Amplicon sequencing of NBS-LRR genes conferring resistance to *Phytophthora infestans* in potato

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Phytophthora infestans is an oomycete responsible for potato late blight (Figure 1), a devastating disease that caused the Great Irish Famine in the 19th century. Nowadays the disease can be controlled by pesticides, but host resistance gains importance as an environment friendly alternative in disease management. All known genes conferring resistance to *P. infestans* (*Rpi* genes) belong to the coiled coil nucleotide-binding site leucine-rich repeat (CC-NBS-LRR) gene family. Currently, more than 70 *Rpi* genes have been identified in *Solanum* species and, nearly 50 *Rpi* genes have been cloned. However, we do not know which *Rpi* genes are present in potato cultivars, especially since the resistance conferred by many of them has been overcome by the rapidly evolving pathogen.

Aim of study

Diversity analysis of 11 potato *Rpi* genes (Table 1) in potatoes varying in ploidy and origin using an Amplicon Sequencing (AmpSeq) approach.

Materials and Methods

- 335 potato genotypes (183 potato cultivars grown in Poland and Norway, 98 breeding lines and 54 genotypes of wild potato species)
- Detection of *Rpi* genes fragments using 1-3 PCR primer pairs per gene (Table 1)
- Amplification of entire coding sequences of the *Rpi* genes, barcoding and sequencing using the PacBio single-molecule real-time (SMRT) circular consensus sequencing (CCS) method

Table 1 List of *Rpi* genes detected in potato genotypes

#	*Rpi* gene	Gene source	Reference sequence
1	*R1*	*Solanum demissum*	AF447489
2	*R2/Rpi-blb3*	*Solanum bulbocastanum*	FJ536346
3	*R3a*	*Solanum tuberosum*	AY849382
4	*R3b*	*Solanum demissum*	JF900492.1
5	*R8*	*Solanum demissum*	KU530153
6	*R9*	*Solanum demissum*	Armstrong et al. 2019
7	*Rpi-phu1 (Rpi-vnt1)*	*Solanum phureja/Solanum venturii*	FJ423044.1
8	*Rpi-blb1*	*Solanum bulbocastanum*	AY426259.1
9	*Rpi-blb2*	*Solanum bulbocastanum*	DQ122125
10	*Rpi-ber1*	*Solanum berthaultii*	MW390806.1
11	*Rpi-chc1.1*	*Solanum chacoense*	MW383255
	Rpi-chc1.2		MW410797

Results

- Using PCR primers the presence of *R3a*, *R3b*, *R1*, *Rpi-phu1*, *R2, Rpi-ber1* and *Rpi-blb1* genes were detected, so far in 69, 45, 40, 40, 24, 18 and 7 potato genotypes, respectively.
- The AmpSeq strategy is efficient, a library of, so far 243 different PCR products (7 *Rpi* genes) yielded more than 4 million reads with an average length of 4.086 bp. Sequencing of the next 4 *Rpi* genes is in progress.
- The presence of *Rpi* genes in different potato genotypes was confirmed and new variants of these genes were detected using PacBio sequencing (Figure 2).
- From 2 to 14 amplicon sequence variants (ASVs) per *Rpi* gene (including pseudogenes) were detected in 243 potato genotypes (Figure 2).
- The highest number of ASVs were detected in wild potato species (26), the lowest number of ASVs were detected in breeding lines (14; Figure 3).
- From 1 to 5 ASVs were obtained per potato genotype.
- The highest number of ASVs per gene was obtained for *R3a*, in total 14, while the lowest number of ASVs was obtained for the *R1* gene (Figure 2).
- The highest numbers of ASVs of different *Rpi* genes were detected in potato cultivars Escort (*R1*, *Rpi-abpt*^{T86}, *R3a*, *R3b*, *R3b*^{G3111}), Klepa (*R1*, *R2-like*, *R3a-like*, *Rpp13-like*, *R3b*^{G3111}) and Rudawa (*Rpi-abpt*^{T86}, *R3a*, *R3a-like*, *R3b*, *R3b*^{G3111}).

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Figure 1 The symptoms of late blight (*Phytophthora infestans*) infection in potato leaves

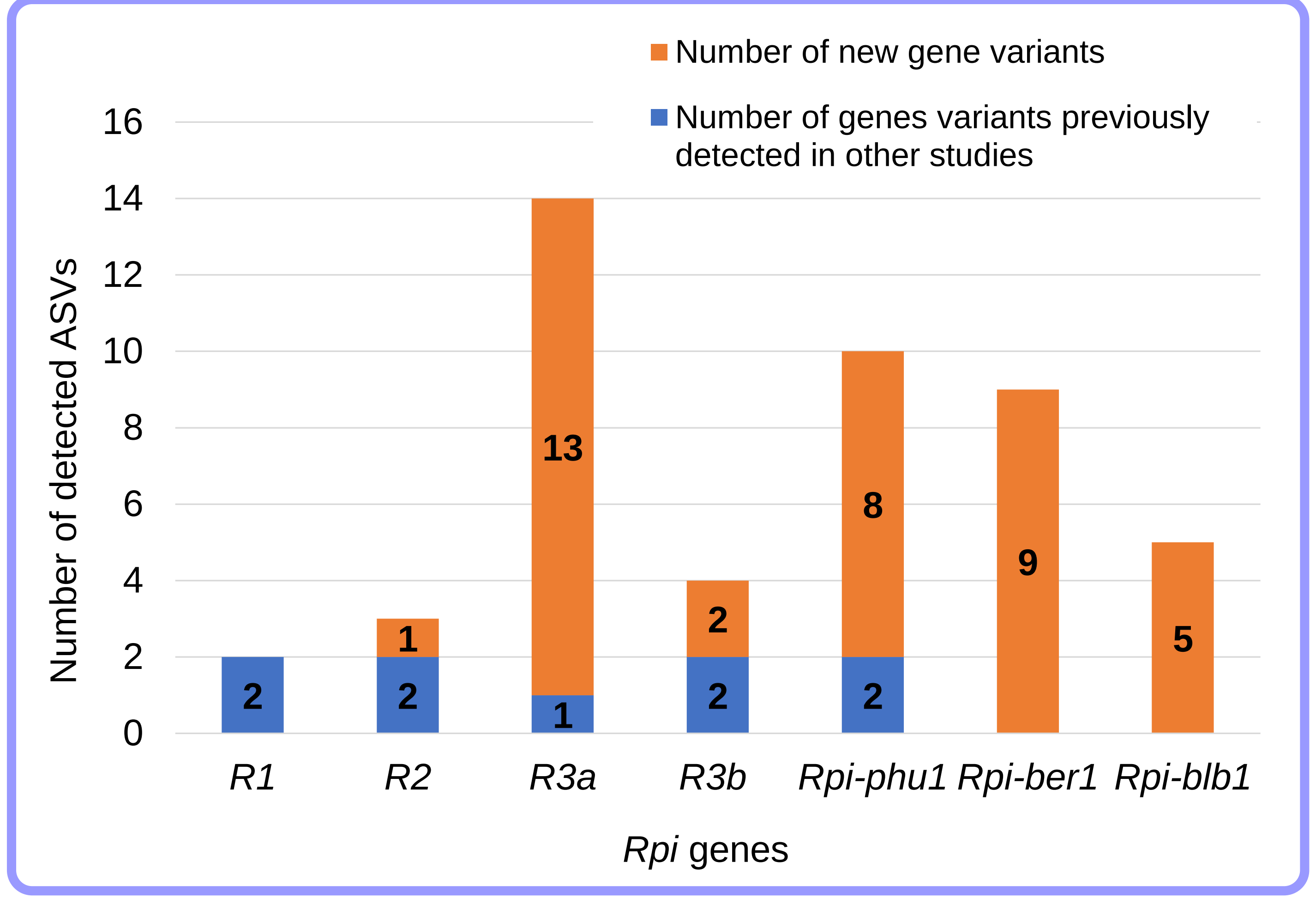

Figure 2 Number of detected ASVs, using PacBio single-molecule real-time (SMRT) circular consensus sequencing (CCS) method

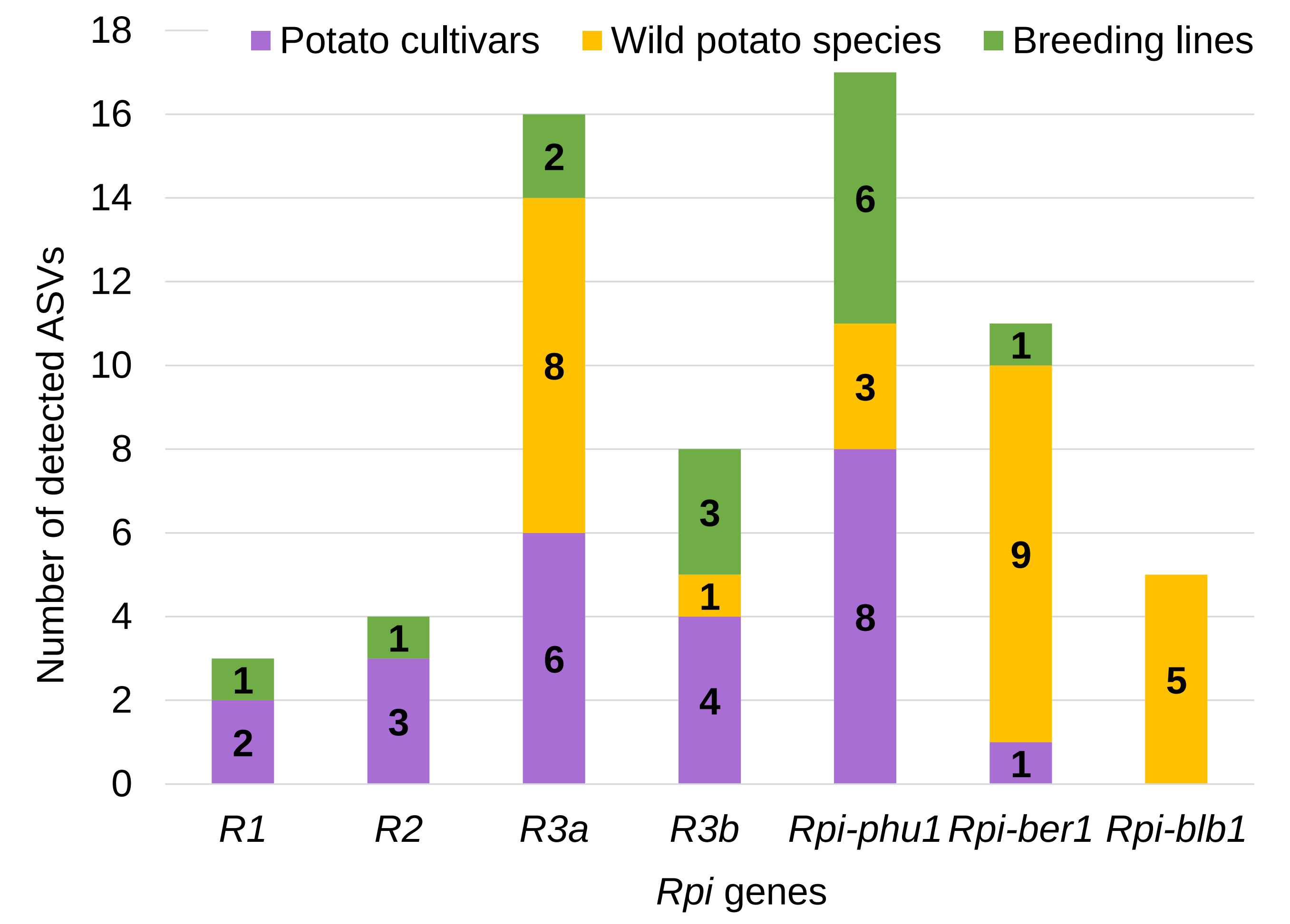

Figure 3 Number of detected ASVs in potato cultivars, wild potato species and breeding lines

Conclusions/Future plans

- The most frequently detected *Rpi* gene was *R3a*, found in 69 potato genotypes. This was also the most diverse gene.
- The least frequently detected *Rpi* gene found in 7 potato genotypes was *Rpi-blb1*.
- The least diverse gene among the 7 sequenced was the *R1* gene.
- Data on the distribution and sequence diversity of the *Rpi* genes can lead to discovery of new *Rpi* variants and facilitate breeding.
- Further sequencing of the *Rpi* genes is in progress.