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CRISPR-CAS9: The mechanism, application and the future perspective in crop plants

Vanessa Eveline Vlaic, Ioana Virginia Berindean*, Andreea Ona, Camelia Munteanu*, Ionut Racz, Alexandru Costin and Leon Muntean

** Corresponding author: Ioana Virginia Berindean e-mail: ioana.berindean@usamvcluj.ro and camelia.munteanu@usamvcluj.ro*

CRISPR-CAS9 technology

Discovered in 1987 unintentionally by a Japanese scientist, this technology is based on a simple defence mechanism against viruses and foreign DNA molecules of bacteria and archaea species.
Since then, researchers have worked on improving this natural process of DNA cleavage and have reached the creation of the most precise tool for modifying the genome.

The Nobel Prize in Chemistry 2020 was won by **Emmanuelle Charpentier** and **Jennifer A. Doudna** **'for the development of a method for genome editing'.**

Importance and aspiration for the future

- Climatic factors and the growth of the global population exert a great pressure on the world economic level.
- **CRISPR-CAS9** technology represents a safe option for revolutionizing agriculture with the development in production quantity but also, sustainability.
- **CRISPR-CAS9** can help scientists deepen their knowledge of the mechanism by which plants interact with different pathogens, thus developing crop plants variants with increased resistance to a wide range of pathogens or pests.

‘…reducing the time required to introduce new traits, provide an alternative method to produce cisgenic modifications, allow genetic editing in crops wherein tissue culture or transformation procedures are not available, permit the targeted introduction or deletion of large genomic regions, allow for alterations in ploidy level, and enable a breeder specified control of gene/metabolite production.’ (Schaeffer and Nakata, 2015)

The working mechanism of CRISPR-CAS9

Utilization of this technology to engineer genomes requires two main components (Schaeffer and Nakata, 2015).
First component, the **Cas9** enzyme with the role of cleaving the DNA and the guide-RNA (**sgRNA**) which guides the enzyme to the specific place from the genome.

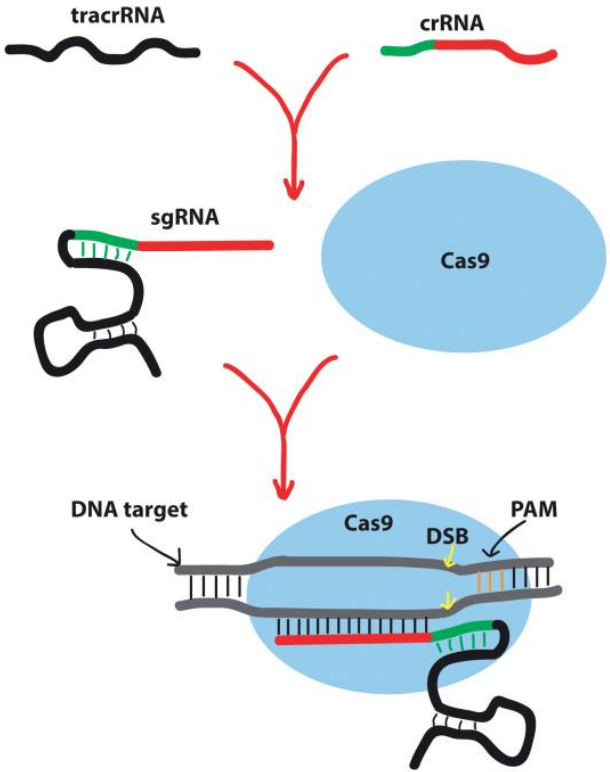


Figure 1; (Zhou et al, 2020)

Applications of the CRISPR-CAS9

According to Peng et al (2017) the mechanism of CRISPR-CAS9 was successfully used in editing the genome of the citrus species *Citrus sinensis* Osbeck and Duncan grapefruit variety with the aim of creating resistance to the most widespread pathogen of citrus crops, ***Xanthomonas citri supsp. citri*** which produces the disease named citrus canker.

- ✓ ‘Resistance of edited plants to canker citrus was further confirmed using in vivo infiltration (Figure 5). No pustules or canker symptoms were detected..’ (Peng et al, 2017)

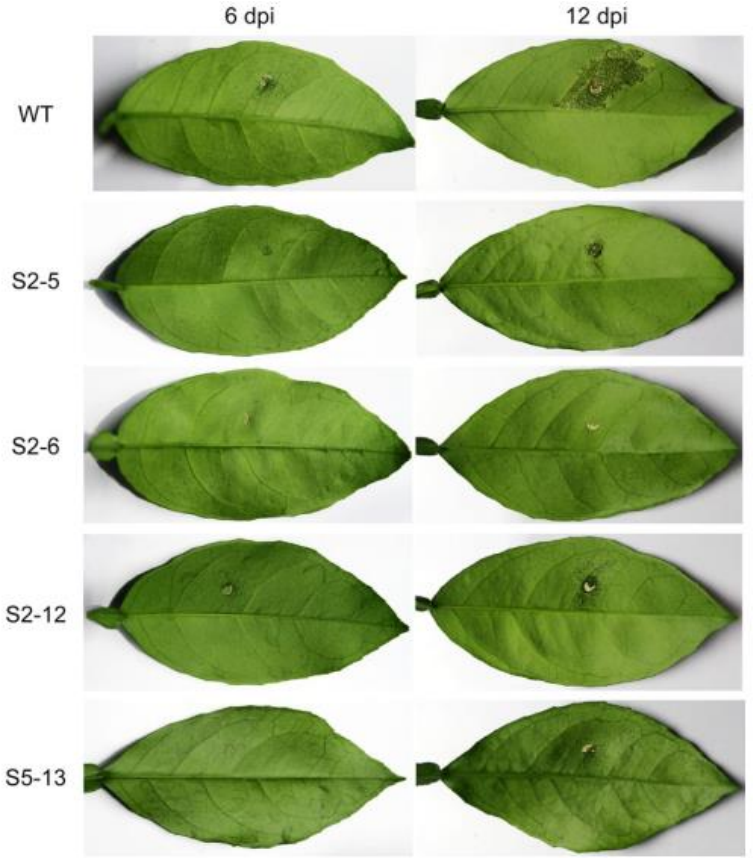


Figure 5 (Peng et al, 2017)

‘we describe a rapid and highly specific method for targeted mutagenesis of soya bean endogenous genes by CRISPR/Cas9 in whole-plant transformation’ (Cai et al, 2017).

- ✓ Another successful application was the induction of mutagenesis of the gene GmFT2a responsible for detecting the photoperiod used during flowering in soybeans (*Glycine max*).

CRISPR-CAS9 was used for **gene deletion/inactivation from the genome**. In order to study future consequences such as the introduction of DNA fragments in other parts of the DNA chain, the official site (http://cbi.hzau.edu.cn/crispr/) that offers such predictions was used. After observations, it was concluded that there were no errors (Cai et al, 2017)

Other experimental applications

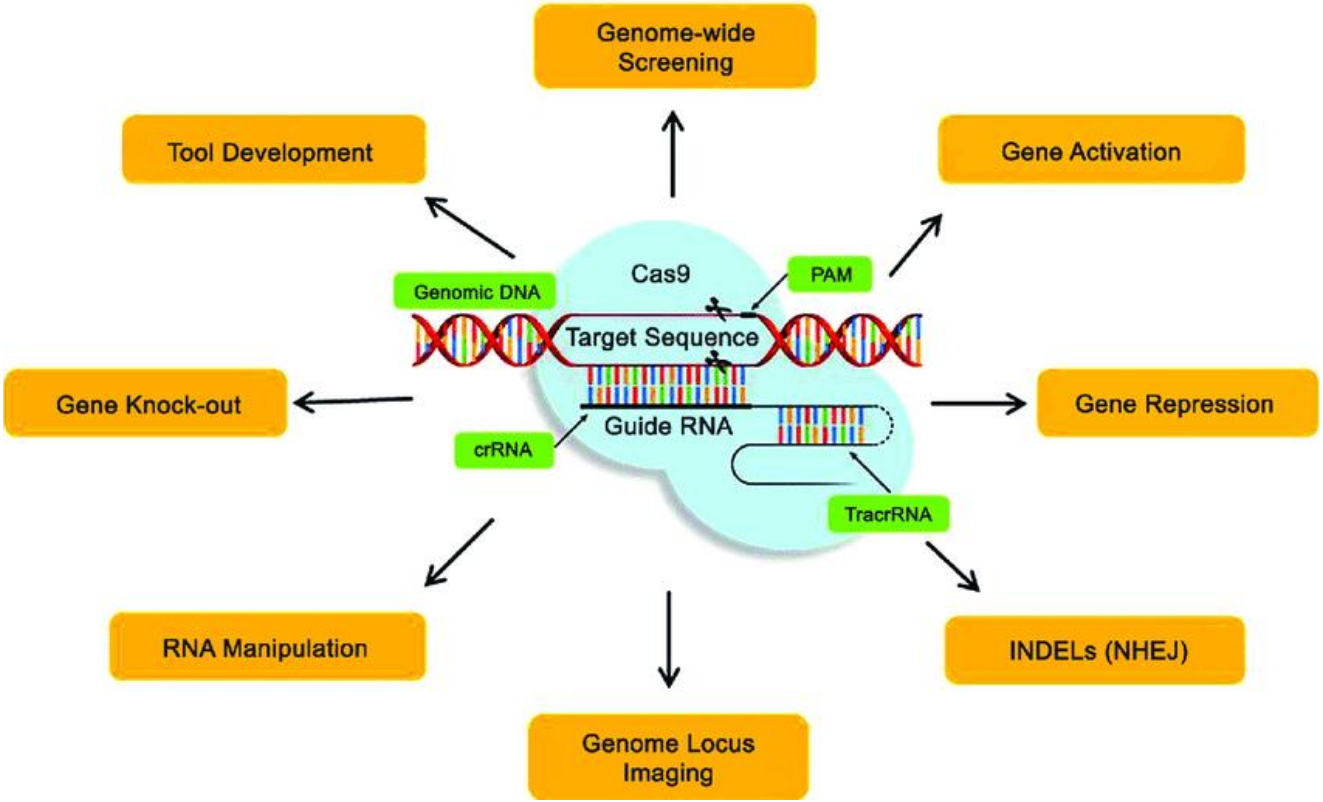
- In the *Cucumis sativus* L. species, CRISPR-CAS9 technology was used to eliminate the CsWip1 gene responsible for inhibiting carpel development. This was achieved with the help of gRNA (guideRNA) molecules and with the specific U6 promoter (Zhou et al, 2020).

- The transfer of a mutant gene DEP1 from wild species of rice *Japonica rice* to commercial species *Indica rice* to increase grain production has been successfully experimented with this technology, thus reducing the time to create new varieties by more than a half (Wang et al, 2017)

Future direction and remarks

- Since the discovery of this genomic editing mechanism, various studies have gained momentum, with researchers reaching results that were thought to be impossible a few decades ago.
- CRISPR can be used to replace the faulty or poor performing R gene (resistance gene) in a cultivated crop variety with the functional R gene from a disease resistant native variety via multiplexed HDR (homology directed repair) methodology (Zaidi et al, 2020)

- The sgRNA targets the plant gene instead of the viral genome, which is more prone to evasion because of its high copy number and high recombination rate. Multiple CRISPR systems have utilized S genes to achieve virus resistance in a number of crop species (Zaidi et al, 2020)



(Anuragi et al, 2018)

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