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Proyecto: Improving heritable virus-induced gene editing (VIGE): how to select the edited seeds?

CRISPR-Cas systems have become pivotal tools for precise genome editing and gene expression regulation. This technology is highly promising in crop science; enabling the creation of more productive and nutritious plant varieties that are resistant to pests and diseases, and better adapted to evolving environmental conditions. In the recent years, the use of viral vectors to facilitate CRISPR-Cas has emerged as a powerful tool for plant genome editing, with advantages, such as high efficiency and a simple DNA-free processing to generate gene-edited plants. However, germline plant cells have specific mechanisms to interfere with viral replication and avoid virus transmission to the next generation. This phenomenon prevents the possibility to obtain seeds carrying the desired edits directly from parental infected individuals. However, in the last years, some strategies have been developed towards overpassing this natural barrier. For this aim, it is important to have an easy reporting system that allows to select edited seeds. We will use plants that constitutively express a fluorescent reporter protein, dsRED. Targeting this gene with the corresponding guide RNA delivered by viral vectors can be qualitative analyzed by observing the plant tissues under a stereomicroscope, including both flowers and seed (Figure 1). In this way, the edited seeds can be selected and germinated in soil. The aim of this work is to develop a multiplexing strategy in which the genes of interest are targeted along the reporter dsRED gene to facilitate selection of edited seeds and avoid tissue culture techniques to produce the desired CRISPR-Cas edited plants.

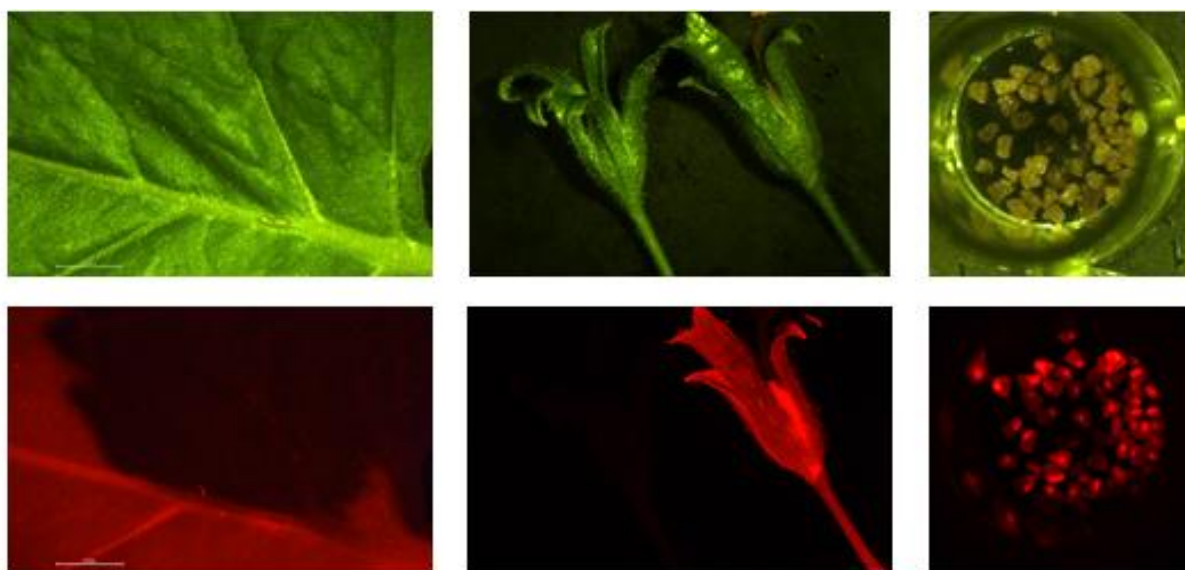


Figure 1. Leaf expressing dsRED with an edited region (left column). Flowers expressing or not dsRED (middle column). Seeds from plants constitutively expressing dsRED (right column)

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