Abstract

Potato (Solanum tuberosum) is the fourth most important crop. As the world's population grows, there will be a need to ensure and maintain stable yields, which are now threatened by advancing climate change, for example by negative impact on tuber initiation. Tuberisation is a complex process governed by a number of regulatory factors, and changes in each of them can affect the success of the whole process. Important tuberigenic factors include the mobile protein SP6A. The aim of this study was to elucidate the role of SP6A in the tuberization process in cultivated potato by characterizing lines with SP6A gene knock-out. For mutagenesis, I used the CRISPR/Cas9 method with specifically designed gRNA so that all SP6A alleles of this tetraploid species have been edited. I stably incorporated the CRISPR/Cas9 components into the plant material via Agrobacterium tumefaciens. Subsequent de novo regeneration did not result in the derivation of a loss-of-function mutant. Although repeated de novo regeneration increased the editing efficiency, I did not obtain a complete knock-out mutant in the lines tested so far. In some of the derived lines, an eight-nucleotide deletion was detected in the sequenced region of the gene of interest, however, in addition to the edited sequences, a substantial portion of the unedited sequences remained in the amplicon. In the preliminary experiment using promising lines, a reduced level of SP6A transcript was determined compared to WT, but in vitro tuberization surprisingly did not show the expected reduction in tuberization potential.

Keywords: *Agrobacterium tumefaciens*, CRISPR/Cas9, cultivated potato, *de novo* regeneration, *StSP6A*, tuberization