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The selection of efficient sgRNAs for CRISPR-Cas9 genome editing of potato (*Solanum tuberosum* L.) aimed to obtain the cultivars with low-amylose starch properties

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Potato (*Solanum tuberosum* L.) is an important starch producing agricultural plant. Starch is the main storage carbohydrate widely used for industrial and nutritional needs. The starch functional properties are mainly defined by the ratio of two polysaccharides: amylose and amylopectin. The aim of the study is development of Russian potato varieties with low- or no-amylose starch properties using the CRISPR-Cas9 genome editing. The *GBSS1* gene is known to be responsible for amylose biosynthesis in potato tubers. The cultivars of choice for editing were popular varieties Nevskiy and Udacha which have good *in vitro* regeneration ability. For precise *GBSS1* gene targeting we made single guide RNA design using the Illumina genomic sequencing data for Nevskiy and Udacha (obtained on NextSeq550 at the ICG SB RAS Core Genome Facility). The obtained reads were aligned to the *GBSS1* from PGSC reference genomic sequence. The resulting *GBSS1* consensus gene sequences were analyzed with CRISPOR, CRISPRdirect, CRISPR-P programmes to pick the candidate sgRNAs. From 109 total output oligos six were selected based on criteria of predicted specificity and secondary structure. The six selected sgRNAs are targeted to *GBSS1* exon 1 and exon 10 sequences. At the next step, 6 sgRNAs were synthesized *in vitro* with the aid of Precision gRNA Synthesis Kit (Invitrogen) and cloning of the corresponding oligos to pUC57-sgRNA expression vector. The obtained sgRNAs were *in vitro* pre-validated as part of Cas9 ribonucleoprotein (RNP) complex to cleave the PCR-produced *GBSS1* sequences of Nevskiy and Udacha. The next step the efficient constructs will be used for DNA-free biolistic transformation of potato protoplasts.
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