AGRICULTURE & ENVIRONMENT
E-NEWSLETTER
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Article ID: AEN-2021-02-06-014

New Era of Plant Genome Editing through CRISPR-Cas9 Technology

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The CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR-associated protein) is replacement term of genome engineering which earlier refer to insertions, deletions, substitutions in the genome of a living organism. The Regularly Interspaced Clustered Short Palindromy Repeats (CRISPR) is an immune system that has been first identified in *E.coli* during 1987 by a Japanese scientist, Yoshizumi Ishino and the term CRISPRs first time coined by Francisco Mojica and Ruud Jansen.

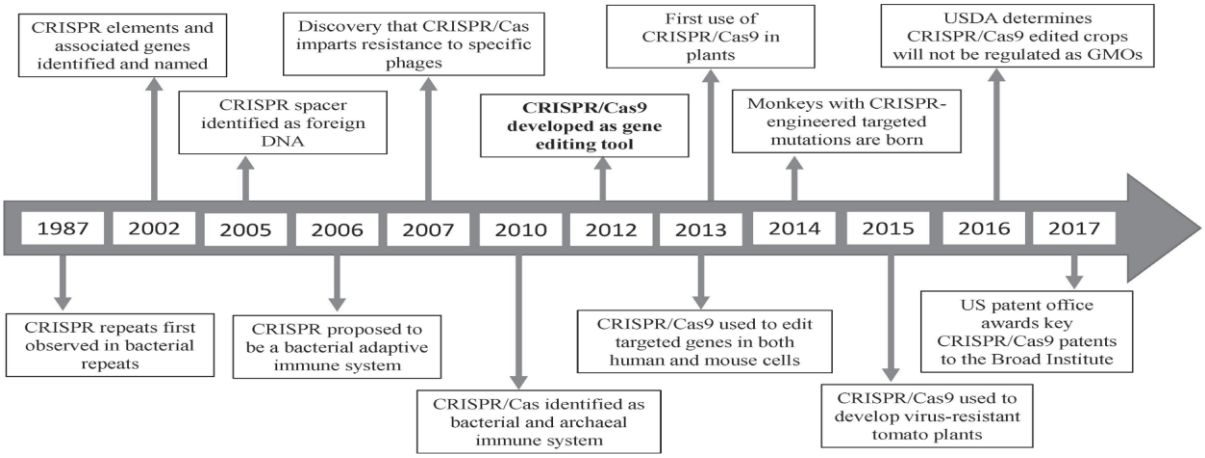


Fig.1 Historical progress of CRISPR-Cas9 (Mushtaq *et al.*, 2018)

Before 2013 three High precision genome editing methods including RNA-guided engineered nucleases (RGENs), Zinc finger nucleases (ZFNs) and Transcription-activator-like effector nucleases (TALENs) were mainly used for gene editing but after 2013 the clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) taken over the genome editing field. In contrast to the above gene editing methods, The CRISPR-Cas9 method is cost-effective, convenient, reliable and user-friendly because CRISPR-Cas9 framework is made up from programmable Cas9 Nuclease and synthetic RNA Short Guide (sgRNA). Whereas, the ZFNs and TALENs is difficult as well as expensive due to requirement of a new protein to conduct an experiment.

Elements of crispr-cas9:

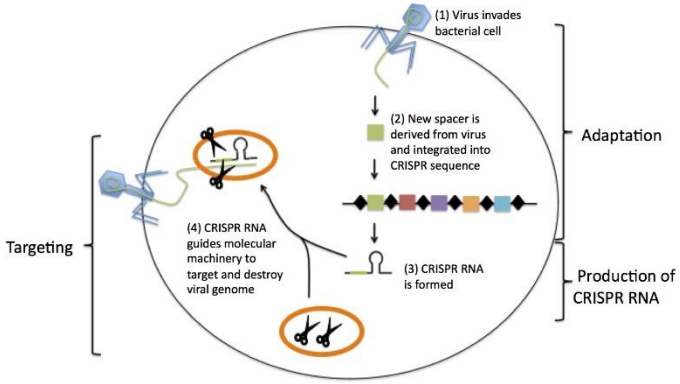
CRISPR-Cas9 Genome Editing requires two main elements: a single-guide RNA (gRNA) and an endonuclease-associated CRISPR (Cas9). When used in CRISPR studies, the sgRNA and Cas9 are coupled into a ribonucleoprotein complex.

a) Cas9: An enzyme of endonucleases that acts as molecular scissors to minimize the diagnostic target DNA sequence. The Cas9 protein is contain six domains viz. REC I, REC II, Bridge Helix, PAM (Protospacers adjacent motif) interacting, HNH and RuvC (**Jinek *et al.* 2014**)

b) The single-guide RNA is a mixture of tracrRNA and crRNA. crRNA includes two main sections, a spacer sequence that leads the complex to the target DNA and a tracrRNA region. As tracrRNA is attached to the crRNA, when the tracrRNA binds to the crRNA, a functional RNA guide is created for Cas9 to be recognized. Several crRNAs can be combined into a crRNA array and then assembled with tracrRNA to form a sgRNA.

Mechanism of CRISPR-Cas9:

CRISPR-Cas9 mechanism is engineered to cause a double-stranded break in the target DNA where a new gene or gene of interest may be inserted. In general, the CRISPR-Cas9 technology can be divided into three steps in order to the invasion of foreign DNA. (1) **Acquisition stage-**In this stage the invasive DNA is recognized and a spacer sequence extracted from target DNA and is injected into the host CRISPR array to create immunological memory; (ii) **Expression stage-** The CRISPR array is translated into a long precursor crRNA (pre-crRNA) which is further translated into mature guide crRNAs containing the memorized sequences of invaders; and (iii) **Interference stage-** The mature CRNA guides the Cas9 protein to identify the suitable DNA target, contributing to the cleavage and degradation of the encroaching foreign DNA (Wang *et al.* 2019 and Haurwitz *et al.* 2010).



Source: https://i2.wp.com/sitn.hms.harvard.edu/wp-content/uploads/2014/07/Pak-Fig-1.jpg

Use of CRISPR-Cas9 in Agriculture:

The rate of world population is increasing as log phase of bacterial growth curve and it is estimated it will cross ten billion till 2050. Besides the population growth, land availability, soil & water quality and increasing biotic and abiotic stresses are significant limitations for farming and food production. Therefore, it is a huge challenge to feed a rapidly increasing population, particularly in the context of food production. So it is important to raise food production with Sustainable agriculture. In last decade the genetic manipulation techniques have been playing major role in sustainable crop production.

Genome editing through manually designed programmable endonucleases is the modern approach. Currently three type of endonuclease (ZFN, TALENs and CRISPR-Cas9) are using for editing the plant genome. The CRISPR/Cas9 is simple and efficient and highly specific promising tool of genome editing and have larger impacts on crop breeding. Gene editing by CRISPR/Cas9 has been implemented in nearly 20 crop species thus far for different traits like yield growth, biotic and abiotic stress control.

Table 1: CRISPR/Cas9 gene editing in agricultural crops

Crop	Target gene	Target trait
Rice	*Gn1a, GS3, DEP1*	Enhanced grain number, larger grain size and dense erect panicles
	OsERF922, OsERF922	Enhanced rice blast resistance
	OsSWEET13	Bacterial blight resistance
	ALS, EPSPS	Herbicide resistance
	OsDERF1, OsPMS3, OsEPSPS, OsMSH1, OsMYB5	Drought tolerance
Wheat	*TaMLOA1, TaMLOB1 and TaMLOD1*	Resistance to powdery mildew
	GW2	Increased grain weight and protein content
	EDR1	Powdery mildew resistance
	TaGW2	Increases seed size
Maize	*Wx1*	High amylopectin content
	TMSS	Thermosensitive male sterile
	ALS	Herbicide resistance
	ARGOS8	Drought stress tolerance
Tomato	*SIMLO1, SIJAZ2*	Powdery mildew resistance, Bacterial speck resistance
	SP, SP5G, CLV3, WUS, GGP1	Tomato domestication
Soybean	*ALS*	Herbicide resistance
Orange	*CsLOB1 promoter*	Citrus canker resistance
Cucumber	*eIF4E*	Virus resistance

*Prabin Adhikari and Mousami Poudel (2020)

Over the last 5 years, it has been actively implemented in crops for functional genome sequencing and for countering biotic and abiotic stress, as well as for enhancing other significant agronomic traits. While some improvements to this technology have contributed to an increase in on-target performance, much of the work carried out is tentative and requires further development.

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