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Decreasing wild potato toxicity by targeted modification of glycoalkaloid metabolism genes

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Wild relatives of crop plants carry a variety of genes controlling resistance to adverse biotic and abiotic environmental conditions and serve as donors of resistance genes. Many of wild potato species accumulate high amounts of steroidal glycoalkaloids (SGAs), which are toxic to humans. This fact impedes application of these wild species as donors for potato breeding. The targeted knockout of genes controlling SGA biosynthesis in wild potato could be a promising strategy to decrease SGA accumulation. The *GAME9* gene plays a key role in SGAs synthesis regulation in potato. It was shown that it is associated with domestication process and decreasing of potato toxicity. The steroidal alkaloid glycosyltransferase (SGT) genes are responsible for the final glycosylation steps in the biosynthesis of the SGAs. The *GAME9*, *SGT1*, *-2*, and *-3* genes were selected as targets for gRNA/Cas9-mediated modification. The fragments of target genes were re-sequenced in few wild potato species and set of gRNAs was designed. The modification of wild potato genome is planned through transformation of protoplasts and subsequent regeneration of mutant plants. The both genetic constructs harboring Cas9 and gRNA genes and RNP Cas9/gRNA complexes will be used for protoplast genome modifications. The methods for gRNA efficiency evaluation and protoplast isolation and transformation have been established for potato. For evaluation of SGA content in plant tissues the HPLC-based protocol has been established.

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