

High-throughput analysis of *Rpi* genes in potato cultivars, breeding lines and wild *Solanum* species

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Phytophthora infestans causes late blight (Figure 1), a highly destructive potato disease which has challenged global agriculture for centuries. Genes involved in resistance to *P. infestans* (*Rpi* genes) have been discovered in wild potato species (*Solanum* spp.), and some of these *Rpi* genes have been introduced into potato cultivars. All known genes conferring resistance to *P. infestans* 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 the many different potato genotypes, especially since the resistance conferred by many of them has been overcome by the rapidly evolving pathogen.



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

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

#	<i>Rpi</i> gene	Gene source	Reference sequence
1	<i>R1</i>	<i>Solanum demissum</i>	AF447489
2	<i>R2/Rpi-blb3</i>	<i>Solanum bulbocastanum</i>	FJ536346
3	<i>R3a</i>	<i>Solanum tuberosum</i>	AY849382
4	<i>R3b</i>	<i>Solanum demissum</i>	JF900492.1
5	<i>R8</i>	<i>Solanum demissum</i>	KU530153
6	<i>R9</i>	<i>Solanum demissum</i>	Armstrong et al. 2019
7	<i>Rpi-phu1 (Rpi-vnt1)</i>	<i>Solanum phureja/Solanum venturii</i>	FJ423044.1
8	<i>Rpi-blb1</i>	<i>Solanum bulbocastanum</i>	AY426259.1
9	<i>Rpi-blb2</i>	<i>Solanum bulbocastanum</i>	DQ122125
10	<i>Rpi-ber1</i>	<i>Solanum berthaultii</i>	MW390806.1
11	<i>Rpi-chc1.1</i>	<i>Solanum chacoense</i>	MW383255
	<i>Rpi-chc1.2</i>		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, each of two libraries (120 and 123 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 3).
- From 2 to 14 amplicon sequence variants (ASVs) per *Rpi* gene (including pseudogenes) were detected in 243 potato genotypes (Figure 3).
- The highest number of ASVs was detected in wild potato species (26), the lowest number of ASVs were detected in breeding lines (14; Figure 4).
- 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 3).
- Highest numbers 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}).

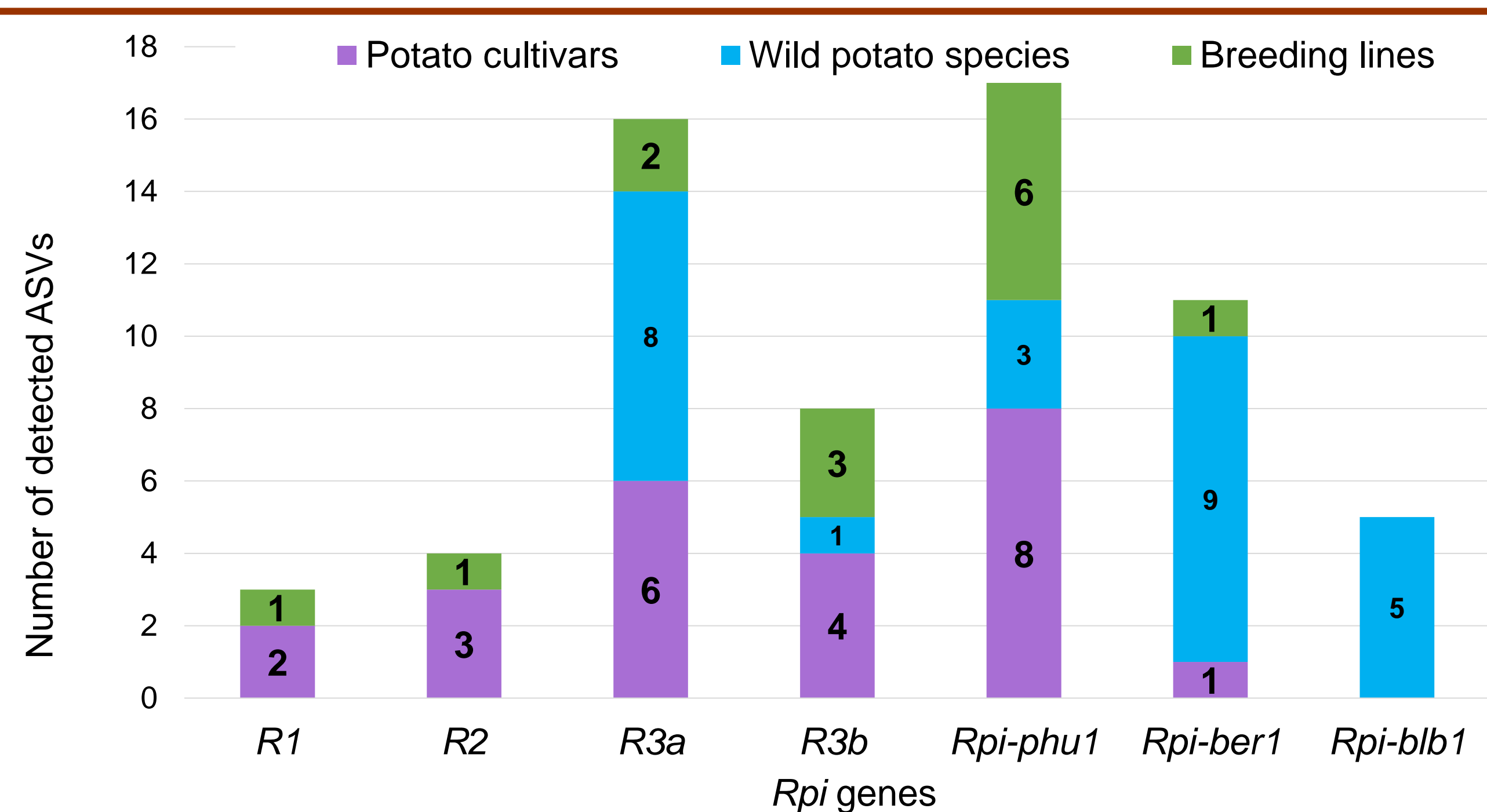


Figure 4 Number of detected ASVs in potato cultivars, wild potato species and breeding lines

AIM: 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 (Figure 2), 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.

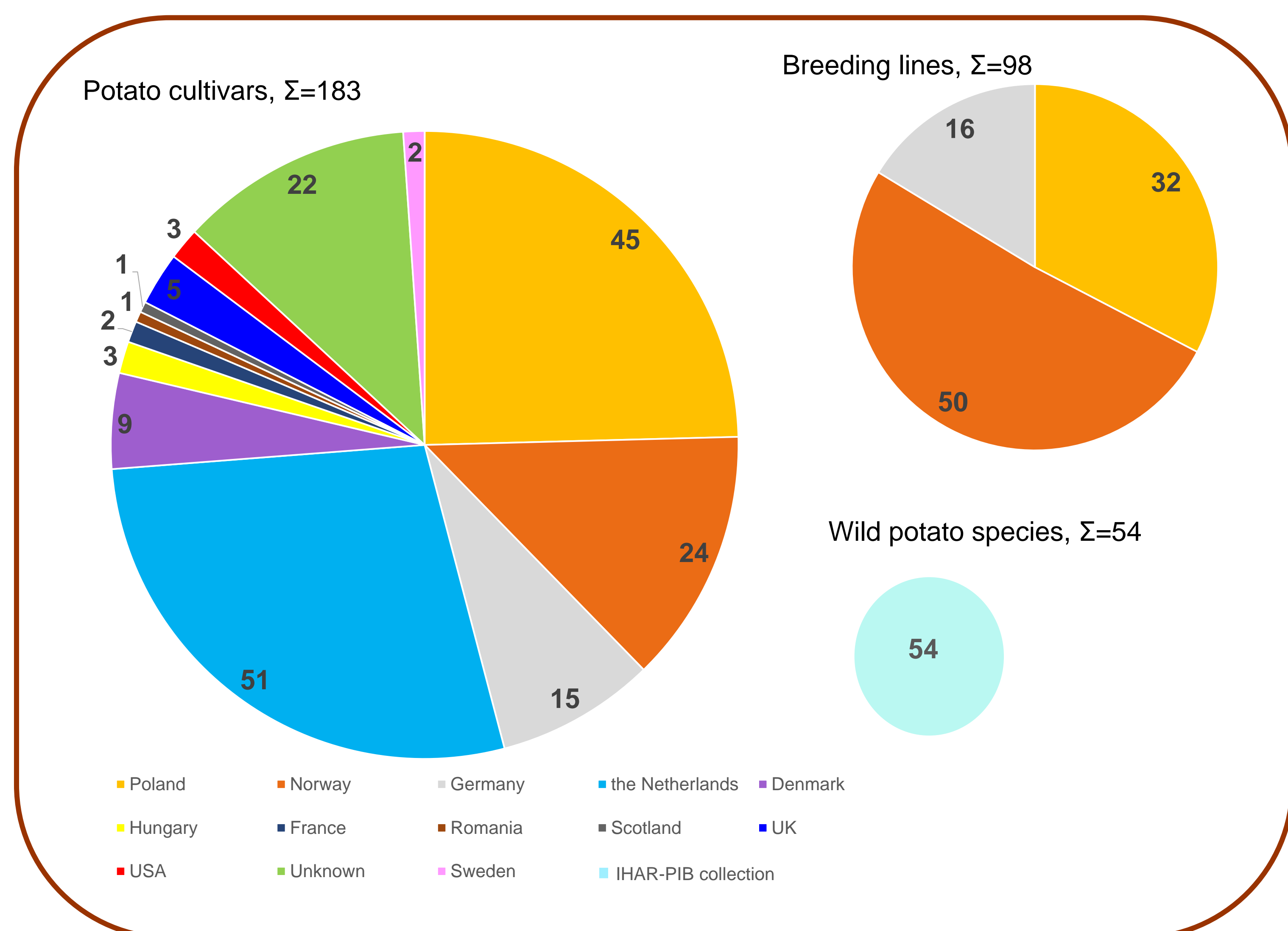


Figure 2 Origin of the potato genotypes used in this study

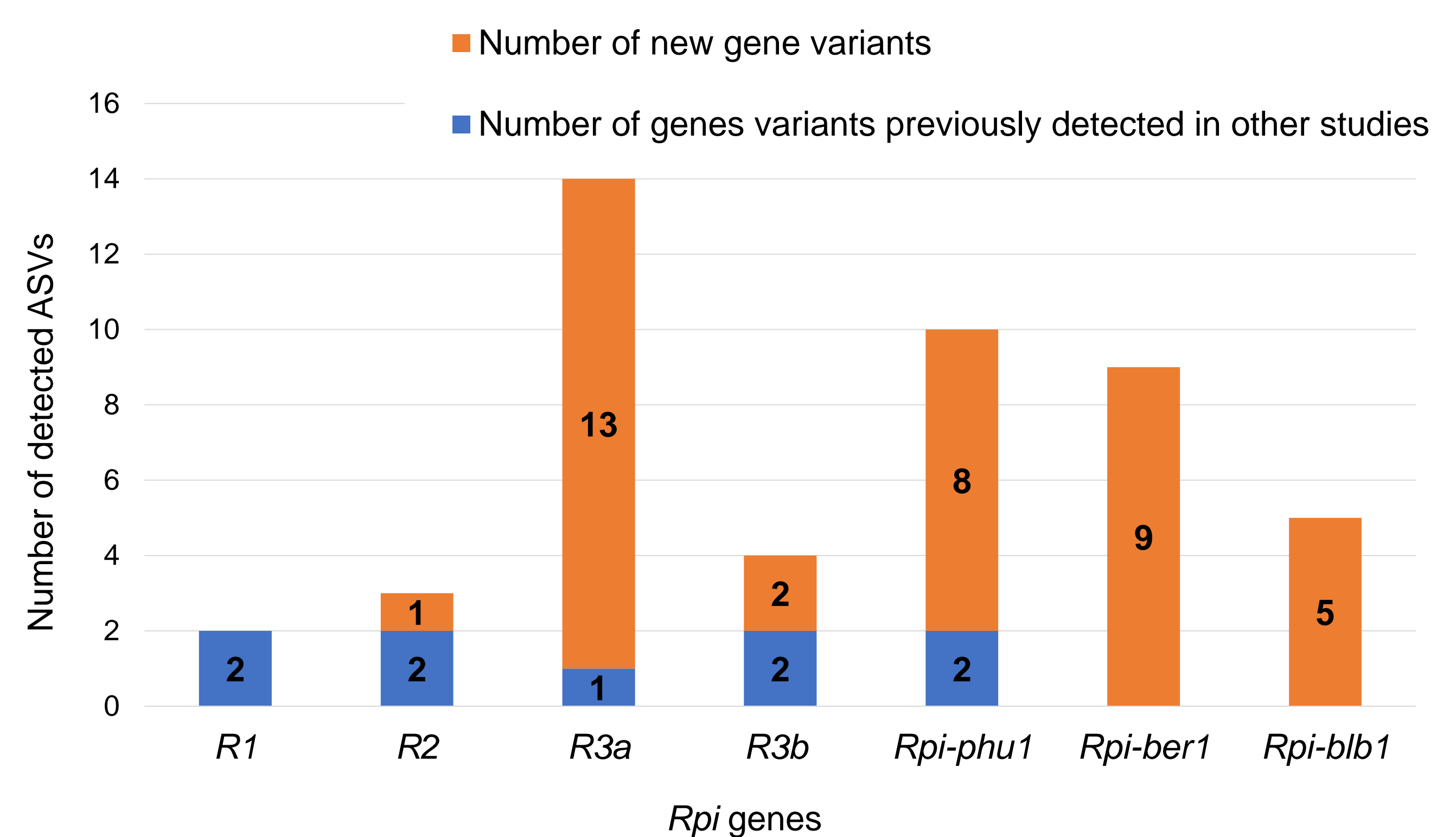


Figure 3 Number of detected ASVs, using PacBio single-molecule real-time (SMRT) circular consensus sequencing (CCS) method

CONCLUSION / 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.

Norway grants



The research leading to these results has received funding from the Norwegian Financial Mechanism 2014-2021, project DivGene: UMO-2019/34/H/NZ9/00559