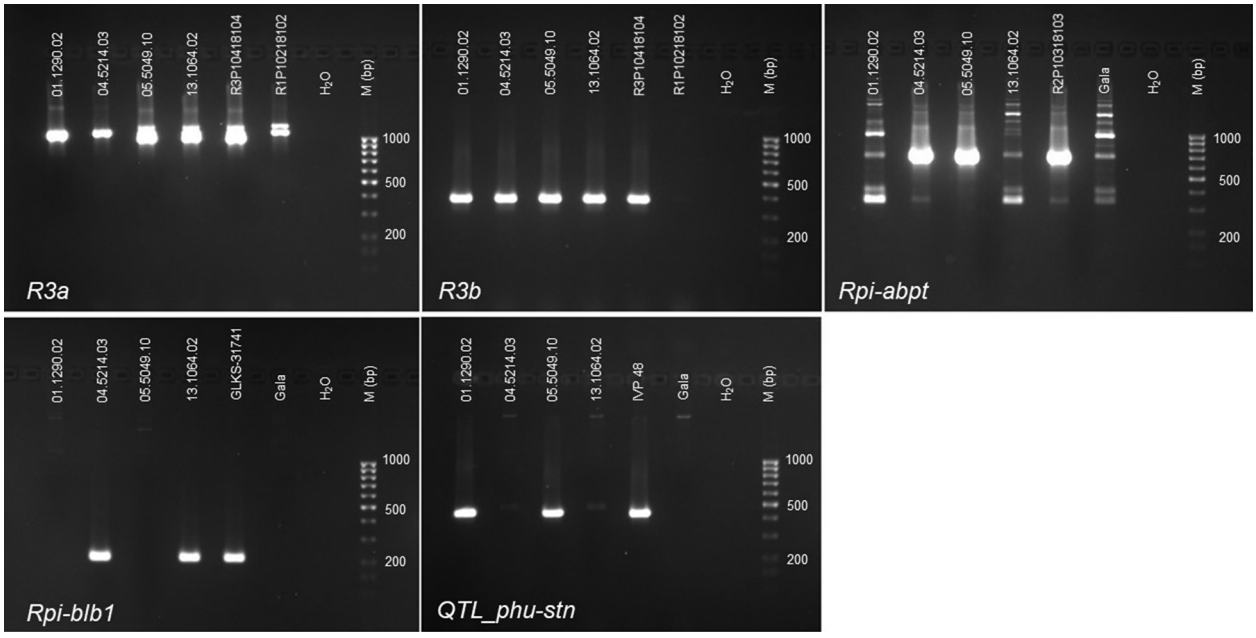

Fig. 1. Detection of *R3a*, *R3b*, *Rpi-abpt*, *Rpi-blb1* and *QTL_phu-stn* in the clones 01.1290.02, 04.5214.03, 05.5049.10 and 13.1064.02 using gene-specific markers. Every last line (M) is a 100 bp ladder (AppliChem; Darmstadt, Germany). *R3a* and *R3b*: positive control R3P10418104, negative control R1P10218102 *Rpi-abpt*: positive control R2P10318103, negative control 'Gala' *Rpi-blb1*: positive control GLKS-31741, negative control 'Gala' *QTL_phu-stn*: positive control IVP 48, negative control 'Gala'.

area under the disease progress curve was calculated and converted into scores from 1 (highly resistant) to 9 (highly susceptible) according to OEPP/EPPO (2021).

Plants for marker analysis were cultivated in a greenhouse for 4 weeks and DNA was extracted from young leaves using the DNeasy Plant Pro Kit (Qiagen; Hilden, Germany). Eleven pairs of gene-specific PCR primers for seven known resistance genes and one QTL allele were used (Table 1). These markers were selected based on the resistance genes coming from the wild progenitors. The PCR reactions of 20 µl consisted of 20 ng template DNA, 0.4 µM of each primer and 10 µl Red HS Taq Master Mix (Biozym; Hessisch Oldendorf, Germany). The PCR products were visualized by agarose gel electrophoresis.

Discussion

In total, 36 of 52 clones tested yielded PCR amplicons for up to four resistance genes for seven of the eight genes tested (online Supplementary Table S1).

The resistance gene *R1* was detected in six clones, eight clones were positive for *Rpi-blb3/R2/Rpi-abpt*, 13 for *R3a* and 27 for *R3b*. All eight cultivars contained the genes *R3a* and *R3b*, in cultivar 'Alanis' the genes *R1* and *Rpi-blb2* were observed as well.

The resistance genes *R1* to *R11* from the wild species *S. demissum* were frequently used in potato breeding due to their early discovery (Vleeshouwers *et al.*, 2011). The hypothesis that they continue to occur in many cultivars for this reason is confirmed by the present study. Markers indicative for *R3* genes were found in many clones and in all cultivars, indicating that these genes have remained for a long time in breeding germplasms after they had been overcome by the pathogen. For example, Rakosy-Tican *et al.* (2020) detected *R3a* and *R3b* in 'Quarta', 'Baltica' and 'Sapro Mira' and *R3b* in 'Romanze'.

Rpi-blb1/Rpi-sto1 was detected in four clones. According to Van der Vossen *et al.* (2003), *Rpi-blb1/Rpi-sto1* provides broad-spectrum resistance and thus makes an important contribution to broaden the genetic base for resistance.

Since *Rpi-phu1* from *S. phureja* does not appear in any of the clones, it may not have entered the gene pool or got lost by selection or genetic drift. *QTL_phu-stn* was detected in five clones. Costanzo *et al.* (2005) first described this QTL and Wickramasinghe *et al.* (2009) developed a marker. The present study is, to our knowledge, the first to investigate the presence of this QTL in potato breeding germplasm. *Rpi-blb2* was determined in only one clone, which is not surprising since crosses between *S. tuberosum* and *S. bulbocastanum* are difficult to achieve.

In the older clones from 2001 to 2003, markers for up to two genes per clone were detected. The 2004 and 2005 clones contained markers for up to four genes per clone. The most recent clones in this study showed markers for up to three genes (Fig. 1, online Supplementary Table S1).

The results indicate that the JKI potato gene pool contains resistance genes introgressed from wild species in the past, which had been maintained over generations of breeding. These genes, with exception of *R3a* and *R3b*, which were also found in susceptible cultivars, in addition to QTLs with smaller effects, are presumably involved in the high resistance properties of a large part of the gene pool. Already broken resistances inherited from *S. demissum* may still contribute to increase the resistance level (Stewart *et al.*, 2003). Additionally, it was shown that durable resistance properties of crop plants can be achieved by stacking of resistance genes (Zhu *et al.*, 2012; Haverkort *et al.*, 2016; Ghislain *et al.*, 2019; Stefańczyk *et al.*, 2020). Rogozina *et al.* (2021) found the resistance level to be correlated to the number of genes. In the present study, some clones carried just one or none of the analysed genes, whilst showing high resistance levels (online

https://doi.org/10.1017/S1479262121000447 Published online by Cambridge University Press

Supplementary Table S1). Late blight resistance of the gene pool under survey appears, thus, not solely based on individual major resistance genes, but also on quantitative effects. In a meta-analysis focused on quantitative *P. infestans* resistance, QTLs for resistance were found to be located on all 12 chromosomes (Danan *et al.*, 2011). Whether a similar situation is present in the JKI potato gene pool remains to be analysed.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262121000447

Acknowledgements. The authors thank the Federal Ministry of Food and Agriculture for funding this project and all potato breeders of the GFPi for providing cultivar material.

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