BioTechnology
ISSN: 0974-7435

BioTechnology: An Indian Journal

Editorial | Vol 12 Iss 12

Crispr-Cas9: A Handy Tool, but Not in Genome-Editing Toolbox of All Plant Species

Gurel E[1*], Pazuki A[1,2], Aflaki F[1,2] and Gurel S[2]

[1]Department of Biology, Abant Izzet Baysal University, 14280 Bolu, Turkey
[2]Sugar Institute, Plant Breeding Department, 06930 Etimesgut, Ankara, Turkey

[*]**Corresponding author:** Gurel E, Department of Biology, Abant Izzet Baysal University, 14280 Bolu, Turkey, Tel: +90 374 254 12 20; E-mail: gurel_e@ibu.edu.tr

Received: November 15, 2016; **Accepted:** November 17, 2016; **Published:** November 23, 2016

Genome modification/engineering, a tool for manipulation of organism's genome using biotechnology, dates back to past century. Its resultant organisms are called genetically modified organism (GMO) in which recombinant nucleic acid (DNA or RNA) techniques are used. However, in genome editing as a newly developed subdivision of genome engineering, molecular scissors (engineered nucleases) are used to insert, remove, or replace target DNA, e.g. zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 (CRISPR–Cas9). Double-stranded breaks (DSBs) created at target sequence by activity of nucleases, will be repaired by either homologous recombination (HR) or no homologous end joining (NHEJ).

Function of CRISPRs and CRISPR-associated (Cas) proteins as a defense mechanism in prokaryotes was identified in 2000s [1,2]. About one decade later, its genome modification potential was realized [3], and growingly was employed in many phyla engineering. The reasons behind CRISPR/Cas9 prevalence over the last few years include easy to use, more versatile, cheaper, quicker and more precise unlike the other mentioned methods. For instance, CRISPR–Cas9 can be ordered at a cost about to 100 times cheaper than that of zinc finger. CRISPR/Cas9 target specificity is in a way that it cuts only certain target DNA sequences. Cas protein using CRISPR spacers is the cutter of given location. Its design, also, is not as complicated as ZFNs and TALENs.

The fundamental question, however, is raised on the possibility of successful delivery of CRISPR–Cas9 into many organism tissues besides model organisms, e.g. mice, fruit flies and arabidopsis. The advancement of improvement in agriculture is not very promising, hence it demands more attempt to keep pace with the most pressing problem, overpopulation of the world [4]. To meet the challenges of agriculture, plant biologists believe that still the technical problems lying in plant genome modification are delivery systems of genome editors into plant cells, which then may face more difficulties in whole plant regeneration from some transformed cells.

Citation: Gurel E, Pazuki A, Aflaki F, et al. Crispr-Cas9: A Handy Tool, but Not in Genome-Editing Toolbox of All Plant Species. Biotechnol Ind J. 2016;12(12):e101.

Many plant species have been tissue cultured *in vitro*. For most of them, the technique is not efficient or transformed plant regeneration is very difficult. Although nearly all genome-editing methods can be applied in plant species, the major bottle-neck is transgene delivery into plant cells, and then the regeneration of transformants [5]. This difficulty is more noticeable when one tries to transform different genotypes, where their response to the same method may differ significantly. The effort can be more tiresome if the genotype is easily regenerated but cannot be readily transformed, and vice versa.

Genotype-independent transformation and regeneration still is a goal. A few numbers of species are amenable to floral dip transformation [6], which sidesteps cumbersome tissue culture methods. A delivery method for many plants is mediated by *Agrobacterium* or other bacteria. However, this technique is species- or genotype-dependent. Another option for plant transgenesis and genome editing is biolistic method, which can be applied for many more plant species, but still suffer from the same problems stemming from tissue culture techniques. In addition, *Agrobacterium* enables researchers to generate single-copy insertions in comparison with multi-copy insertions when using biolistic methods. On the other hand, by using biolistic methods a clean gene technology can be expected [7], whereas *Agrobacterium* integrates its vector backbone sequences into host genome.

Even if both above-mentioned problems (i.e. delivery and regeneration techniques) are addressed, a new one may compound, that it is relatively large size of Cas coding sequence. Routinely, a plasmid in *Agrobacterium* shuttles Cas protein and its guidance RNA (sgRNA) genes into plant cells, which means the introduced genes into plant cells are normally integrated into the host genome. Researchers have recently adopted an alternative approach, direct protein delivery, enabling them to tackle the big size issue and at the same time, they would clear the hurdle imposed by current GMO regulations.

Recently, direct protein delivery, instead of DNA, into plant cells by biolistic method allowed a transient presence of the protein to modify genome in maize (*Zea mays*) [8]. Similarly, Cas9 enzyme and sgRNA are firstly assembled outside a plant tissue, and then the complex, not the corresponding genes, is introduced into the plant. This method seems superior to clean gene delivery technology, since in addition to its improved efficiency, owing to transgene-free delivery of the complex into plant cells, the biosafety regulations on GMO can be removed.

A mini-Cas9 found in *Staphylococcus aureus*, 25% smaller than the conventional Cas9s, was used in mice for gene therapy. Using the mini-Cas9 is expected in plant genome editing as well. Inducing site-directed gene modifications in plants with no off-target effects is one of the areas researchers focus on [9].

Genome editing methods have been changing and improving constantly. One of the other methodological approaches is searching for new enzymes except Cas9 in various microbes with different properties. One of them is called Cpf1, which is smaller with different sequence requirements. It can efficiently cleave target DNA sequence immediately followed by a short 5′ T-rich protospacer adjacent motif (PAM), whereas a G-rich PAM follows the target in Cas9 systems. In addition, the targeted DNA is cleaved as a 5-nt staggered cut distal to the T-rich PAM [10]. The other one called LshC2c2 from *Leptotrichia shahii* is specialized in targeting single-stranded RNA, while other ones target DNA [11]. LshC2c2 application

in plant sciences may help researchers in understanding and manipulation of plant transcriptomes and in counteracting virulent viruses having RNA as their genomes.

In conclusion, over growing population are seeking for more and better foods and feeds. Achieved progress in plant improvement does not seem sufficient. Newly discovered technologies can be employed to advance agricultural products to address consumer needs and to convince regulators. CRISPR/Cas9 aided genome editing technology has appeared very promising. Based on CRISPR/Cas system, many different methods and approaches have been tested so far [12]. It has shown a wonderful and fast evolution from the date it was identified in a bacterium immune system to delivering with no transgene (CRISPR/Cas) integration into host genome [13]. Moreover, a CRISPR-edited crop is likely to bypass USDA oversight [14]; and surprisingly, a genetically engineered mushroom has previously been free of USDA regulation [15]. Despite the progress achieved in transgenesis, plant tissue culture still is very efficient and reliable technique for a few model plants. However, for many other major crops this technique is relatively applicable only for a few genotypes, which are not always commercial ones. Therefore, plant tissue culture lags behind transgenesis techniques, and still needs to be subjected to many experiments to keep apace with demand for secure food. CRISPR/Cas dawn promises a bright and sunny noon, if tissue culture clouds let it shine.

REFERENCES

1. Mojica FJ, Díez-Villaseñor C, Soria E, et al. Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria. Molecular Microbiology. 2000;36(1):244-46.
2. Jansen R, Embden JD, Gaastra W, et al. Identification of genes that are associated with DNA repeats in prokaryotes. Molecular Microbiology. 2002;43(6):1565-75.
3. Garneau JE, Dupuis M, Villion M, et al. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature. 2010;468(7320):67-71.
4. Ludewig F, Sonnewald U. Demand for food as driver for plant sink development. Journal of Plant Physiology. 2016;203:110-15.
5. Altpeter F, Springer NM, Bartle LE, et al. Advancing crop transformation in the era of genome editing. The Plant Cell. 2016;28(7):1510-20.
6. Lu C, Kang J. Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by *Agrobacterium*-mediated transformation. Plant Cell Rep. 2008;27:273.
7. Breitler JC, Labeyrie A, Meynard D, et al. Efficient microprojectile bombardment-mediated transformation of rice using gene cassettes. Theoretical and Applied Genetics. 2002;104(4):709-19.
8. Martin-Ortigosa S, Peterson DJ, Valenstein JS, et al. Mesoporous silica nanoparticle-mediated intracellular Cre protein delivery for maize genome editing via lox-P site excision. Plant physiology. 2014;164(2):537-47.
9. Osakabe Y, Watanabe T, Sugano SS, et al. Optimization of CRISPR/Cas9 genome editing to modify abiotic stress responses in plants. Scientific reports. 2016;6:26685.
10. Zetsche B, Gootenberg JS, Abudayyeh OO, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell. 2015;163(3):759-71.
11. Abudayyeh OO, Gootenberg JS, Konermann S, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science. 2016;353(6299):aaf5573.

12. Ding Y, Li H, Chen LL, et al. Recent advances in genome editing using CRISPR/Cas9. Frontiers in Plant Science. 2016;7:703.
13. Mojica FJ, Montoliu L. On the origin of CRISPR-Cas9 technology: From Prokaryotes to Mammals. Trends in Microbiology. 2016;24(10):811-20.
14. Waltz E. CRISPR-edited crops free to enter market, skip regulation. Nature Biotechnology. 2016;34(6):582.
15. Firko MJ. Request for confirmation that transgene-free, CRISPR-edited mushroom is not a regulated article. USDA, APHIS. April 13, 2016.