

Efficient targeted mutagenesis by endogenous promoter controlled tRNA-gRNA array of CRISPR/Cas9 in potato and application in *Solanum tuberosum*

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Research Article

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Abstract

Here, we developed an endogenous promoter controlled polycistronic tRNA-gRNA (PTG) array of CRISPR/Cas9 in potato for efficient multiplex mutagenesis. The editing efficiency was significantly improved in potato combined with short-term heat treatment. In addition, our use of CRISPR/Cas9 to engineer mutations in wild *solanum* species demonstrates a high editing efficiency of the developed PTG/Cas9 in *Solanum etuberosum*, which contributes to develop *Solanum etuberosum* as a new model for functional genetics research in *solanum*.

Key Message

Development of endogenous promoter driven CRISPR/Cas9 for high efficient gene editing in potato and *Solanum etuberosum* in combination with heat treatments.

Full Text

Potato (*Solanum tuberosum* L.) is the most important food crop after wheat and rice around the world, and has been an important part of human food and nutrition security. Currently, potatoes are already a staple for 1.3 billion people, and are becoming increasingly popular in the developing countries (Stokstad 2019). Breeding better potatoes helps to keep up with the rising demand for potato, and to feed a growing population under the threat of climate change. To breed a better potato, it benefits to have loads of genetic material. And there is still an abundance of genes related to food security traits in potatoes whose endogenous functions have yet to be understood. The well-developed clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9)-mediated genome editing technique has already proven to be powerful in functional genetics analysis and creating genetic diversity (Gao 2021). Due to four copies nature of their chromosomes in commercial varieties and low gene-editing efficiency, there are a limited number of publications in potato. Here, we developed an efficient CRISPR/Cas9-mediated gene editing protocol in potato using endogenous promoter controlled polycistronic tRNA-gRNA (PTG) array of CRISPR/Cas9. Combined with short-term high temperature treatment, the mutation efficiency of the potato transformants was significantly increased. In addition, the developed PTG/Cas9 system showed highly efficient gene editing in wild *Solanum* species *Solanum etuberosum*. Thus, the combination of endogenous promoter-driven PTG/Cas9 and heat treatment provide a reference for the efficient targeted mutagenesis of potato and potentially wild *Solanum* species.

To develop an endogenous promoter controlled PTG/Cas9 editing vector in potato, the PTG/Cas9 expression cassette of pRGE32 (Xie, et al. 2015), was cloned to pJCV55. Next, to replace the OsU3 promoter, 200 bp potato U6 promoter reported by (Guerineau and Waugh 1993), was synthesized and inserted into pJCV55. StUBI10 promoter was further cloned to pJCV55 to substitute the OsUBI10 promoter. Finally, the vector was named pJCV55-StU6-200-StUBI10-EV (Fig. S1, Fig. S2). To test the use of it in potato, firstly, two guide RNAs were designed to target phytoene desaturase (*PDS*) (Fig. 1a-b). We obtained 37 transgenic lines in diploid AC142 by rooting selection in kanamycin-containing medium. We

found that only 6 lines of them showed mosaic phenotype, and the others were indistinguishable from AC142 (Fig. 1c). Because it has been reported that the mutation efficiency of CRISPR/Cas9 in plants was significantly improved by heat treatments (LeBlanc, et al. 2018). Thus, these transgenic plants were subjected to heat at 37°C to improve the mutation efficiency. We found that 20 lines of them showed albino or mosaic phenotypes after high temperature (HT) treatment (Fig. 1c). When these plants were further propagated and grown at room temperature (RT), new albino lateral leaf-shoots formed on the base of heat-treated plants (Fig. 1d). Additionally, Sanger sequencing of those albino plants also demonstrated an increased editing efficiency after heat treatment (Fig. 1e). To further test whether this editing vector is useful in tetraploid potato. We obtain 4 transgenic lines in E-potato 3 (E3) cultivar background. All four lines grown at room temperature were indistinguishable from E3. But 3 lines of them showed mosaic phenotypes after HT treatment, and new albino lateral leaf-shoots also formed when these mosaic plants were propagated and grown at RT (Fig. S3, Fig. S4). Our results was consistent with the previous report on sweet orange (Tang, et al. 2021).

Understanding the endogenous functions of genes in wild relatives will enable us to introduce new traits into cultivated potatoes using molecular engineering technologies, and to engineer the domestication of wild species with agricultural potential (Li, et al. 2018). Thus, it was also tested in *Solanum etuberosum* to see if the developed PTG/Cas9 system could be translated to wild *Solanum* species. To test the use of PTG/Cas9 gene editing in *Solanum etuberosum*, *SELF PRUNING 5G* (*SP5G*) gene was selected to engineer mutations in this long-day plant (Fig. S5), because it has been shown that this florigen paralog function as a flowering repressor in both *Solanum lycopersicum* (tomato) and *Solanum tuberosum* (Abelenda, et al. 2016; Soyk, et al. 2017; Zhang, et al. 2018). And the variation in *SlSP5G* is critical for the domestication of cultivated tomatoes (Zhang, et al. 2018).

Two previously reported guide RNAs (Zhang, et al. 2018), were used to engineer mutations in this study (Fig. 1f). Using *Agrobacterium*-mediated transformation of leaf explants (Fig. 1g), we obtained 23 independent first-generation (T0) transgenic plants by rooting selection. Genomic DNA of these transgenic plants were directly amplified using primers specific to *SeSP5G*, and sequenced to screen editing events without heat treatments. We found that all transgenic plants were edited, and five of them were homozygous mutants based on sequence analysis (Fig. 1h, Fig. S6). Furthermore, four of these homozygous mutants, harbor a mutation near the target 2, generated PCR bands resistant to *Stu*I digestion (Fig. 1i), indicating a high efficient gene editing of our developed PTG/Cas9 system in *Solanum etuberosum*. Notably, null mutant *CR-Sesp5g-15* plants flowered in short days but *CR-Sesp5g-6* (one amino acid deletion) and wild type never produced any flowers (Fig. 1j-k), suggesting *SeSP5G* is also responsible for the photoperiodic flowering in *Solanum etuberosum*.

In conclusion, we developed an endogenous promoter controlled PTG/Cas9 in potato for efficient multiplex mutagenesis. The endogenous promoter is efficient for *Agrobacterium*-mediated genetic transformation using both leaf explants and micro-tuber discs. The editing efficiency is significantly improved in the tested diploid and tetraploid potato cultivars combined with short-term heat treatment. Importantly, our use of PTG/Cas9 to engineer *SeSP5G* mutations confirms a conserved flowering

repressor role of *SP5G* in several *solanum* species, also demonstrates a high editing efficiency of the developed PTG/Cas9, which strongly helps to develop *Solanum etuberosum* as a new model for functional genetics research in *solanum*, because it possess the diploidy, self-compatibility, and most importantly, quick and easy genetic transformation.

Declarations

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Author contribution statement S.J., J.D. and B.S. conceived and designed research. S.J., W.X., H.L., F.D. conducted experiments. S.J., W.X., and B.S. analyzed the data. S.J. wrote the manuscript. K.C. and J.D. directed the study and revised the manuscript. All authors read and approved the manuscript.

Conflict of interest the authors declare no conflict of interest.

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Figures

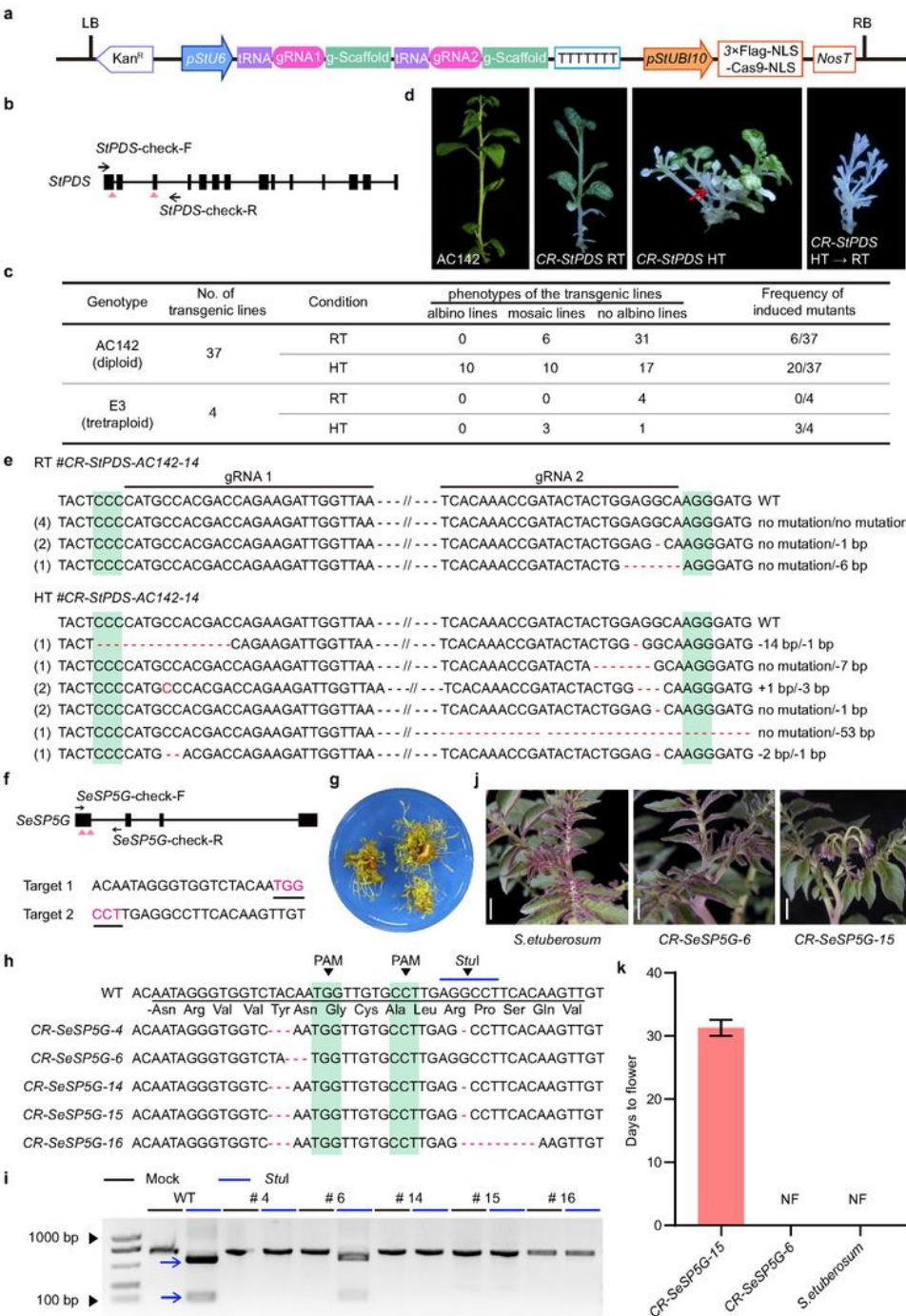


Figure 1

Endogenous promoter controlled tRNA-gRNA array of CRISPR/Cas9 in potato and application in *Solanum etuberosum*. **a** Schematic map of the constructed PTG/Cas9 expression cassette. StU6, potato U6 snoRNA promoter. StUBI10, potato ubiquitin 10 promoter. G-Scaffold, gRNA Scaffold. **b** The first and third exons were targeted by CRISPR/Cas9 using two single-guide RNAs (red triangle). Black arrows indicate forward and reverse forward primers used for PCR genotyping and sequencing. **c** Mutation efficiency induced PTG/Cas9 in potato cultivar AC142 and E3. **d** Phenotyping induced by PTG/Cas9 in transgenic potato AC142. RT, room temperature at 22°C; HT, High temperature at 37°C. **e** Sequences of *StPDS* alleles identified from first-generation (T0) transgenic line *CR-StPDS-AC142-14*. Protospacer-adjacent motif sequences are highlighted in light blue. Insertion and deletion are indicated by + and –, respectively. **f** The first exon was targeted by CRISPR/Cas9 using two single-guide RNAs (red triangle). Black arrows indicate forward and reverse forward primers used for PCR genotyping and sequencing. **g** Image of regeneration shoots from leaf explants. **h** Sequences of *SeSP5G* alleles identified from first-generation (T0) transgenic homozygous mutants. Protospacer-adjacent motif sequences are highlighted in light blue. The restriction enzyme sites of *Stul* used for genotyping are indicated by blue line. Mutations are shown in red. Insertion and deletion are indicated by + and –, respectively. **i** Restriction digestion screen of T0 homozygous mutants using the enzyme *Stul*. Wild type *Solanum etuberosum* generate digested PCR bands (blue arrow), and plants with mutations generate PCR bands resistant to *Stul* digestion. **J, k** *CR-SeSP5G* mutants flowered in short day conditions, while the wild type could not flowered in short days. Scale bars: 1 cm; NF, no flower.

Supplementary Files

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