Generation of CRISPR/Cas9-Based Gene Editors to Inactivate Susceptibility Genes to Potato Virus Y (PVY) in Potato (*Solanum tuberosum*) cv. Patagonia

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Solanum tuberosum is one of the most important crops for human consumption. The local variety Patagonia-INIA significantly supplies the local market, with optimal use for oven cooking. Patagonia stands out for its resistance to oomycetes but is susceptible to Potato virus Y (PVY), which utilizes the molecular translation machinery to carry out its infection processes and is the main virus affecting the species. To multiply, PVY hijacks and utilizes initiation of translation factors (eIFs), so mutations in eIFs could generate resistant individuals; unfortunately, natural mutations in eIF genes have not been described in potato. The emergence of editing (GE) tools such as CRISPR/Cas9 makes feasible to obtain PVY-resistant potato genotypes by this technique. In this work we aimed the development of GE in Patagonia due to its relevance. Previously, we characterized the complete set of eIF4E candidate genes (named eIF4E1, eIF4E2, eIF(iso)4E, and nCBP); from these and considering very recent findings, GE by double cut of the eIF4E1 isoform was prioritized. Guide RNA (gRNA) couples for eIF4E1 were designed and cloned into pVH-X vectors; a home-made technology based on T-DNA modified plasmids containing geminivirus genome modules and expression cassettes for Cas9, GFP, hygromycin resistance, and multiple gRNAs. The functional evaluation of these vectors and gRNA couples was carried out by transient leaf agroinfiltrations using the EHA105 strain. Infections were monitored by GFP expression, and 7 days post-infection (the higher reporter expression), explants were selected for target gene analysis. Among the evaluated vectors, we identified one construct able to induce a clear double cut in the Patagonia eIF4E1 gene. This identified gene editor construct allowed for long-term experimentation from which completely regenerated have been already obtained. Propagation and molecular analyses of these materials are currently under progress towards the identification of eventually PVY-resistant Patagonia individuals.

Materials and methods

Fig 1. Gene editors for virus susceptibility genes.

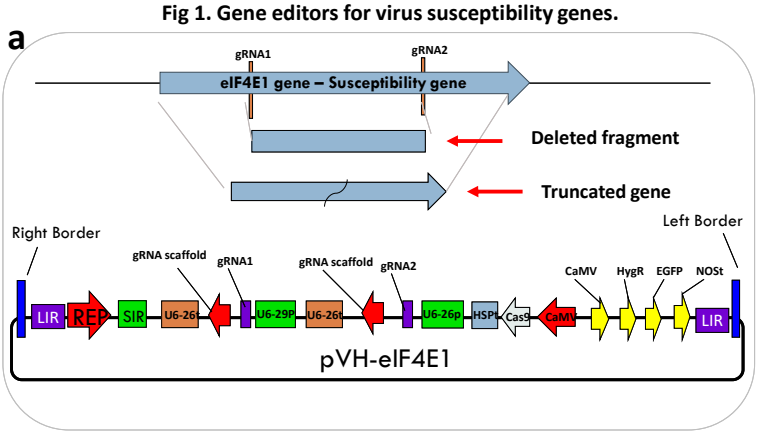
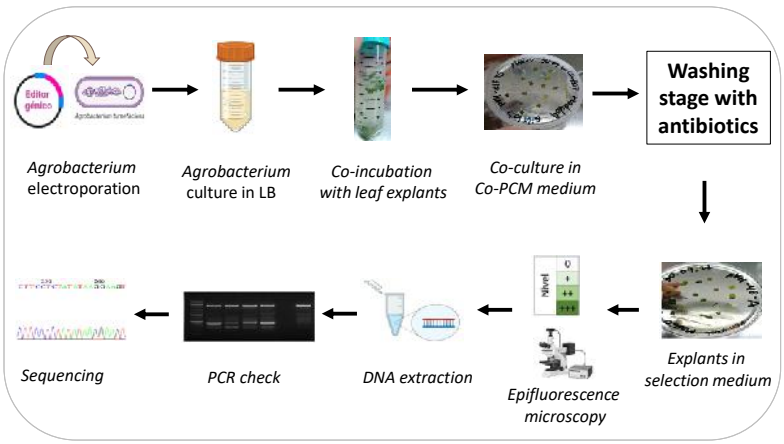
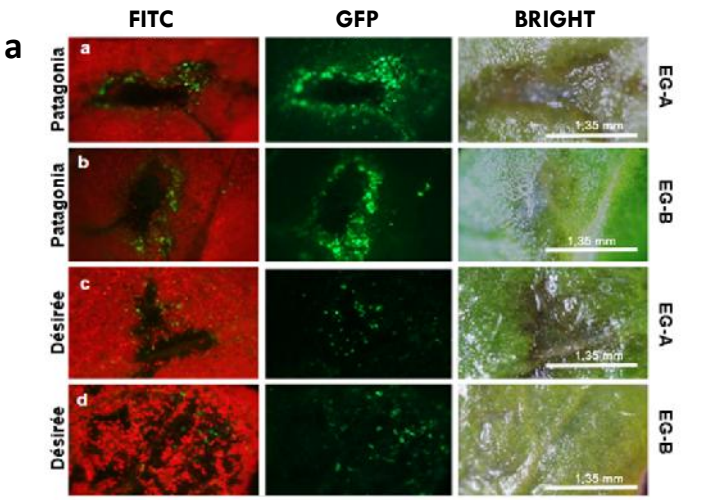


Fig 2. *Solanum tuberosum* gene transfer process



Results



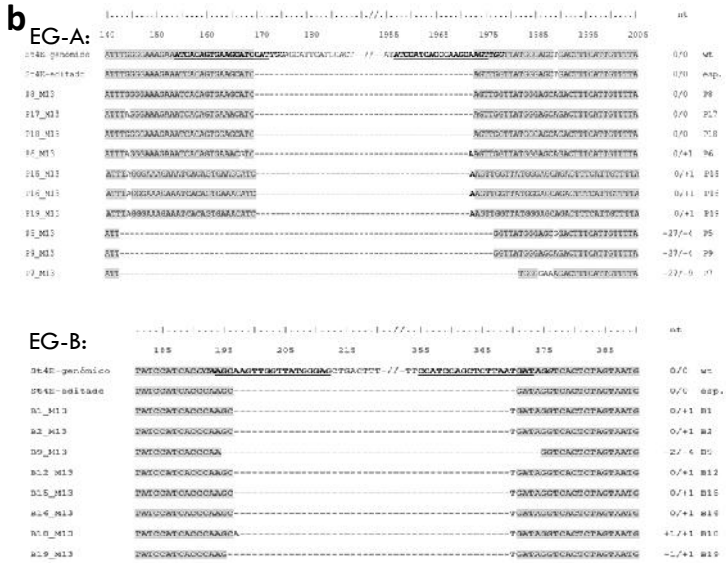


Fig 3. Evaluation of gene editors. a) Visual assessment of GFP expression in Patagonia explants. **b)** Sequencing of the amplicons derived from PCR amplification of eIF4E1 gene with both gene editors.

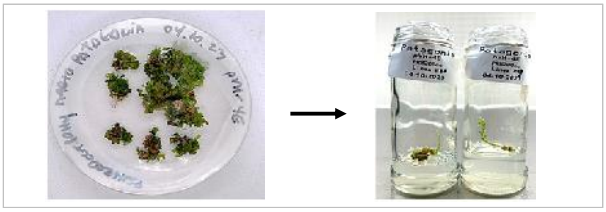


Fig 4. Regeneration of explants. Putative individuals for the double cut edited eIF4E1 gene.

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