Genome Editing Technology in Citrus

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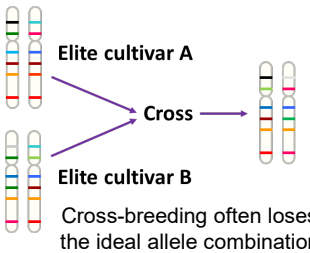
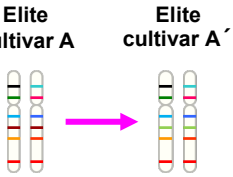
Importance of Genome Editing



Cross-breeding often loses the ideal allele combination

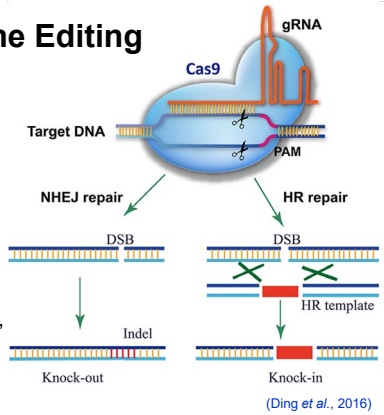


Genome Editing in Clonal Population

Genome editing is the best way to introduce minor changes into the genomes of citrus cultivars with commercial importance.

Principle of Genome Editing

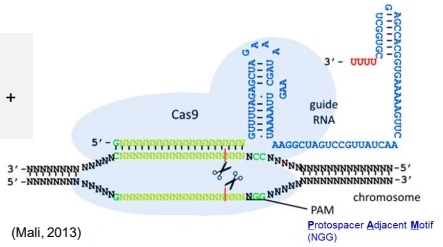
Genome editing is achieved by the introduction of double-stranded DNA breakage (DSB) and its repair through the error-prone non-homologous end-joining (NHEJ) or homologous recombination (HR) with a DNA fragment with desired sequence, resulting in gene mutations.



(Ding *et al.*, 2016)

Technology for Genome Editing

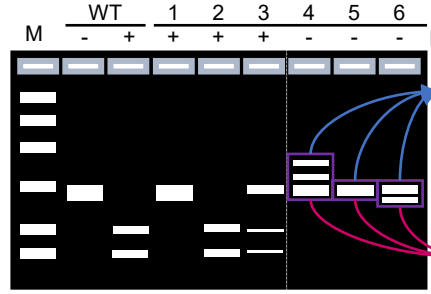
- Cas9, the nuclease
- Guide RNA (gRNA)
  20bp target sequence + gRNA scaffold



(Mali, 2013)

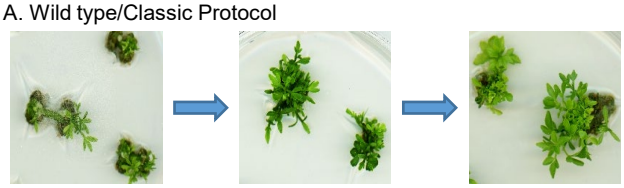
CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 (CRISPR-associated protein 9) technology needs only two components, Cas9 nuclease and guide RNA (gRNA).

Detection of Mutations: CAPS and Amplicon-seq



Difficulty and Break Through in Genome Editing of Trifoliate Orange

A. Wild type/Classic Protocol

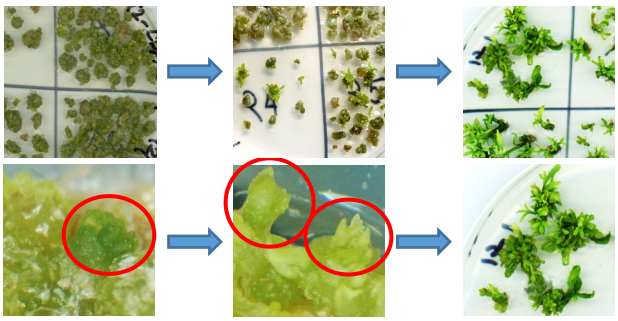


Medium A → Medium B → **Shoot growth**

B. Transformed & genome-edited/Classic Protocol

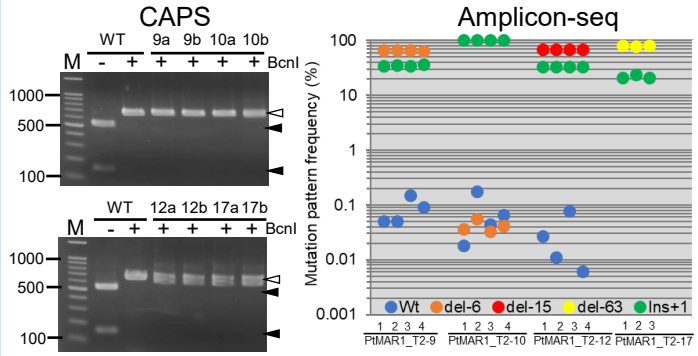
Media A → Media B → **No shoot regeneration**

C. Transformed & genome-edited/New Protocol



Medium C **Shoot primordia formation** → Medium D **Shoot regeneration** → Medium E **Multiple shoots regeneration**

Nearly Clonal Propagation of Genome-Edited Trifoliate Orange Shoots



Genome editing of the *MAR1* Gene conferred Antibiotics Resistance to Trifoliate Orange