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CRISPR/Cas Technology: A Novel Approach for Plant Genome Modification

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Abstract:

Cultivar development through plant breeding has reached to new horizon of transgenic technology to meet the current need for the crop development. CRISPR/Cas technology has made a breakthrough in the field of genome editing which has added new stars in transgenic technology. This article mainly deals with basic concept of CRISPR/Cas technology and its mechanism along with various types of CRISPR/Cas system. Advantage of CRISPR/Cas technology over other existing tools for genome editing has also been outlined. Applications of CRISPR/Cas technology in genome editing also has been summarized at the end of this article.

Introduction:

Genome editing of model organisms is essential for gene function analysis and is thus critical for human health and agricultural production. The current technologies used for genome modification include ZFN (zinc-finger nuclease), meganucleases, TALEN (Transcription activator- like effecter nucleases), *etc.* Recently, a new technology for genome modification, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/ Cas (CRISPR-associated) systems, has been developed. CRISPR/Cas systems are adaptive defense systems in prokaryotic organisms to fight against alien nucleic acids. Compared with protein-guided technologies like ZFN and TALEN, CRISPR/Cas system is much easier to implement, as only short guide RNAs need to be customized to target the genes of interest. Up to now, the CRISPR/Cas system has been successfully applied to efficient genome editing in many eukaryotic organisms including human, mice, zebra fish, fly, worm, yeast and few plants. (Miao *et al.*, 2013).

Mechanism of CRISPR/Cas System:

CRISPR–Cas system recognizes and targets the alien genetic material through stepwise processes, namely acquisition, expression, and interference. The **acquisition** process involves recognition and integration of foreign DNA as spacer within the CRISPR locus. In the **second step**, *i.e.* **expression**, the long pre-crRNA is actively transcribed from the CRISPR locus and processed into crRNAs with the

help of Cas proteins (Cas1, Cas2, Cas9, and Cas4/Casn2) and the tracr-RNA molecule. The tracr-RNA pairs with the repeat region of crRNA via base complementarity and the processed crRNAs enter into the CRISPR-associated complex for antiviral defence (CASCADE). During the **interference step**, the crRNA guides the Cas protein complex to the specific target region of the foreign DNA for cleavage and provides immunity against pathogen attacks (Kumar and Jain, 2014).

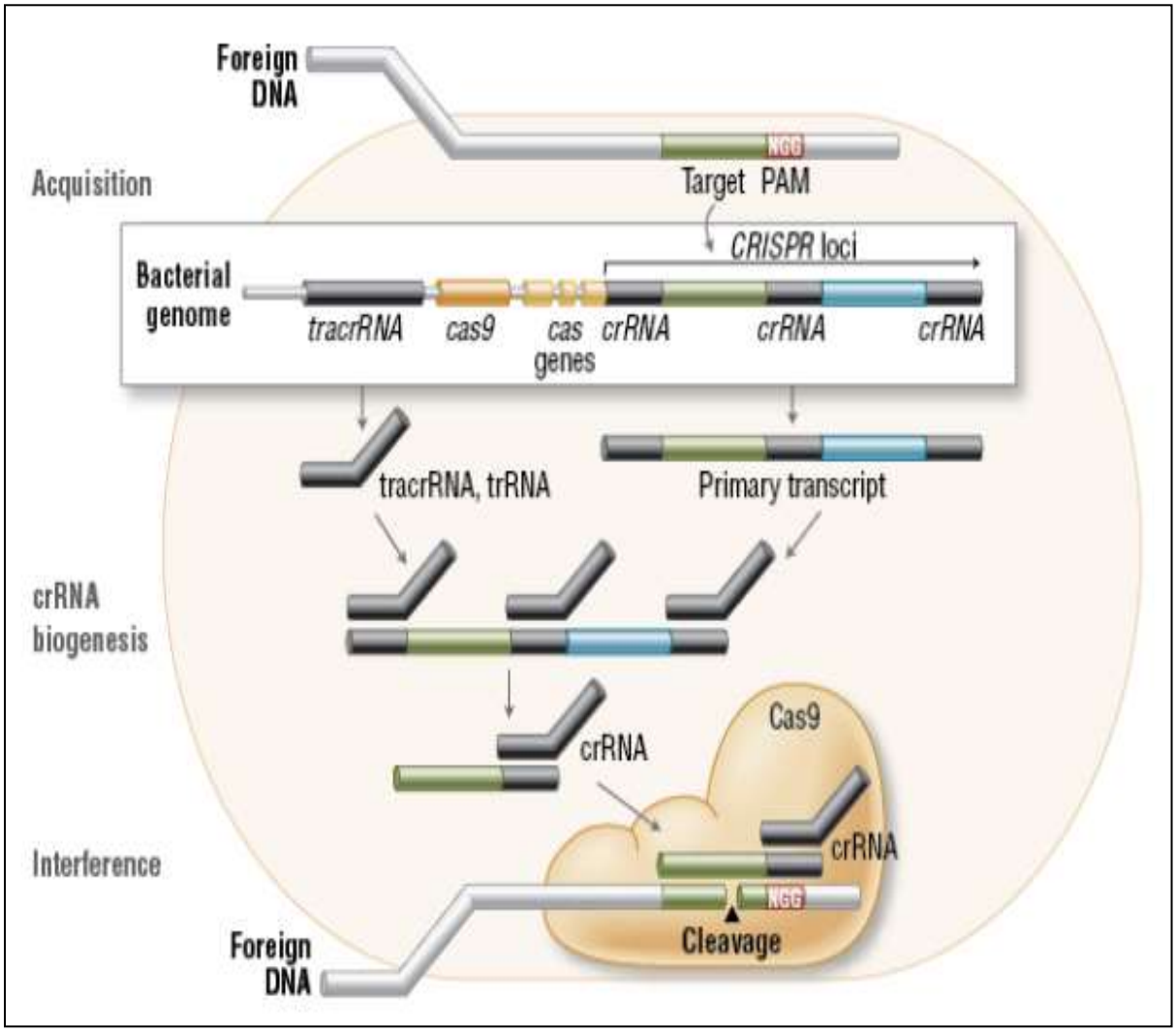

Figure 1. Cas9 in vivo: Bacterial Adaptive Immunity (Jinek *et al*., 2012)

Types of CRISPR - Cas Systems

Based on this classification that integrates phylogeny, sequence, locus organization, and content, three types have been distinguished, Type I, Type II, and Type III CRISPR-Cas systems.

Type I CRISPR-Cas System

In addition to the presence of the conserved Cas1 and Cas2 proteins, Type I is defined by the ubiquitous presence of a **signature protein, the Cas3** helicase/nuclease. Cas3 is a large multi domain protein with distinct DNA nuclease and helicase activities. In addition, there are multiple Cas proteins that form CASCADE-like complexes that are involved in the interference step. The Type I CRISPR-Cas system in *E. coli* is one of the best characterized.

Type II CRISPR-Cas System

This system is typified by the **Cas9 signature protein,** a large multifunctional protein with the ability to generate crRNA, as well as target phage and plasmid DNA for degradation. Cas9 appears to contain two nuclease domains, one at the N terminus (RuvC-like nuclease) and an HNH (McrA-like) nuclease domain in the middle section (which might be involved in target cleavage based on its endonuclease activity). The best-studied Type II system is that of *Streptococcus thermophilus,* which has been shown to provide defence against bacteriophage and plasmid DNA.

Type III CRISPR-Cas System

This system has a number of recognizable features, including the **signature RAMP protein, Cas10,** which is likely involved in the processing of crRNA and possibly also in target DNA cleavage, and is somewhat functionally analogous to the Type I CASCADE. The Type III system also contains the **signature Cas6,** involved in crRNA processing and additional RAMP proteins likely to be involved in crRNA trimming. The universal cas1 and cas2 genes are mostly in operon-like structures with the rest of the cas genes but are not always in the same operon as the RAMP proteins in the Type III systems. So far, two type III systems have been distinguished (Type IIIA and IIIB). In *Pyrococcus furiosus,* a Type IIIA system, the target of CRISPR interference is mRNA, whereas in *Staphylococcus epidermidis,* a Type IIIB system, the target is DNA.

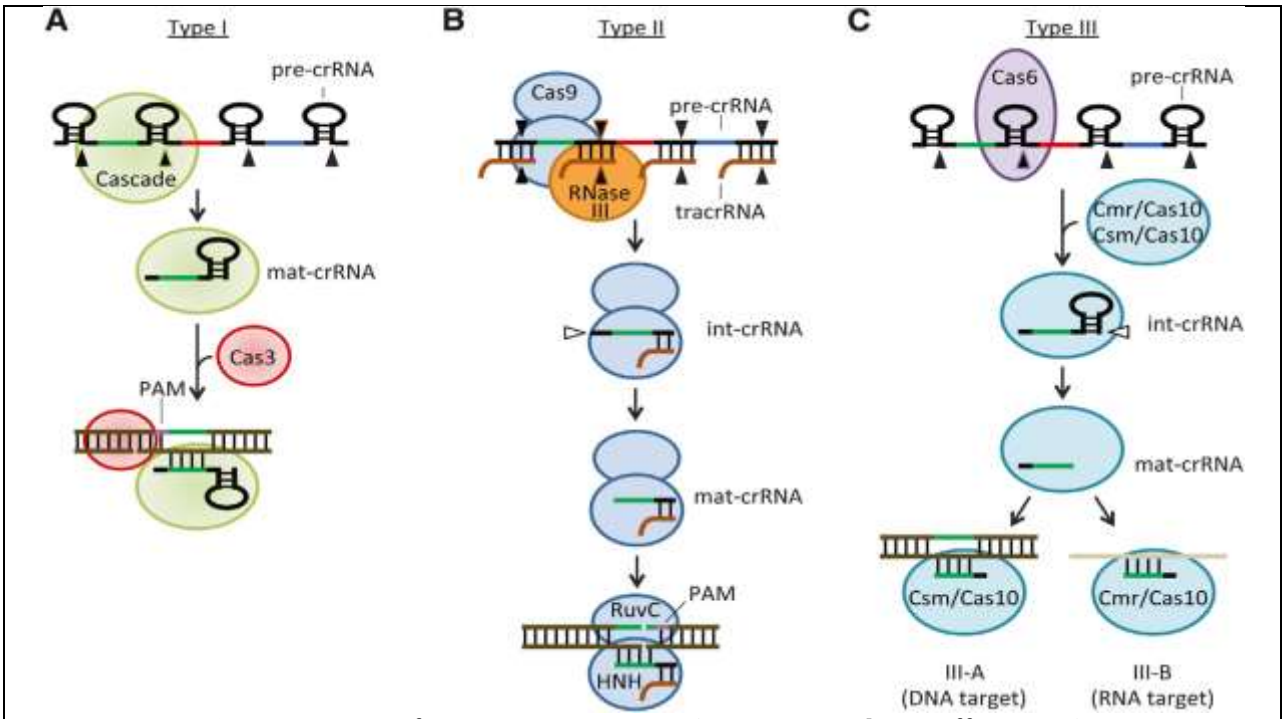

Figure 2. Types of CRISPR/Cas System. (Barrangou and Marraffini, 2014)

Difficulties with existing technology

- Both ***ZFNs*** and ***TALENs*** have tandem repeats in their DNA-binding domains that can be engineered to recognize specific DNA sequences
- The resulting chimeric nucleases can thus be **guided through protein** to the desired target sequences in the genome to generate DSBs
- Hence, a new ZFN or TALEN chimeric **protein** needs to be **engineered** for every new target
- This has been a major hurdle in the wide use of these two gene-editing systems because engineering a new protein is no trivial task
- ***Costly:*** ZFN or TALENs are much more expensive
- ***Reliability:*** Skip bases, off - target toxicity
- ***Long Turn-over Time:*** Difficult to Scale up, ZFN : Several weeks to build a few pairs for expert, TALEN : One week to build a few pairs

Advantages of CRISPR over Other

- By contrast, ***Cas9*** is a nuclease **guided by small RNAs** through **Watson-Crick base pairing** with target DNA, Hence,
- **Easier to design** and **highly specific**
- **Efficient and well-suited** for high-throughput
- **Multiplexed gene editing** is possible for a variety of cell types and organisms
- So only a new sgRNA is needed for a new target site

RNAi Vs CRISPRi

RNAi	***CRISPRi***
• **Target/Object:** ➢ RNA (post-transcriptional control) ➢ RNA Degradation ➢ Object(s) in cytoplasm • **Design/Construct:**	• **Target/Object:** ➢ DNA (transcriptional control) ➢ Block transcription initiation or transcription elongation ➢ Both template and non-template strands ➢ Object(s) in nucleus • **Design/Construct:** ➢ Easy and flexible ➢ Design algorithm available ➢ PAM dependence limited its target sequence • **Efficiency:** ➢ Variable ➢ Off-target effects (maybe less)

Applications of CRISPR/Cas System

- ***Genotyping*** of Microorganisms
- Building ***Resistance to Viruses***
    - To obtain ***phage-resistant mutants***
    - ***To vaccinate bacterial strains***
- ***Genome Engineering***
- ***Gene Therapy***
- ***To regulate endogenous gene expression***
- To label ***specific chromosomal loci*** in living cells or organisms

Outline of generating a CRISPR/Cas9-mutagenised plant line (Belhaj *et al*., 2015)

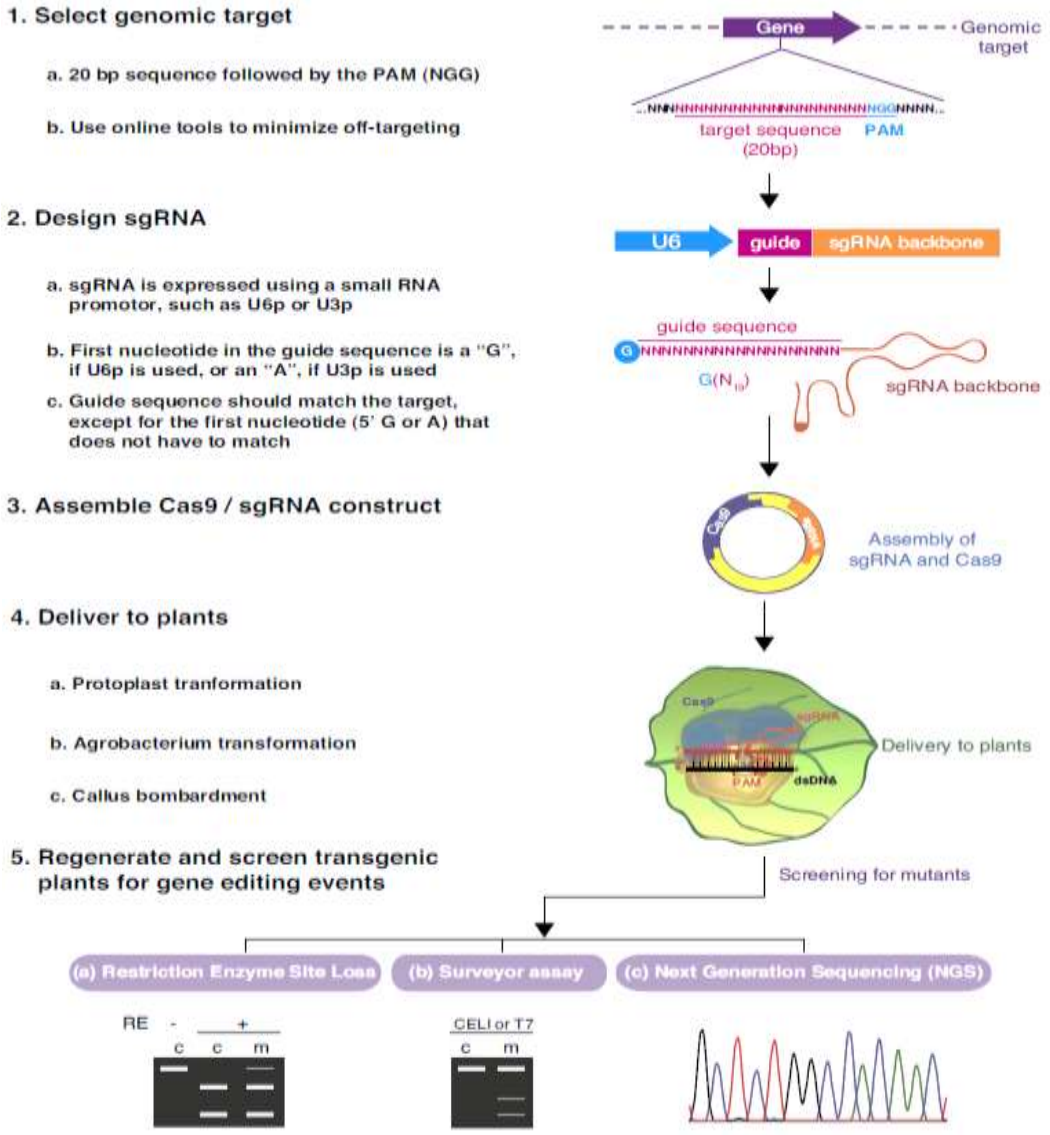

Table 1: List of top genes targeted by CRISPR/Cas system (Kaur *et al.*, 2015)

Genes	Number of entries	Organism
Tyr	252	*Mus musculus, Rattus norvegicus, Xenopus tropicalis, Danio rerio*
ADH1	238	*Arabidopsis thaliana, Nicotiana benthamiana*
PDS	155	*Nicotiana tabacum, Nicotiana benthamiana, Oryza sativa, citrus sinensis*
Prkdc	125	*Rattus norvegicus, Mus musculus*
Tet1	118	*Rattus norvegicus, Mus musculus*
TT4	108	*Arabidopsis thaliana*

B2m	95	*Rattus norvegicus, Mus musculus*
YSA	92	*Oryza sativa*
Tet2	88	*Rattus norvegicus, Mus musculus*
DDM1	87	*Glycine max*
CCR5	86	*Homo sapiens*
PCSK9	81	*Mus musculus*
DMD	80	*Homo sapiens*
Fh	72	*Danio rerio*
Pcdh	72	*Homo sapiens*
HBB	70	*Homo sapiens*
PDS3	66	*Arabidopsis thaliana, Nicotiana benthamiana*

Conclusion:

- The CRISPR/Cas9 technology is versatile method for efficient genome modification of eukaryotes and found useful to study the effect of genome modification on plants through the gene knockout technique.
- The CRISPR/Cas9 system requires only the redesigning of the crRNA which makes it **far convenient** and **more promising** as compare to ZFN and TALEN.
- It has been successfully used in various crop plants like rice, wheat, sweet orange, tomato, etc.; for the development of mutagenised plant lines through genome modification to improve quality parameters.

Future Thrust:

- More efficient CRISPR system should be identified, characterised and developed for better utility and **expanding the targeting range** of RNA-guided Cas9.
- The field urgently needs to develop unbiased strategies to globally assess the **off-target effects** of Cas9 nucleases or paired nickases.
- **Multiplex** genome modification strategies should be developed
- Efforts should be initiated to improve crop plants for **biotic stress resistance**, **nutritional quality** *etc*…

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