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Transplastomic plants — new approaches to solving "old" problems

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Transplastomic plants are capable to accumulate the significant amounts (up to 70% of TSP) of target recombinant proteins in tissues. However, the production of such forms is severely limited by the low yield of initial transformants. This problem requires the development and optimization of new approaches to the delivery of transgenes into chloroplasts and an increase in the frequency of their integration into the plastome. Transplastomic tobacco plants expressing the *gfp* reporter gene and the *aadA* selectable marker under the control of the *PrrnG10L* promoter and the *TpsbA* terminator were obtained in the laboratory of plant bioengineering. It is known that the selected promoter and insertion region (between the tRNA genes of isoleucine and alanine) are capable to provide a high yield of recombinant proteins in the leaves of transplastomic plants [1]. However, the content of recombinant GFP in the leaves of the obtained transplastomic plants was determined at the level of 0.12%, and the variability for this trait was minimal and ranged from 0.09 to 0.16% of TSP. Insufficient accumulation of the target protein in transformants is not associated with transcription disorders or the presence of non-transgenic copies of the plastome. Probably, the low frequency of transformation and the lack of variability between the transformants are the reasons that make it difficult to select highly productive forms. It is proposed to increase the efficiency of targeted delivery of genetic constructs to plastids using single-walled carbon nanotubes loaded with recombinant DNA. This process can also be facilitated by our proposed approach to increase the frequency of DNA double-strand breaks in target regions of the plastome through the use of the CRISPR-Cas9 genome editing system.

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Keywords: chloroplasts; plastome; transformation; editing; carbon nanotubes.

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