PacBio amplicon sequencing of *Ry$_{sto}$* homologues in wild potato species

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Potato virus Y (PVY) is among the top ten economically important plant viruses. It causes potato tuber necrotic ringspot disease, leading to decreased yield and tuber quality. Wild relatives of potato are valuable sources of genes involved in resistance to many pathogens that attack potatoes, including PVY. The gene *Ry$_{sto}$* derived from *Solanum stoloniferum* (Fig. 1) confers extreme resistance to PVY.

AIM:To screen *Ry$_{sto}$* homologues and to analyze their diversity in wild relatives of potato.

PLANT MATERIAL: 298 genotypes representing 29 accessions of 26 tuber-bearing *Solanum* species, IHAR-PIB's collection.

METHOD: PacBio amplicon sequencing of the full coding sequences of *Ry$_{sto}$* homologues using barcoded primer pairs V, U and T (Fig. 2). The sequencing service was provided by the Norwegian Sequencing Centre (www.sequencing.uio.no).

Fig. 1 *Solanum stoloniferum* donor of gene *Ry$_{sto}$* (https://ics.hutton.ac.uk/germinate-cpc/#/home)

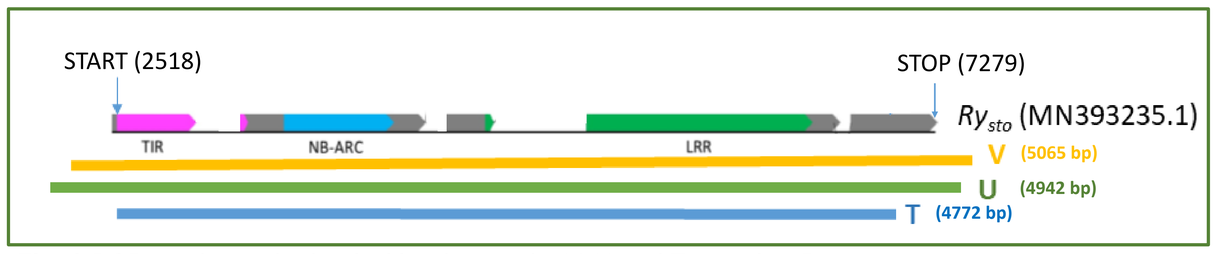

Fig. 2 PCR products obtained with primer pairs V, U and T covering *Ry$_{sto}$* gene

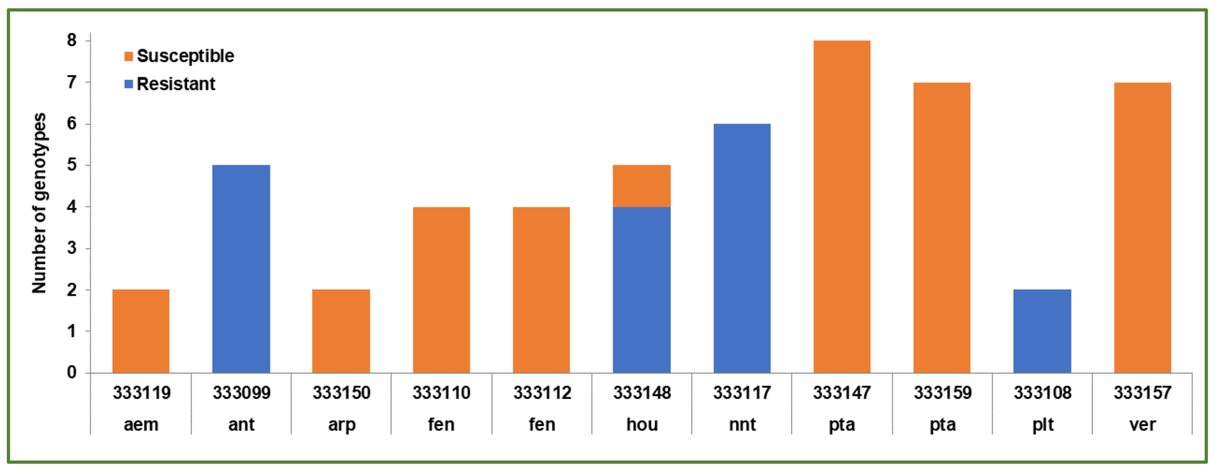

Fig. 3 Resistance of tuber-bearing *Solanum* species to potato virus Y (PVY). aem, *S. aemulans*; ant, *S. antipovichii*; arp, *S. arrac-papa*; fen, *S. fendleri*; hou, *S. hougasii*; nnt, *S. neoantipovichii*; pta, *S. papita*; plt, *S. polytrichon*; ver, *S. verrucosum*.

RESULTS:

- A total of 55 unique *Rysto-like* sequences were identified in 72 genotypes representing 12 accessions of ten tuber-bearing Solanum species and six resistant controls (potato cultivars Alicja, Bzura, Hinga, Nimfy, White Lady and breeding line PW363) (Figs. 1 and 3).
- From 1 to 13 *Rysto-like* sequences were detected per *Solanum* sp. accession, between 1 and 8 *Rysto-like* variants were detected per potato genotype. In resistant controls, the number of *Rysto-like* sequences varied from 1 to 3.
- Nucleotide identity of the obtained 55 *Rysto-like* sequences to that of the reference *Ry$_{sto}$* gene (MN393235.1, Grech-Baran et al. 2020) ranged from 89.87% to 99.98%. The *Rysto-like* sequences originated from primer pair T showed higher levels of nucleotide diversity than that originated from primer pairs V and U, with the greatest diversity found in the third intron (Fig. 4).
- In total 45 unique protein sequences were predicted (Fig. 5). Two of them, Rysto-like26 identified in potato cultivars Alicja, Bzura, White Lady and Rysto-like16 in potato clone PW363, showed 100% identity to the Ry$_{sto}$ reference protein. Identity of the remaining 43 predicted Rysto-like proteins to the reference protein ranged from 76.41 to 99.92 %. TIR and NB-ARC domains showed the highest level of conservation, while LRR and C-JID domains were found to be most variable.

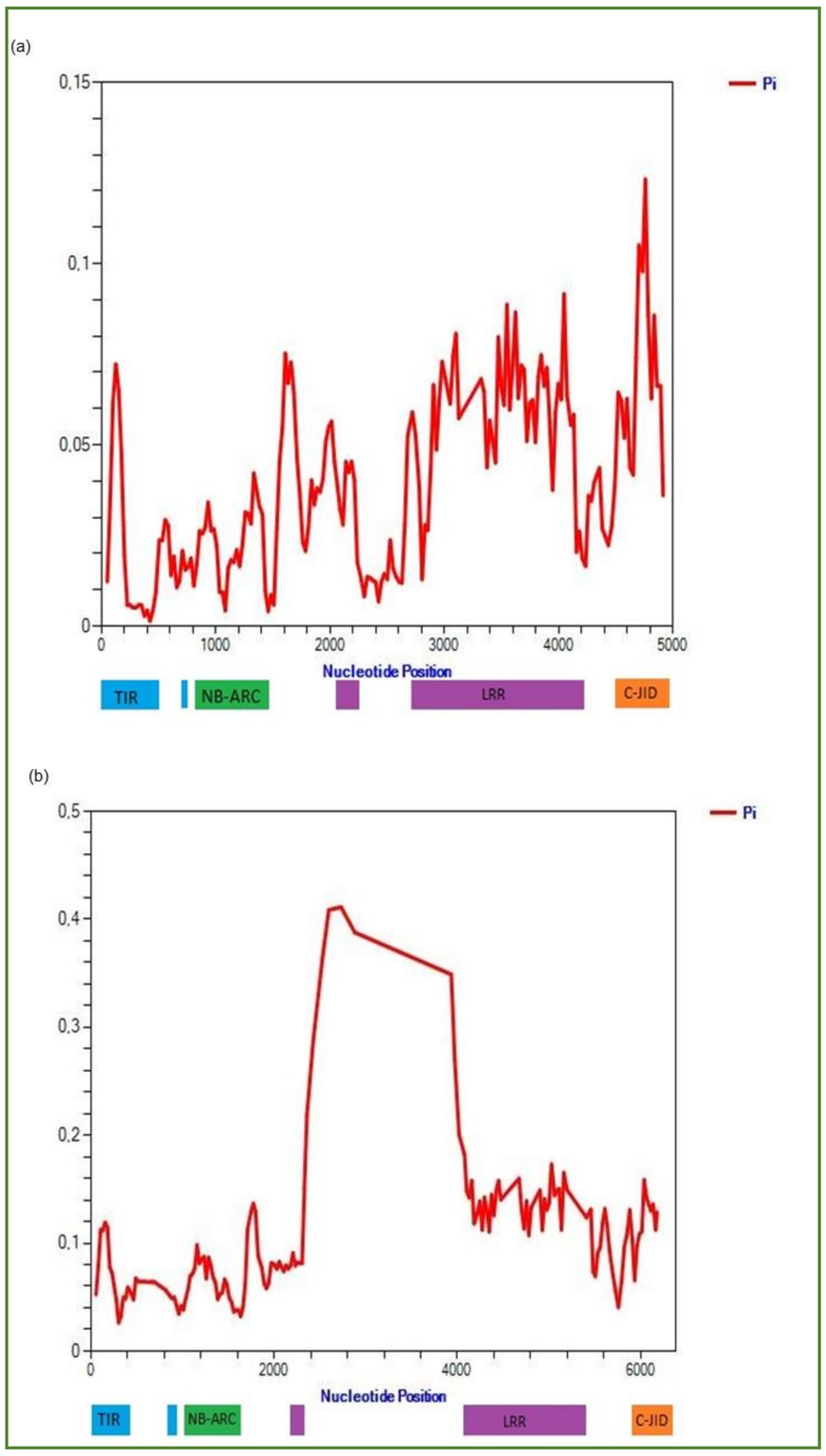

Fig. 4 Nucleotide diversity (Pi) of the *Ry$_{sto}$* homologues. Pi values range between 1 (very diverse) and 0 (conserved). (a) 25 variants obtained with U and V primers; (b) 30 variants obtained with T primer (without the ATG start codon). N-terminal domain homologous to the Drosophila Toll domain and human interleukin-1 receptor (TIR; blue); leucine-rich repeats (LRR; purple); nucleotide-binding domain (NB-ARC; green); C-terminal jelly roll/Ig-like domain (C-JID; orange).

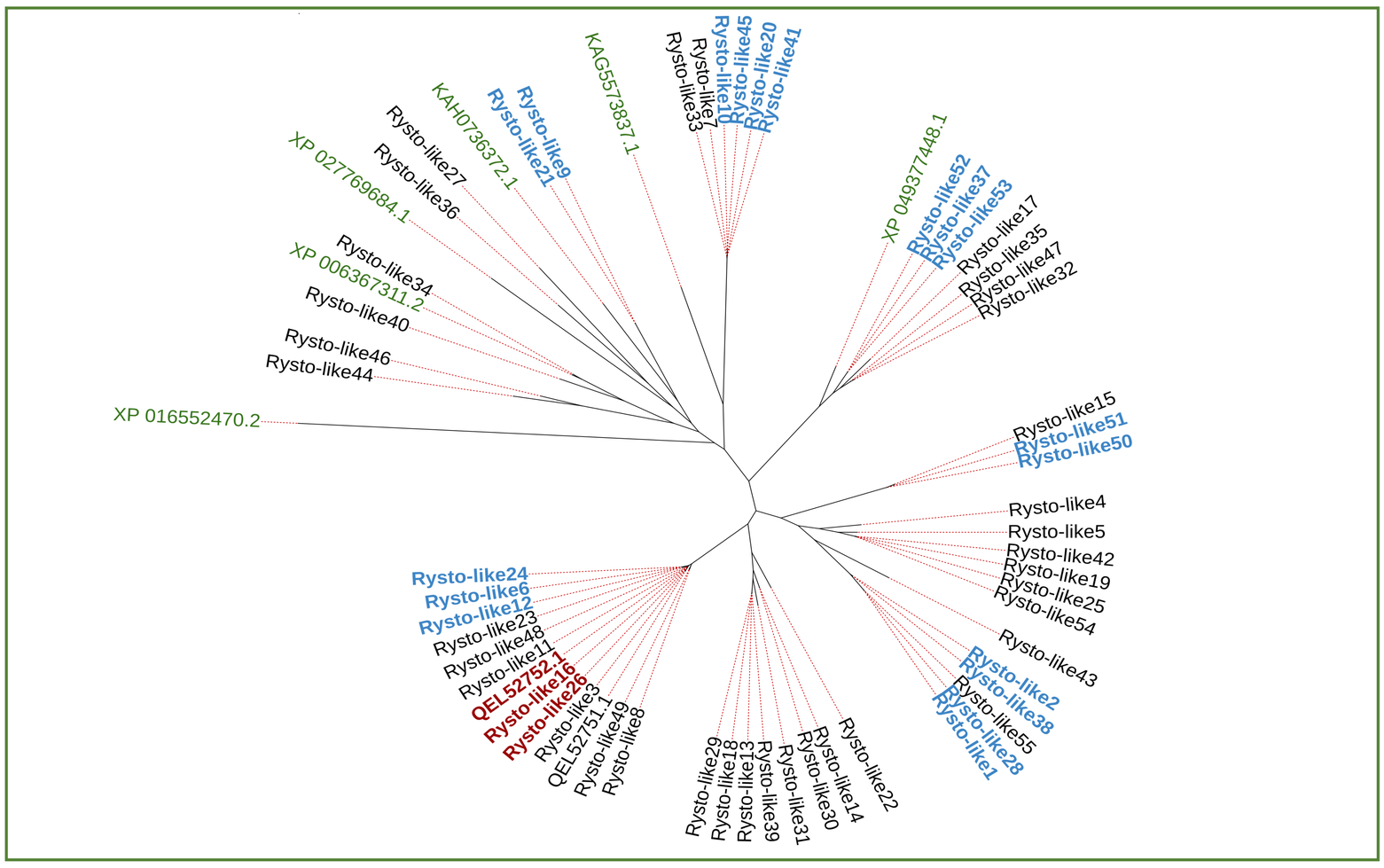

Fig.5 Phylogenetic tree of Ry$_{sto}$ homologous proteins. Ry$_{sto}$ protein and the obtained Rysto-like proteins with 100% identity are marked in bold (red). The Rysto-like homologous proteins with 100% identity to each other within a branch are marked in bold (blue). Other proteins from *Solanaceae* are marked with green. Protein sequences were aligned using the ClustalW program. The phylogenetic tree is constructed using FastTree2 tool used minimum-evolution subtree-pruning-regrafting (SPRs) and maximum-likelihood nearest-neighbor interchanges (NNIs). XP016552770.2 - *Capsicum annum* /disease resistance protein Roq1-like; XP006367311.2 - *S. tuberosum*/TMV resistance protein-like; XP027769684.1 - *S. pennellii*/TMV resistance protein-like; KAH0736372.1 *S. tuberosum*/hypothetical protein; KAG5573837.1 - *S. commersonii*/hypothetical protein; XP049377448.1- *S. stenotomum*/disease resistance protein Roq1-like; QEL52751.1/QEL52752.1- *S. stoloniferum* / Ry$_{sto}$ protein – dominant/less abundant isoform.

CONCLUSION:

- This study confirmed the presence of *Ry$_{sto}$* gene in potato cultivars Alicja, Bzura, White Lady and breeding line PW363. Two variants were found in Alicja in addition to variant described by Grech-Baran et al. (2020).
- Higher levels of diversity of the *Rysto-like* sequences were found in the wild relatives of potato than in the tested potato clone and cultivars.
- Inter-species and inter-genotype variability of the *Rysto-like* sequences in the studied tuber-bearing *Solanum* species were observed, in respect to the number of variants identified, composition of the variants and content of individual variants.

References:
Grech-Baran M, Witek K, Szajko K, Witek AI, Morgiewicz K, Wasilewicz-Flis I, Jakuczun H, Marczewski W, Jones JDG, Hennig J. (2020) Extreme resistance to Potato virus Y in potato carrying the Rysto gene is mediated by a TIR-NLR immune receptor. Plant Biotechnol J. 18:655-667.

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