Transgene-free CRISPR-Cas9 inducing multiallelic mutations in tetraploid potato (Solanum tuberosum)

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Abstract

Potato is ranked as one of the most important food crops in the world. Potato is also one of the major crops grown for starch production in northern Europe. Starch produced from potatoes has many uses, both in food and technical applications, and is often chemically or physically modified to reach certain specifications. To increase the portfolio of "green-labelled" starch products, we would like to replace the down-stream processing modified starches by starch modified *in planta*. Potato is a tetraploid crop with tetrasomic inheritance and high heterozygosity, making traditional cross-breeding a long term process. Therefore, breeding technologies where only one or a few traits can be introduced into an elite background is of major interest.

Genome editing through targeted endonucleases facilitating chromosomal double or single strand breaks are an emerging tool for plant breeding. The most common method to deliver the mutagenesis components into plant cells are in DNA vectors, either targeting stable integration in the genome or with the intension of transient expression. Outcrossing of DNA inserts are preferably avoided in potato, hence a transient expression is favoured. The use of CRISPR-Cas9 to produce site-specific double strand breaks, via transient expression of plasmid DNA in potato protoplasts, was found to introduce unintended random parts of vector DNA in the genome to a rather high frequency[1-2]. The inserts were proven located in the Cas9 cut site and hence associated with the double strand break repair.

We therefore implemented transgene-free mutagenesis method through CRISPR-Cas9 ribonucleoprotein delivery[2]. The method was successfully applied to develop amylopectin starch potatoes by knocking-out a granule bound starch synthase (GBSS). The amylopectin starch can be used, without further down-stream modification, in for example food and paper applications.

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References

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