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CRISPR/Cas9: Utilising CRISPR/Cas9 to Enhance Crop Quality

Komal Choudhary[1] and Sanju Choudhary[2] Arti sharma[3]

Abstract

The alterations (insertions, deletions, and substitutions) made to a living organism's genome are referred to as genome engineering, genome editing, and gene editing. These days, the most popular method for editing genomes is based on CRISPR-Cas9, which stands for Clustered Regularly Interspaced Short Palindromic Repeats and associated protein 9. CRISPR-Cas9 is an adaptive immune system found in prokaryotes that inherently shields cells from DNA viral infections. Through modifications, CRISPR-Cas9 has been transformed into a flexible genome editing tool with a plethora of uses in fundamental research on gene functions, medicine, and agriculture. More and more monocot and dicot plant species are using CRISPR-Cas9 to improve production, quality, and nutritional value as well as to introduce or improve resistance to biotic and abiotic challenges, among other uses. Here, we go over the fundamentals, recent developments, and agricultural enhancement uses of CRISPR-Cas9-based gene editing.

Introduction

During several decades, modern agricultural methods have led to a large increase in crop productivity. As a source of several nutrients, including proteins, fibre, vitamins, minerals, and bioactive substances, crops have come under increased consumer scrutiny due to their direct correlation with human health.

A range of techniques, such as molecular marker-assisted breeding, genetic engineering breeding, chemical and radiation-mediated mutation breeding, and conventional crossing breeding, have been effectively employed to enhance diverse agricultural attributes. The precise and predictable modification of plant genomes by genome editing (GE) technology is shown clear

Komal Choudhary[1] and Sanju Choudhary[2] Arti sharma[3]

[1]Research Scholar, Department of Plant Breeding and Genetics, Shri Karn Narendra Agriculture University, Jobner, Rajesthan (303329)

Author Mail- komalchoudhary522001@gmail.com

[2]Ph.D Scholer, Department of Plant Pathology, SKNAU, Rajasthan Agriculture Research Institute, Durgapura, Jaipur, Rajasthan (302018)*

[3]Ph.D Scholer Department of Entomology, (RARI) Durgapura Jaipur, Rajasthan

benefits in crop breeding lately. With the least chance of being off-target and no integration of external gene sequences, genome editing can produce predictable and inheritable mutations in certain genomic locations. CRISPR/Cas systems have become to be the most widely used GE technology in recent years. When it comes to genome editing, CRISPR/Cas systems are more effective and simple to employ than other SDNs because the editing's specificity is determined by the guide RNA's nucleotide complementarity to a particular sequence, rather than requiring intricate protein engineering. As a result, CRISPR/Cas technologies have been used by numerous researchers for gene functional study.

CRISPR/Cas9 System for Gene Editing

CRISPR-Cas9 is a revolutionary technology in the field of molecular biology that allows for precise and targeted genome editing. The term "CRISPR" stands for Clustered Regularly Interspaced Short Palindromic Repeats, which are specific DNA sequences found in the genomes of bacteria and other microorganisms. These sequences have been adapted for use in genetic engineering.

The two primary parts of the CRISPR-Cas9 system are the Cas9 protein and CRISPR RNA (crRNA). While the Cas9 protein functions as a molecular scissors, cutting the DNA at the desired site, the crRNA contains a guide sequence that is complementary to a particular target DNA sequence.

This is a condensed explanation of how CRISPR-Cas9 functions:

1. Designing the Guide RNA (gRNA): Scientists design a synthetic RNA molecule (gRNA) with a sequence complementary to the target DNA they want to modify.
2. Formation of CRISPR-Cas9 Complex: The gRNA is combined with the Cas9 protein to form a functional complex.
3. Target Recognition: The gRNA guides the Cas9 protein to the specific DNA sequence in the target organism's genome. The guide RNA is engineered to precisely match the target sequence.
4. DNA Cleavage: Once the CRISPR-Cas9 complex locates the target DNA sequence, the Cas9 protein acts as molecular scissors and induces a cut in the DNA at that precise location.
5. Cell Repair Mechanism Activation: After the DNA is cut, the cell's natural repair mechanisms come into play. There are two primary repair pathways: Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR).

- NHEJ: This frequently results in minor insertions or deletions (indels) at the cut site, which might disrupt the gene

and perhaps cause it to become inactive.

- HDR: By supplying a DNA template with the required sequence throughout the repair process, it is possible to add particular modifications or "edits"

Crop quality has proven to be a crucial factor in establishing the crops' market worth. Both internal and exterior characteristics generally influence crop quality. Size, colour, texture, and aroma are examples of physical and aesthetic qualities that make up the exterior

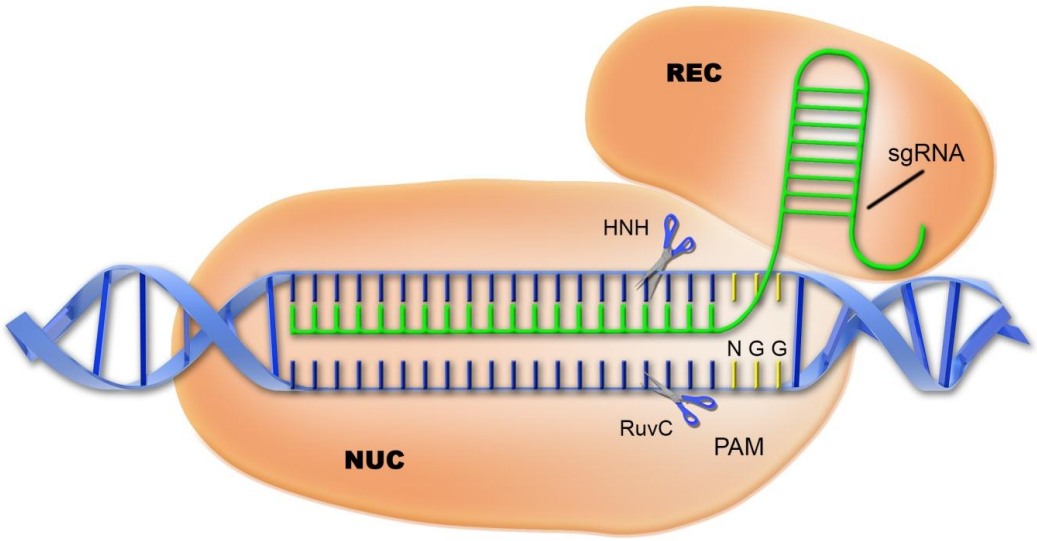

Figure 1. RNA-guided cleavage by the Cas9 protein

CRISPR-Cas9 has a wide range of applications, including gene therapy, functional genomics, agriculture, and biotechnology. Its versatility and relative ease of use have made it a powerful tool for researchers and scientists working in various fields. However, ethical considerations and potential off-target effects are also subjects of ongoing discussions and research within the scientific community.

Molecular Breeding Assisted by CRISPR/Cas9 Accelerates Crop Quality

quality traits. On the other hand, the internal quality factors consist of bioactive molecules like lycopene, carotenoids, flavonoids, γ-aminobutyric acid, and others, as well as nutrients like protein, carbohydrate, and fats. Crop quality was improved using CRISPR/Cas9 with an emphasis on fruit texture, edible quality, physical beauty, and nutritional content.

1. Enhancing the Physical Appearance of the Crop

1.1. Changes in Dimensions and Form

Rice and tomatoes have yielded the greatest information regarding the management of fruit shape and size. In five kinds of Japanese rice, GS3 (GRAIN SIZE 3), the first QTL found to regulate grain length, has been effectively eliminated. In comparison to wild type, the T1 lines' grain lengths have grown across all genetic backgrounds. Grain weight (GW) is influenced by grain shape as well as quality. For instance, disruption of several GW negative regulators, including GW2, GW5, and GW6, has raised rice GW.

1.2. Alteration of Colour

For example, Americans and Europeans choose red tomatoes, but Asian customers favour pink tomatoes. Research has shown that the lack of flavonoid pigments in the peel was the cause of the pink phenotype. Therefore, using CRISPR/Cas9 to delete genes involved in the pigment manufacturing pathway, it is possible to manipulate the colour of fruits. As a transcription factor involved in the flavonoid biosynthesis pathway, MYB12 influences the accumulation of flavonoids and controls the phenotypic of pink skin. It has been successfully accomplished to create pink-fruited tomatoes by knocking down SlMYB12.

2. Enhancing the Texture of Crops

- **Increasing the Shelf Life**

The technology known as CRISPR/Cas9 has the potential to significantly increase the shelf life of tomatoes and bananas.

Numerous naturally occurring mutant genes, including Nr, alc, rin, nor, and Cnr, have the ability to increase shelf life. These mutations do, however, come with minimal nutritional content, an unpleasant flavour, and lack of colour. According to one study, alc mutation preserved the colour and scent of the fruit in addition to extending shelf life.

3. Enhancing Tastiness

3.1. Enhancing the Quality of Eating and Cooking

Amylose production requires the Waxy (Wx) gene, which codes for granule-bound starch synthase I (GBSSI). Asian consumers tend to favour rice varieties with a somewhat low amylose level (7–10%) because they cook up to be soft and sticky. Using the CRISPR/Cas9 system, several genetic improvement experiments have effectively mutated the Wx gene in the japonica background rice accessions, producing those with a grain amylose content of 5–12% without sacrificing other desirable features.

3.2. Improving Flavor

According to research, the majority of aromatic rice varieties are particularly high in 2-acetyl-1-pyrroline (2AP) chemical, which is also essential for fresh bread and popcorn and gives food products a scent similar to that of popcorn and crackers. Studies on genetics have demonstrated the co-segregation of fragrance synthesis with BADH2, which codes for a

betaine aldehyde dehydrogenase. It has been found that whereas non-functional mutations of BADH2 convert GABald into 2AP, functional BADH2 assisted in the conversion of γ-aminobutyraldehyde (GABald) into GABA

4. Nutrient Elements Biofortified

Numerous nutrient components found in fruits and vegetables have anti-oxidant, anti-inflammatory, and anti-cancer properties. Breeding programmes have been put into place to biofortify different crops with carotenoid, γ-aminobutyric acid (GABA), iron, and zinc concentrations. It has been attempted to use gene editing for biofortification to satiate the "hidden hunger" with high-quality nutrients.

4.1. Raising the Content of Carotenoid

Rice, tomato, and banana carotenoid biofortification has been achieved by the use of CRISPR/Cas9-mediated genome editing. Since there is no foreign gene integration in the host genomes, the ones generated by this method show promise for escaping a genetically modified regime. Generally speaking, carotenoid biofortification was accomplished using two different approaches. Firstly, carbon flow is imposed into the carotenoid biosynthesis pathway by overexpressing phytoene synthase genes via CRISPR/Cas9-mediated knock-in. This allows rice to have marker-free gene-edited mutants with 7.9 μg/g of β-carotene in dry weight. It also allows for the integration of a carotenogenesis cassette containing the CrtI and PSY genes into the rice target site.

4.2. Micronutrient Biofortification

One potential application of CRISPR/Cas9 in rice is the suppression of Vacuolar Iron Transporter (VIT) genes, including OsVIT2, to increase the iron content of the grain. According to a recent study, OsVIT2 mutation enhanced the distribution of iron in the grain's embryo and endosperm and, ultimately, raised the amount of iron in the polished grain without having an adverse effect on yield.

4.3. Improving the Content of Fatty Acids

The most produced and consumed edible oil is soybean oil, which has a far lower oleic acid content (20%) than olive oil (65–85%). To control the fatty acid content of soybeans, several fatty acid desaturase genes, including FAD2 and FAD3, were specifically targeted and altered. By modifying two homeologous genes of GmFAD2, scientists were able to raise the amounts of oleic acid from 20% to 80% in 2019, whereas the levels of linoleic acid decreased from 50% to 4.7%.

4.4. Eliminating Anti-Nutrients

CRISPR/Cas9 has been used to eliminate an ITPK gene that codes for an enzyme that catalyses the penultimate step of phytate production, thereby lowering the phytic acid content of rapeseeds. Plant

performance was unchanged despite a 35% decrease in phytic acid in the ITPK mutants.

Conclusion:

The most popular and adaptable tool in crop breeding and functional genomics now is the CRISPR/Cas9 system. Its unparalleled capacity to manipulate genes aided in the development of a large number of crop types with the intended agronomic traits. But most efforts to improve crops through gene editing are still in the early stages of figuring out how the genome functions and what the regulatory mechanisms are. Commercialization of crops modified with genes is still a ways off. Furthermore, not all of the conditions for modifying the plant genome have been fulfilled by gene-editing technologies. The application of CRISPR/Cas in plants will require more development since multiple QTLs govern several quality-related variables, and changing a single gene might not have a major impact on phenotypic change. The development of an effective CRISPR/Cas mediated chromosomal rearrangement technique is achievable.

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