

CRISPR: FROM EARLY DISCOVERIES TO APPLICATIONS IN PLANT SCIENCE

1987

Yoshizumi Ishino, Osaka University, discovers repeated sequences of DNA in *E. coli*

1995

Mojica finds similar repeated sequences in *H. volcanii* and speculates these structures have important functions in prokaryotes^[1]

2001

Mojica proposes the name 'Clustered Regularly Interspaced Short Palindromic Repeats' (CRISPR)^[2]

2005

First paper reporting the discovery of CRISPR is published^[3]. French scientists discover new applications of CRISPR as defence mechanism against bacteriophages

2008

E. coli CRISPR system is inserted into an *E. coli* strain lacking of endogenous system^[5]. Luciano Marraffini discovers CRISPR can block plasmids^[6]

2011

By transferring the entire CRISPR locus, scientists discover Cas9 is the only protein required to commence target interference against plasmid and bacteriophage DNA^[8]

2014

CRISPR/Cas9 is firstly applied in wheat^[11] and a Cas9 tool used for transient expression in sweet orange is developed^[12]

2016

CRISPR/Cas9 is used to develop photo and thermo-sensitive male sterile rice to enhance breeding^[15] and to obtain bi-allelic rice mutants^[16].

1993

Francisco Mojica, Alicante University), discovers multiple copies of palindromic repeated sequences of 30 bp in *H. mediterranei*

2000

These DNA repeated sequences are named 'Short Regularly Spaced Repeats' (SRSR)

2002

Researchers discover CRISPR-associated (cas) genes in proximity of the CRISPR complex able to cut DNA like scissors

2007

Genetic selections to isolate phage-resistant bacteria is performed by using *S. thermophilus* strain and genes cas7 and cas9 are investigated^[4]

2010

Cas9's nuclease activity is found to cut DNA at precise positions encoded by specific sequences of crRNAs. tracrRNA is recognised as an essential component in processing CRISPR function^[7]

2013

CRISPR is firstly used in mammalian cells^[9] and a range of binary vectors carrying Cas9 in Arabidopsis, tobacco, rice and sorghum are constructed^[10]

2015

First application of CRISPR/Cas9 in *H. vulgare* and *B. oleracea*^[13] and development of Cas9 carrier targeting the RIN gene regulating tomatoes ripening^[14]

