

# Diversity analysis of *Rpi-ber1* and *Rpi-vnt1* genes determining broad-spectrum resistance to *Phytophthora infestans*

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*Phytophthora infestans* is an oomycete that causes the infamous potato late blight disease. Costs associated with crop losses and chemical control of late blight are estimated to be more than € 9 billion per year. Wild *Solanum* species are valuable sources of genes conferring resistance to *P. infestans* (*Rpi*). To date more than 70 *Rpi* genes have been discovered in potato and wild *Solanum* species. Many *Rpi* genes have become ineffective due to the rapidly evolving pathogen, but genes such as *Rpi-ber1* from *Solanum berthaultii* and *Rpi-vnt1* (*Rpi-phu1*) from *Solanum venturii* and from still confer resistance to many *P. infestans* strains (Figure 1).

**AIM:** Diversity analysis of *Rpi-ber1* and *Rpi-vnt1* (*Rpi-phu1*) genes in potatoes using amplicon sequencing (AmpSeq) approach.

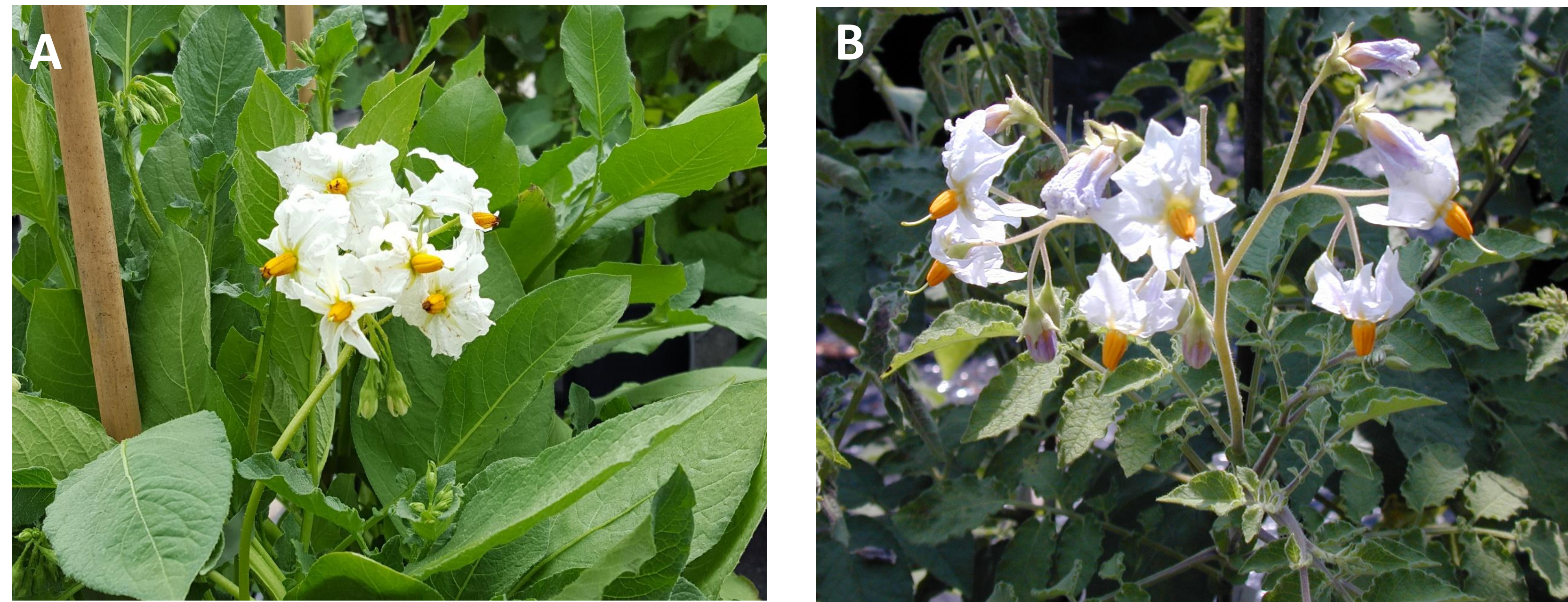
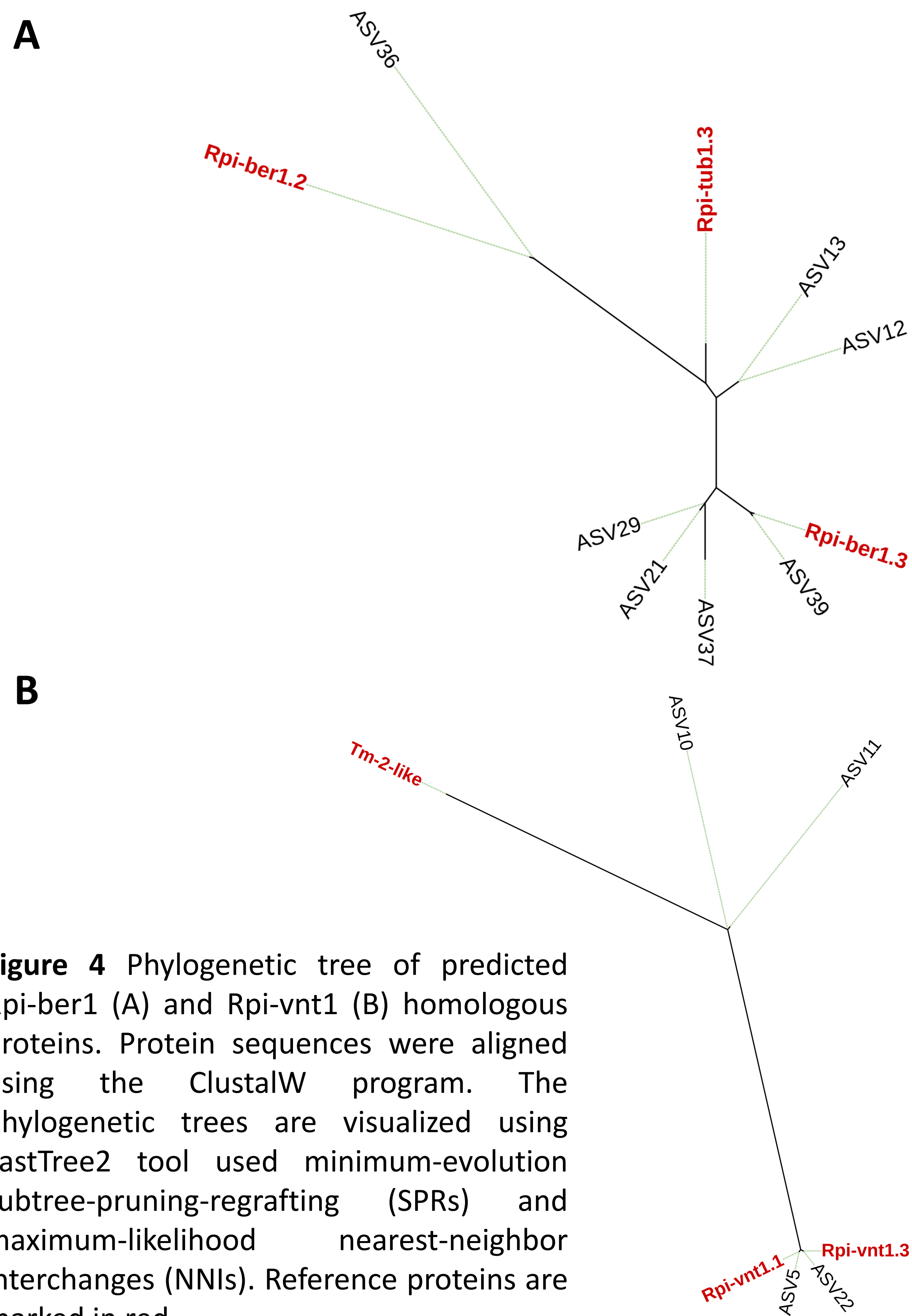


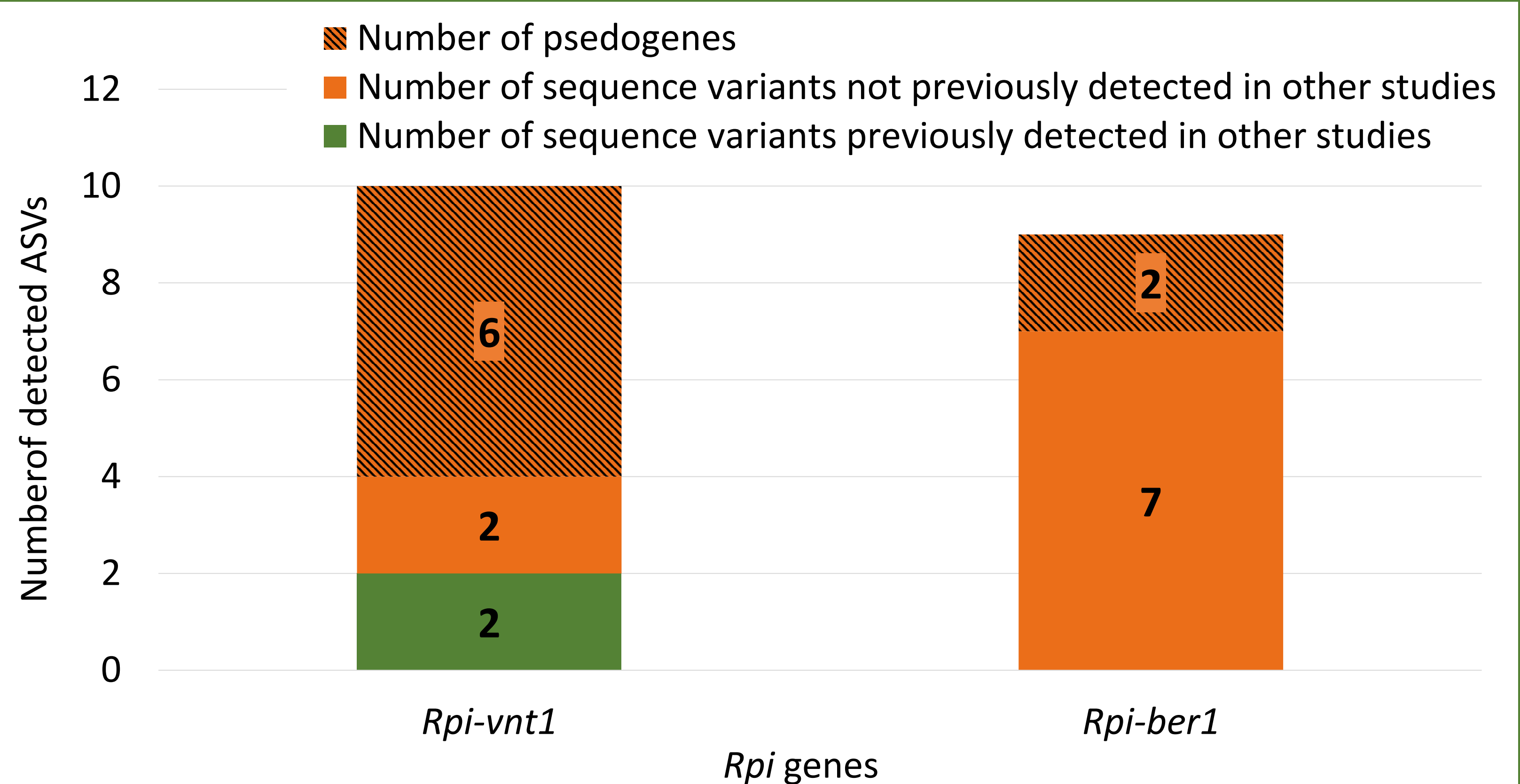
Figure 1 *Solanum venturii* (A) and *Solanum berthaultii* (B) <https://ics.hutton.ac.uk/germinate-cpc/#/home>

## MATERIALS AND METHODS:

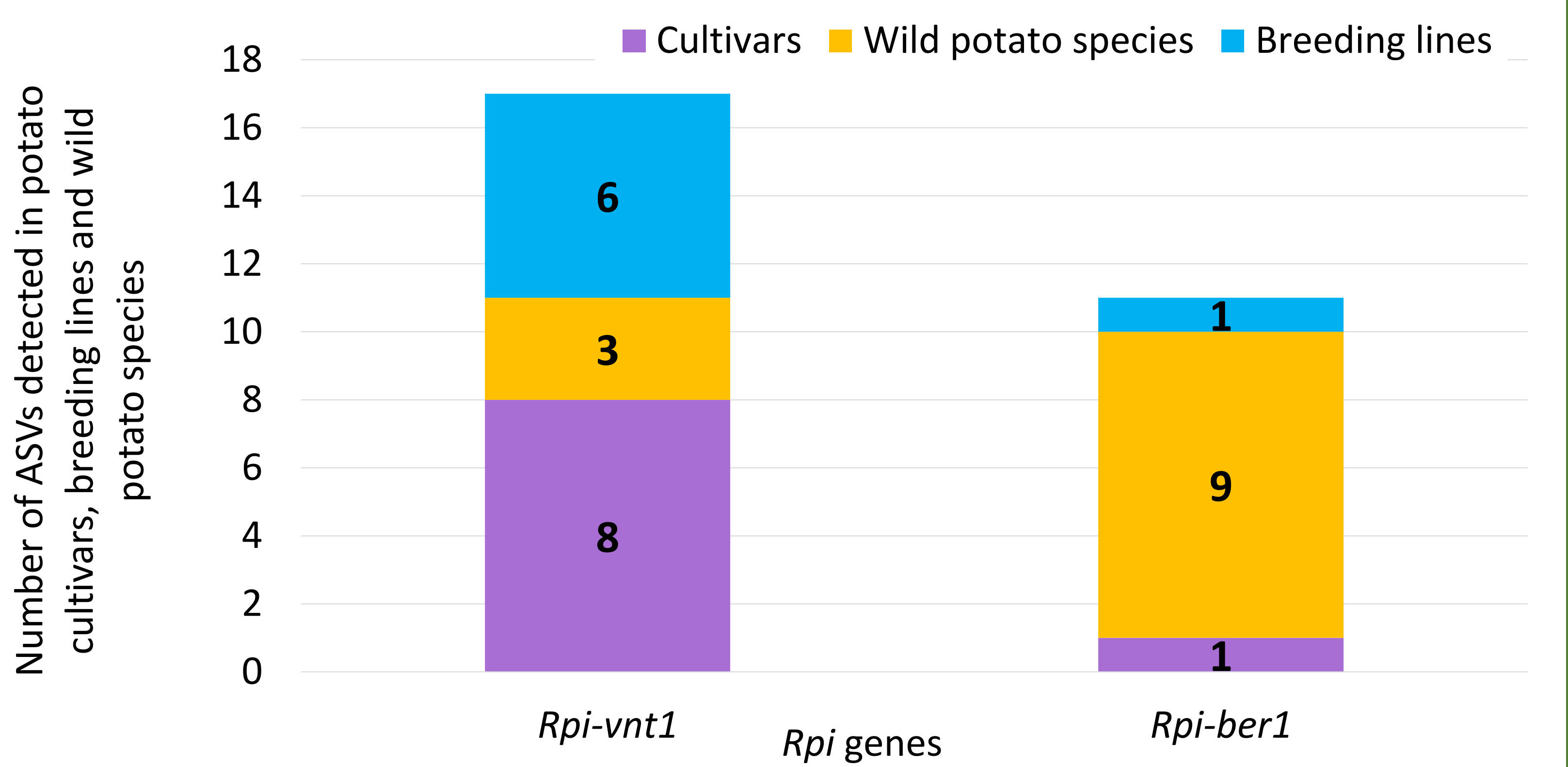
- 335 potato genotypes (183 potato cultivars grown in Poland and Norway, 98 breeding lines and 54 genotypes of wild potato species).
- Detection of *Rpi* genes fragments using 1-3 PCR primer pairs per gene.
- Amplification of entire coding regions of the *Rpi* genes and sequencing using the PacBio single-molecule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



**Figure 4** Phylogenetic tree of predicted *Rpi-ber1* (A) and *Rpi-vnt1* (B) homologous proteins. Protein sequences were aligned using the ClustalW program. The phylogenetic trees are visualized using FastTree2 tool used minimum-evolution subtree-pruning-regrafting (SPRs) and maximum-likelihood nearest-neighbor interchanges (NNIs). Reference proteins are marked in red.



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



**Figure 3** Number of amplicon sequence variants (ASVs) detected in potato cultivars, wild potato species and breeding lines

## RESULTS & CONCLUSIONS:

- Using PCR primers, the presence of *Rpi-ber1* and *Rpi-vnt1* genes was detected in 47 and 78 potato genotypes, respectively.
- So far, full length *Rpi-ber1* and *Rpi-phu1* genes, were sequenced from 17 and 40 potato genotypes, respectively.
- For the *Rpi-vnt1*, 10 amplicon sequence variants (ASVs) were detected, of which 8 were new variants not previously detected in other studies (including 6 pseudogenes; Figure 2).
- For the *Rpi-ber1*, 9 new ASVs were detected (including 2 pseudogenes; Figure 2).
- 9 ASVs in wild potato species and 8 ASVs in potato cultivars were detected for *Rpi-ber1* and *Rpi-vnt1* genes, respectively (Figure 3).
- We confirmed the presence of the *Rpi-vnt1.1* and *Rpi-vnt1.3* in 14 potato genotypes.
- In 7 potato genotypes, 2 variants of *Rpi-vnt1* showed approx. 88% protein identity to the Tm-2-like from *S. verrucosum* and approx. 86% identity to *Rpi-vnt1* protein (Figure 4).
- Rpi-ber1* variants showed >99% identity to *Rpi-ber1.2* protein (ASV36) and >98% identity to *Rpi-ber1.3* (ASV21, 29, 37 and 39) and *Rpi-tub1.3* (ASV12 and 13) (Figure 4).
- Rpi-vnt1-like* pseudogenes are widespread (6 variants detected in more than half of the tested genotypes).
- Rpi-ber1-like* pseudogenes were detected in 11 potato genotypes.
- Rpi-ber1-like* genes were found in *S. punae*, *S. albicans*, *S. arrac-papa*, *S. sparsipilum* and *S. kurtzianum*. These wild potato species may be new sources of resistance to *P. infestans*.
- The AmpSeq strategy proved to be reliable and efficient and will allow us to obtain data on the diversity of genes crucial for the potato defence against *P. infestans*.

### REFERENCES:

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Foster SJ, et al. 2009. *Rpi-vnt1.1*, a *Tm-2(2)* homolog from *Solanum venturii*, confers resistance to potato late blight. Mol Plant Microbe Interact. 22(5):589-600.

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