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Gene Editing in Plants

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Abstract--- *Gene editing or genome editing is a type of genetic engineering in which the DNA is introduced, deleted, modified or replaced into the genome of a living organism. It is the deliberate alteration of a selected DNA sequence in a living cell. In this, a strand of DNA is cut by using the molecular scissors at a specific point and the broken DNA strands are fixed together with the help of DNA ligase by naturally existing cellular repair mechanism. It was first accomplished by Herbert Boyer and Stanley Cohen in 1972. It is an important technology for making changes in DNA, which includes the changes in physical traits such as eye color or risk of any disease. Genome / gene editing using Engineered Endonuclease (GEEN) systems have been well established over the fields of plant biotechnology. Till now, gene editing have been implicated in various different plants such as Arabidopsis including the main crops like rice, wheat and maize and some less important crops such as strawberry, cucumber, etc. To improve the safety of food, the research in plant biology aims at improving the yield of crops and various other factors such as biotic and abiotic stress, along with the enhancement in the nutritional content of food.*

Index Terms--- *Gene editing, Genetic engineering, Molecular scissors, DNA ligase, Cellular repair mechanism, Genome editing using engineered endonucleasre (GEEN), Plant biotechnology, Biotic and abiotic stresses.*

I. INTRODUCTION

Through slowly embracing chemical substances, improved plant varieties and advanced equipment, agriculture has benefitted from science and technology. Increased food and feed production as well as cost reductions for producers and consumers are amongst the economic benefits of such advances. In reality, plant-based innovations gradually expand in agriculturally intrinsic innovations.

Over the last three decades, research in agriculture has been extended beyond the input traits of GM crops [1][2][3] and spread to the marketing of output-traits of GM products. This development is due to several new techniques for breeding (NBT)[4][5], such as gene editing. But like most biotechnological developments and particularly, food-related ones, countries are assimilating or rejecting them on the basis of distinct socio-economic and political realities [6].

Genome editing is commonly used both for the fundamental research and the direct enhancement of desirable characteristics in market crops in the field of plant science for the targeted gene improvement.

Most of the tools used for genome editing have been targeted so far. In addition, the Oligonucleotide Directed Mutagenesis (ODM)[7][8], the source of which was formed at the beginning of the 1980s and which found its way in plant science 115 years ago, primarily engineered nuclease (ENs) is used. The engineered nucleases are divided into four types – (i) Zinc – Finger Nucleases[9][10], (ii)Meganucleases, (iii) Transcription Activator-like Effector Nucleases

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(TALENs)[11]–[14] and (iv) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) – Systems[15].In most of the cases, the introduction of the genome editing tools is achieved by the incorporation of stable integrated plants into the plant genome (figure 1). The plants are self-pollinated or are crossed with each other to get free from the incorporated DNA; the only feature that remains is intended mutation. However, in some cases, it is seen that the transient expressions of the genome editing tools via plasmid initiate these mutations in the plants, but all of these techniques make use of the recombinant technology to use the recombinant DNA in the intermediate step. At later stages, the tools were well developed by using the "solely RNA, preassembled Cas9 protein-gRNA ribonucleoproteins (RNPs) or TALEN – proteins" for inducing the mutation in plants (figure 2). All these tools are completely exempted from the DNA, so the integration of DNA into the genome of a plant can be excluded.

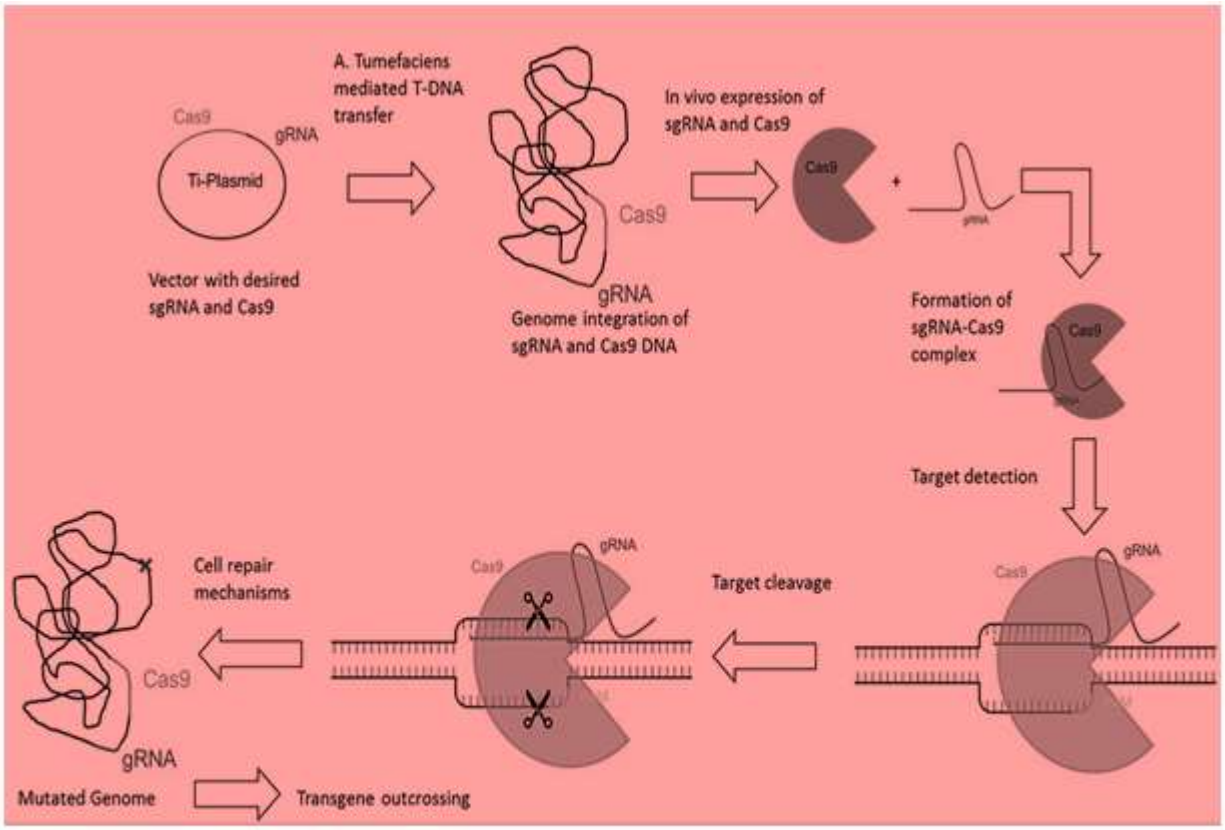

Fig.1: Exemplary comparison of classic CRISPR/Cas9and DNA-free CRISPR/Cas9. Comparison of classic CRISPR/Cas9 through the example of tumefaciens transformation and DNA-free CRISPR/Cas9 exemplified by PEG-mediated protoplast fusion.

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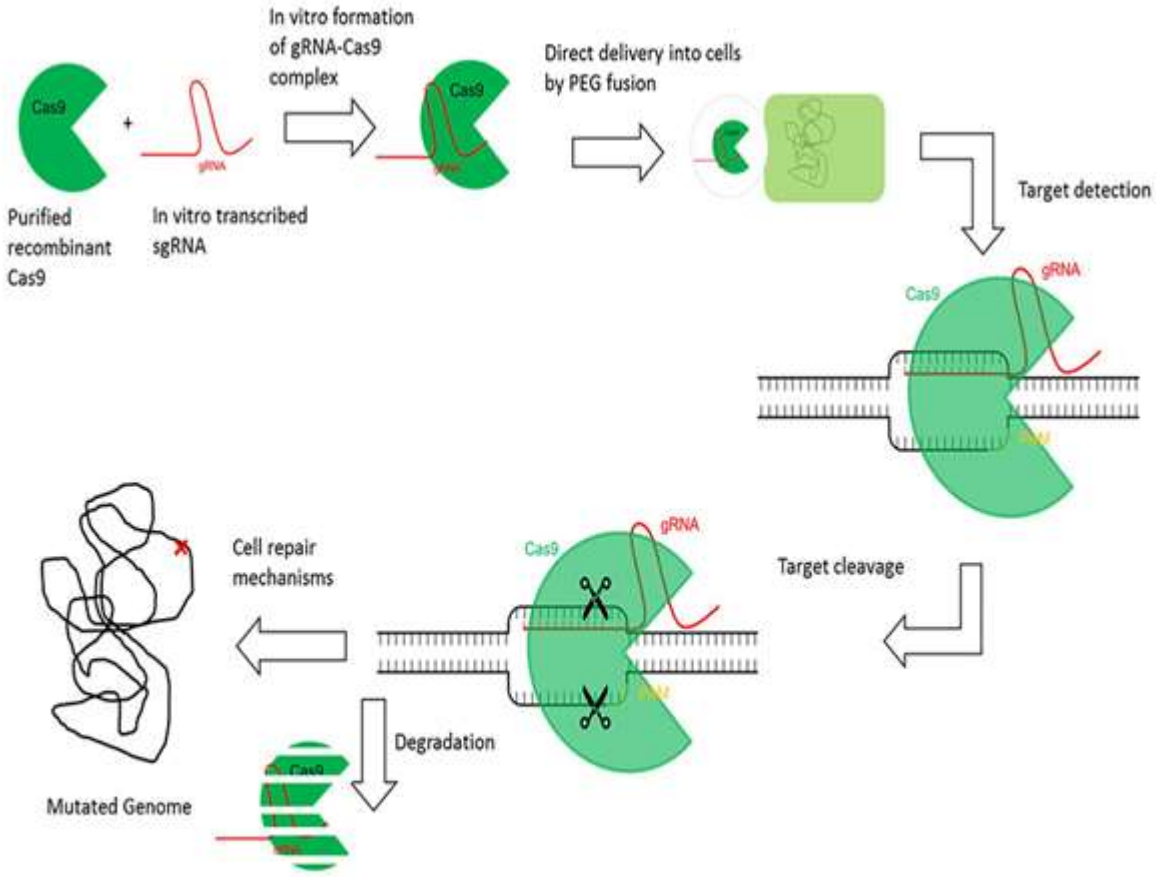

Fig.2. Exemplary comparison of classic CRISPR/Cas9 and DNA-free CRISPR/Cas9. For DNA-free CRISPR/Cas9 recombinant Cas9 and in vitro translated gRNA are required.

"CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) genome editing has the potential to enhance the improvement of crops and the production of food as opposed to the imprecise and lengthy conventional (CONV) breeding. The technology for gene editing makes it possible to re-arrange plant genome with the targeted and high precision. Several people belonging to the scientific community hope that the genome editing will contribute considerably to the precision breeding by means of preliminary applications and the biological principles underlying genome editing, thereby increasing the product development costs as opposed to CONVs and genetic modifications. The techniques which are based upon the concept of gene editing provides new strategies and opportunities to develop crops with good improvements at low costs by clearly and properly inserting the favorable traits or removing the undesirable traits from the genes of the plants.

The gene edited crops have the potential to provide good benefits for the consumers. Some of the examples of the gene edited crops include – soybean, tomatoes, potatoes, etc. Although, diverging the social expectations on health and possible environmental threats of gene edited crops and foods in and between the main consuming markets do not, however, bode well on acceptance of the product and further regulatory approval.

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II. HISTORY OF GENOME EDITING OF PLANTS:

The genes of the plants have been altered by nature from several years, with the natural selection enabling the genome - edited plants to survive even in the extreme temperatures. However, all the crops growing and used by the people have been altered by the genetic modifications. These genetic modifications are the significant factors for the improvement in the genes of the crops, but earlier when these genetic modification practices were not available, the naturally occurring mutations were used to modification of genes in plants, but these mutations were harmful for the crops as these induces various diseases in the genes of the crops.

The discovery that Agrobacterium tumefaciens, the bacterium that causes the crown gall disease, is a natural genetic engineer which introduces a piece of its own DNA in the genome of an infected plant, theoretically bearing a DNA sequence that is provided by a scientist, has been a major advancement in the genetic modification. This bacterium introduces a tumor-inducing (Ti) plasmid into the genome of a plant cell. The plant biotechnology base was the development of "binary vectors" that are derived from the Ti-plasmid, which can replicate in E. coli and Agrobacterium and still can be incorporated in the plant genomes. With these devices, even the genes which are derived from the organisms located at the distant location can be introduced into the plant genome and this process is known as "transgenesis", while if the genes are derived from the similar species of plants, then this process is known as the "cisgenesis". Though, this strategy has many disadvantages, such as the nature of the introduction of gene, the potential to kill functional genes, public concern regarding GMOs, and the inefficiency in using the plant's native genetic range.

The gene-targeting technology was first established by Mario Capecchi in 1980, together with the idea of harnessing double-strand breaks (DSBs) for gene editing. The ability to produce site-specific double-strand breaks (DSBs) was developed later. The DSBs are produced to determine the genetic performance via an imprecise repairing of the "non-homologous end joining (NHEJ)" or the exact process of repairing the homological repair (HDR) by harvesting the cell's own repair machinery (figure 3). NHEJ has the property of insertion, and deletion of few bases or removal of the genes. More than one DSB allows for even more types of changes such as chromosome deletion, gene inversion and chromosomal translocation, with DSBs on two different chromosomes. Unlike NHEJ and HDR which allows the repairing and enables the user-defined rewriting of the sequence (figure 3). The genome editing can be achieved by using HDR which precisely alters the genome using various types of genome repair models, ranging from short oligonucleotides to hundred base pairs of the genes with homologous ends or arms flanking the DSB site.

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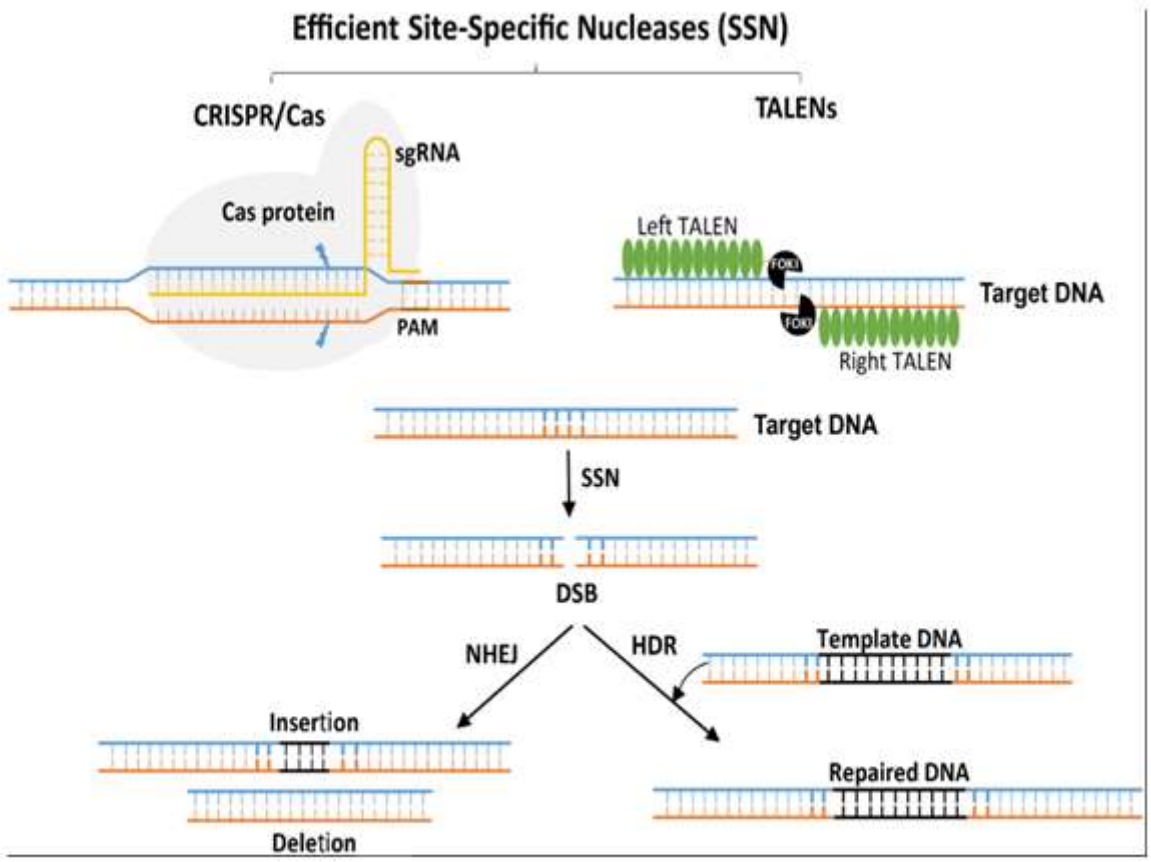

Fig.3. Site-specific nuclease-induced genome editing.

III. CURRENT APPLICATIONS OF GENOME-EDITING IN PLANTS:

The authors currently promote their work as transgenically-free in a series of publications, but by examining those publications, which shows that transgenicity is the only end product. In many cases, the transient plasmid CRISPR-DNA expression or steady integration with subsequent backbone transition has led to the mutation. DNA can still be incorporated into the host genome for both techniques, as plasmids in the cells are degraded and integrated into the sites that have been cut off. DNA-free editing originates in the editing and frequent adaptation of animal cell lines or embryos for most of the species. As there was concern raised that the plants which are transformed by using the DNA might be covered by the genetic technology and this concern was raised due to the DNA-free editing of plants, which is a newest and most emerging field.

IV. CONCLUSION

With high efficiency, easy engineering and robustness, CRISPR/Cas systems have revolutionized plant genome technology and democratized its application. The current state of this technology allows various applications to improve crop production, disease resistance and climate change adaptation. There is still a need for several

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technological improvements; particularly accurate editing and delivery of reagents from genome technology in germ cells to avoid tissue culture requirements.

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