DEVELOPMENT OF A PROTOPLAST SYSTEM FOR NON-TRANSGENIC, TARGETED GENOME EDITING IN S. TUBEROSUM

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Genome editing technologies based on the CRISPR-Cas9 programmable nuclease are emerging as invaluable tools for research and plant breeding. Crops modified using this technology have recently been deemed non-regulated by U.S. regulatory agencies, provided that any transgenes used during editing are removed from the final product. For clonally propagated crops such as potato (*Solanum tuberosum* Group Tuberosum), transgene removal remains particularly challenging and time-consuming. Non-transgenic genome editing by transfecting protoplasts with preassembled Cas9 ribonucleoprotein complexes offers a strategy for efficiently editing clonally propagated crops.
To knock-out selected genes and to modify others *in loco,* we have established a protoplast regeneration platform in *Solanum tuberosum.* This system should provide a rapid method of testing new guide RNAs and ability to quickly and easily introduce multiple edits in a crop genome at a single time. Here, we report on our protoplast isolation and plant regeneration from two potato clones: *S. tuberosum* Group Tuberosum *cv.* Desiree and *S. tuberosum* Group Phureja *cv.* DM1-3, as well as our gene target design strategy. We observe efficient protoplast isolation frequencies in both *S. tuberosum* Group Tuberosum cv. Desiree and in *S. tuberosum* Group Phureja cv. DM1-3. Contrary to DM1-3, Desiree successfully regenerates callus and plant shoots, making it an ideal candidate to further explore the potential of this genome editing technology for crop modification. We anticipate that edited lines produced from our pipeline will provide useful material for potato functional genomics.