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DNA-free gene editing in plants: a brief

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Abstract :

- ❖·**The conversion of bacterial CRISPR/Cas defense system into a simple and efficient tool for genome manipulations brought experimental biology into new dimensions.**
- ❖·**In plant biology and biotechnology, CRISPR/Cas gene editing became the second most important technology after plant transformation**
- ❖·**The main obstacle is that they include DNA delivery and, frequently, its subsequent integration into cellular genome. For this reason novel methods to achieve gene editing without the need of stable transformation and even without DNA delivery were developed. These new approaches include *in vitro* ribonucleoprotein complexes formulations use of virus-like particles and employment of bacterial secretory systems for Cas/gRNA delivery.**

Introduction:

- ❖·**DNA-free gene editing became a new and fast-developing trend in biological research due to its obvious advantages.**

- ❖·**Historically, genome editing became available in the 1990s upon the development of efficient plant transformation techniques. The first experiments used heterologous DNA (either relatively long fragments or oligonucleotides) for allele replacement *via* homologous recombination.**

- ❖·**The discovery of the bacterial immunity system based on RNA-guided endoDNAses opened a new possibility for precise genomic modifications.**

- **Gene editing became capable of reaching almost all research groups including those with minimal equipment and facilities.**

- **Since then CRISPR/Cas has been successfully applied on a number of species and for different purposes. In addition, almost all main plant transformation methods have been employed. Soon after a new problem emerged that needed to be addressed. The main obstacle was that the original gene editing includes DNA delivery (encoding either Cas or gRNA or both) and, frequently, its subsequent integration into the cellular genome.**

Related Works:

- ***Another Study focused its attention on vegetatively propagated plants. They used a cytidine based editor (CBE) to edit the acetolactate synthase (ALS) gene in potatoes and tomatoes via Agrobacterium infection. Point mutations in the ALS gene can lead to different types of resistance in plants. They successfully produced transgene-free (12.9%) chlorsulfuron-resistant tomatoes with a very high base editing efficacy (up to 71%). In potatoes, the transgene frequency was a little bit lower (10%). The main drawback of this method was the off-target effects, therefore there is a need for further protocol optimization.***