World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:12, No:03, 2018

Optimization for Guide RNA and CRISPR/Cas9 System Nanoparticle Mediated Delivery into Plant Cell for Genome Editing

Authors : Andrey V. Khromov, Antonida V. Makhotenko, Ekaterina A. Snigir, Svetlana S. Makarova, Natalia O. Kalinina, Valentin V. Makarov, Mikhail E. Taliansky

Abstract : Due to its simplicity, CRISPR/Cas9 has become widely used and capable of inducing mutations in the genes of organisms of various kingdoms. The aim of this work was to develop applications for the efficient modification of DNA coding sequences of phytoene desaturase (PDS), coilin and vacuolar invertase (Solanum tuberosum) genes, and to develop a new nanoparticles carrier efficient technology to deliver the CRISPR/Cas9 system for editing the plant genome. For each of the genes - coilin, PDS and vacuolar invertase, five single RNA guide (sgRNAs) were synthesized. To determine the most suitable nanoplatform, two types of NP platforms were used: magnetic NPs (MNPS) and gold NPs (AuNPs). To test the penetration efficiency, they were functionalized with fluorescent agents - BSA * FITS and GFP, as well as labeled Cy3 small-sized RNA. To measure the efficiency, a fluorescence and confocal microscopy were used. It was shown that the best of these options were AuNP - both in the case of proteins and in the case of RNA. The next step was to check the possibility of delivering components of the CRISPR/Cas9 system to plant cells for editing target genes. AuNPs were functionalized with a ribonucleoprotein complex consisting of Cas9 and corresponding to target genes sgRNAs, and they were biolistically bombarded to axillary buds and apical meristems of potato plants. After the treatment by the best NP carrier, potato meristems were grown to adult plants. DNA isolated from this plants was sent to a preliminary fragment of the analysis to screen out the non-transformed samples, and then to the NGS. The present work was carried out with the financial support from the Russian Science Foundation (grant No. 16-16-04019).

Keywords : biobombardment, coilin, CRISPR/Cas9, nanoparticles, NPs, PDS, sgRNA, vacuolar invertase
Conference Title : ICANA 2018 : International Conference on Applications of Nanotechnology in Agriculture
Conference Location : Tokyo, Japan
Conference Dates : March 27-28, 2018