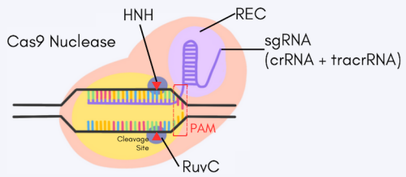
CRISPR-CAS9 IN SOYBEAN

INTRODUCTION TO CRISPR-CAS9

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)- CRISPR Associated Protein 9 (Cas9) is a genome editing tool consisting of the Cas9 enzyme that cleaves DNA, and the guide RNA (gRNA) that can recognise the sequence of the DNA to be edited.

Cas9 Complex

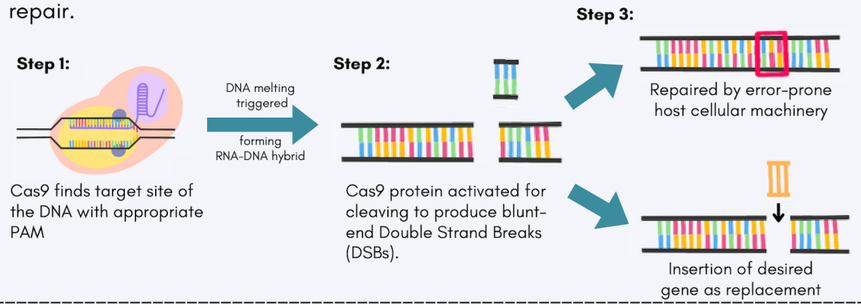


The REC lobe is responsible for binding guide RNA.
The NUC lobe consists of RuvC, HNH, and Protospacer Adjacent Motif (PAM) interacting domains.

- The RuvC and HNH domains cleaves the complementary and non-complementary strand of target DNA respectively.

Mechanism

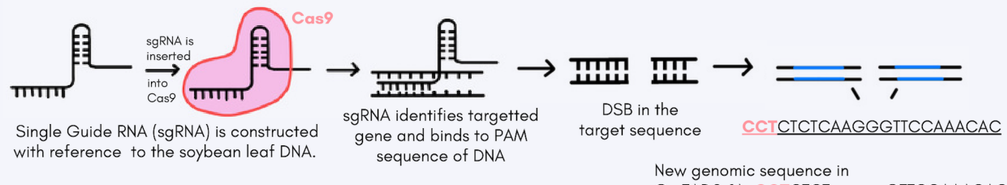
CRISPR-Cas9 can be broken down into three steps: recognition, cleavage, and repair.



THE SCIENCE AND TECHNOLOGY BEHIND CRISPR-CAS9 EDITED SOYBEAN

IMPROVING QUALITY OF SOYBEAN OIL

The high level of polyunsaturated fatty acids renders soybean oil unstable and thus susceptible to the development of unpalatable flavors and trans fatty acids. Hence, CRISPR-Cas9 is used to silence the soybean fatty acid desaturase (regulates unsaturation of fatty acids) encoding genes' (GmFAD2-1A and GmFAD2-1B) expression, reducing the formation of polyunsaturated fatty acids.

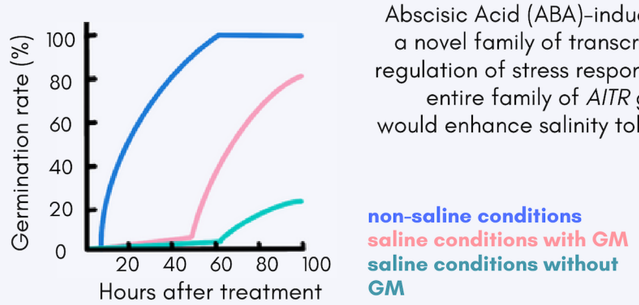


In the cell's attempt to repair the DSB, the GmFAD-1A and GmFAD-1B genes will be silenced.

New genomic sequence in
GmFAD2-1A: CCTCTCT - - - - - GTTCCAAACAC
GmFAD2-1B: - - - - - - - - AAGGGTTCCAAACAC

IMPROVING SALINITY TOLERANCE OF SOYBEAN

High Salinity Level → Inhibition of water and nutrient uptake → Leaf and Stem Area Index reduced Plant growth retarded

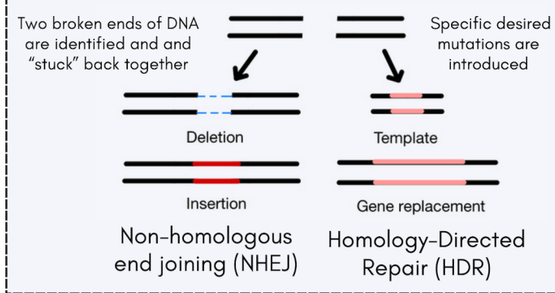


Abscisic Acid (ABA)-induced transcription repressors (*AITRs*) is a novel family of transcription factors that are involved in the regulation of stress responses by the plant. The silencing of the entire family of *AITR* genes using CRISPR-Cas9 in soybean would enhance salinity tolerance, thereby reducing the effects of high salinity on plant growth.

Graphs depicts the germination rate of GM and non-GM soybean under non-saline and saline conditions.

Graph is an adaptation of that of Wang T, Xun H, Wang W, Ding X, Tian H, Hussain S, Dong Q, Li Y, Cheng Y, Wang C, Lin R, Li G, Qian X, Pang J, Feng X, Dong Y, Liu B, Wang S. under the journal, Mutation of GmAITR Genes by CRISPR/Cas9 Genome Editing Results in Enhanced Salinity Stress Tolerance in Soybean, from Front Plant Sci, published on 2021 Nov 26.

HOW ARE THE DSBS REPAIRED?



IMPACTS OF GMO SOYBEANS

Total processed soybean usage by %

80% MEAL — Animal Feed and Food Products

20% OIL — Cooking Oil and Biofuel

HEALTH BENEFITS

Monounsaturated fats are healthier than polysaturated fats as monounsaturated fats have the additional benefit of being high in Vitamin E, and helping to maintain and develop new cells in the body.

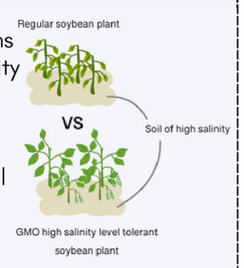
GmFAD2 silencing using CRISPR-Cas9 thus improves the health content of soybean oil and soybean.

INHERITANCE OF BENEFICIAL TRAITS

The parent soybean plant can pass down these beneficial traits to their offspring, increasing the population of good quality soybeans.

HIGHER CROP YIELD

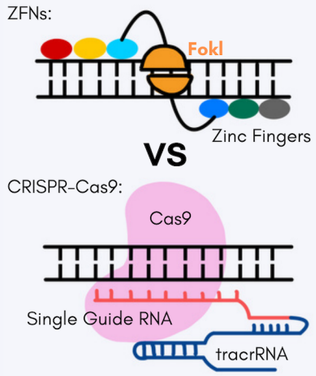
GM technology to make soybeans more tolerant towards high salinity levels allow more soybeans to reach the mature stage, increasing and stabilising the soybean supply which is essential as the demand for soybean is rising with the increase in global populations.



BENEFITS AND RISKS OF APPLICATION OF CRISPR-CAS9

ADVANTAGES

1. **Multiplexed Gene Engineering:** Since CRISPR-Cas9 relies on the guide RNA (gRNA) to direct the Cas9 enzyme, introducing multiple gRNAs targeting various genes allowing for edits at the same time. These gRNAs guide the Cas9 enzyme to multiple desired genomic locations, inducing double-strand breaks and enhancing efficiency.
2. **Simpler Gene Editing:** Compared to CRISPR-Cas9, the first-generation gene editing tool, Zinc Finger Nucleases (ZFNs) require two important domains: a DNA-binding domain and a DNA-cleavage domain known as FokI, in order to perform genetic engineering. In contrast, CRISPR-Cas9 does not need to be paired with a separate cleaving enzyme as the Cas9 is capable of cleaving DNA strands, making the process simpler.



DISADVANTAGES

1. **Potential for Off-Target Genome Editing:** namely unwanted changes to the genome.

A popular method to detect off-target sites is GUIDE seq. This technique relies on the delivery of double stranded oligonucleotides (dsODNs) with known sequences. It integrates into double stranded breaks and provides templates for polymerase chain reaction (PCR) amplification that allows for detection of mutants.

GLOBAL FOOD SECURITY LANDSCAPE

Definition

"Food security is the state of having reliable access to a sufficient quantity of affordable, nutritious food…The four pillars of food security include **availability**, **access**, **utilization**, and **stability**."

Contributing factors

1) Ongoing conflicts around the world

- Reduced food exports from the countries involved in these conflicts, especially those who are major food exporters, lead to supply shortages and inflation in food prices globally.

2) Climate Change and Shocks

- Growth of crops require specific conditions but erratic weather and more frequent natural disasters makes it harder for these crops to grow.
- Increasing evaporation and transpiration rates due to higher temperatures affects natural soil salinity rate which limits crop growth.

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This infographic poster, entitled "CRISPR-Cas 9 in Soybeans", was created by Jaelyn Chua, Cherise Chua, Chloe Heng and Renae Chua of CHIJ St. Nicholas Girls' School, as an entry to the GMAC Students Challenge 2024.

The contents of this infographic may not necessarily reflect the views of GMAC.