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Cultivating the Future: Next-Generation Agriculture Biotechnology

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Abstract
Agricultural sector is facing many new challenges such as climate change, rapidly increasing world population and the scarcity of resources due to ever increasing population. With climate change, population growth and resource depletion pose an unprecedented threat to global food security. While with the technological advances with time, new techniques have emerged which helped researchers to analyze and develop new cultivars in crops. The future of agriculture biotech is swift to transform the farm industry, offering new-age solutions for world issues. The present chapter deals with recent advancements in Agricultural biotechnology (specifically cutting-edge genome editing techniques and their role in crop improvement) by giving detail about the up-to-date gene editing tools like CRISPR-Cas9, ZFN and TALEN regarding its mechanisms, advantages of disadvantages of each type with respect to recently developed prime-editing technique which is considered as generation-next advancement. These new techniques provide unprecedented accuracy during genetic modifications. Various aspects of synthetic biology including 'omics' approaches are also discussed here. The present techniques may be useful to improve crop traits such as disease resistance, abiotic stress tolerance and nutritional content in different crop genotypes. The pros and cons of using these technologies to speed up breeding programs, develop new traits or to solve important agricultural issues are also discussed.

Keywords: Agriculture Biotechnology, CRISPR-Cas9, Genome editing, TALEN, ZFN

1. Introduction
The agricultural industry is going with a transition phase and at present standing at a critical juncture to feed a growing population of India and World as well. The farming sector is changing continuously with the advancement of technology, and this change brings a series of interrelated consequences to enhance the traits and finally the yield. World is facing environmental issues such as sudden surge of temperature, occurrence of erratic rain, flood due to changing in the climatic conditions. Another issue is biotic stress to plants such as diseases etc. The best way to prevent the harmful effects of climate change on food security and crop productivity is by not allowing it in firsthand as this directly impacts growth with minute weather patterns around higher temperatures. With an estimated 9.7 billion people on the planet by 2050, our food systems are under tremendous strain! Water scarcity, new diseases and pests that impact sustainable agriculture, and the loss of arable land are challenges. With the promise of strong instruments capable of resolving these intricate problems, biotechnology shines like a bright light in this dismal situation.

Since its inception, biotechnology has primarily benefited agriculture, even outperforming traditional applications of genetically modified organisms (GMOs). Even in our present endeavors to build robust, fruitful, and sustainable agricultural systems, it continues to serve as a foundation. When combined with conventional breeding methods and information used to guide variety release decisions, biotechnology offers a set of tools that can be used to improve crop varieties. These tools include improved nutritional content, increased yield potential, resistance to pests and diseases, and the ability of plants to adapt to changing environmental conditions.

The field of next-generation agricultural biotechnology is vast and teeming with innovative concepts, instruments, technologies, and research approaches. High-precision gene-editing tools like CRISPR-Cas9, which allow for precise modifications to plant genomes to create new varieties, are at the forefront of this endeavor. Synthetic biology goes even farther with gene-editing, enabling us to create not only biological systems but also new metabolic pathways in crops. The creation of large sets of high-throughput data from proteomics, metabolomic, and genomics techniques allows for a thorough understanding of plant biology and supports clever crop production techniques. Agricultural biotechnology will also advance in tandem with other technological advancements. Data-driven farming has become a reality with the advent of biotechnology and the use of artificial intelligence.

Technology has a wide range of potential uses, from developing climate-proof crops to genetically altering plants to produce more nutritious food or even serving as bio-factories for crucial molecules that would otherwise be difficult for humans to obtain.

Next-generation agricultural biotechnology holds great promise but realizing it will take a comprehensive strategy that includes not only scientific advancements, but also public acceptance and economic viability that far outweigh the success of early genomics tools like genetically modified crops. Environmental stewardship must also become an equal partner. The focus of this chapter is on the cutting edge of next-generation agricultural biotechnology and how it can transform farming practices, ensure food security, and usher in a sustainable future. The most recent developments in next-generation agricultural biotechnology will be covered in this chapter, along with how they have the potential to transform agriculture, increase food security, and create a sustainable future.

2. Methodology

The present review was prepared through a comprehensive and systematic survey of scientific literature focusing on recent advancements in agricultural biotechnology. Relevant research and review articles were collected from major databases including Scopus, Web of Science, PubMed, and Google Scholar. The search was performed using combinations of keywords such as “next-generation agriculture,” “biotechnology,” “genome editing,” “synthetic biology,” “CRISPR,” “plant transformation,” and “sustainable agriculture.”

3. Key Technologies Driving Next-Generation Agriculture

3.1. Zinc Finger Nucleases (ZFN)

A class of modified DNA-binding proteins called zinc finger nucleases is used to cause eukaryotic cells to either add or delete genes. ZFNs are chimeric restriction enzymes that are made by fusing the cleavage portion of the FNL gene with a zinc finger DNA-binding domain. Custom-designed ZFNs that specifically target any desired DNA sequence within the framework of a complex genome can be created because zinc finger domains can be made to recognize particular DNA sequences[1]. By using endogenous DNA repair pathways, these reagents allow for the precise manipulation of higher eukaryotic genomes. ZFNs are created by joining a cleavage domain (often the restriction enzyme FokI) to a zinc finger DNA-binding domain, which can be modified to target user-specified DNA sequences. Double-strand breaks (DSBs) at user-specified genomic locations are produced by zinc finger

nucleases (ZFNs), opening the door to gene editing.

3.1.1. Process of ZFN

i) Design: Engineering a zinc-finger domain to recognize specific DNA sequences. ZFs are modular protein domains that bind to DNA sequences; typically, each ZF recognizes three base pairs of DNA and multiple ZFs can be linked together recognizing longer sequences.

ii. Binding: A pair of ZFNs, each with a specific DNA-binding domain, binds to the target DNA sequence on opposite strands.

iii. Cleavage: FokI endonuclease domains dimerize and cleave both DNA target strands causing a double-strand break

iv. Repair: The cell's DNA repair machinery assembles on the break and then mends it, frequently causing small insertions or deletions (indels) that disrupt gene function. Instead, researchers may provide a template DNA to insert or replace particular genes during the repair.

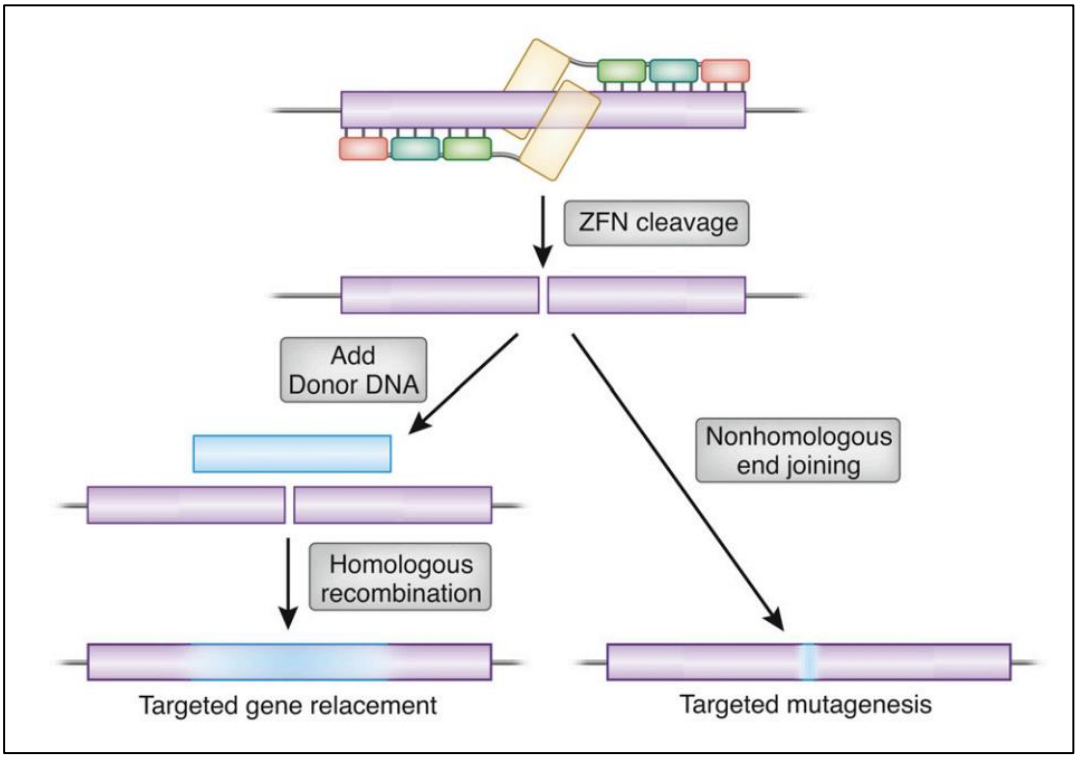

Figure 1: Schematic representation of Zinc Finger Nucleases[1]

3.1.2. Applications of ZFN in agriculture

ZFNs have various applications in agriculture, including:

Targeted Mutagenesis: ZFNs can be utilized to knock out/disrupt or modify gene through the introduction of target mutations leading to the development of traits in crops.

Disease Resistance: Same as with insects, ZFNs can also be used to build well disease resistant plants by altering or knocking out genes which aid in either susceptibility (preferred) and defense mechanisms.

Herbicide Tolerance: The ZFNs help create herbicides resistant to crops by targeting key genes important for herbicidal metabolism.

Improved Nutritional Value: ZFNs can alter transgenes involved in nutrient biosynthesis, resulting in a more nutritious plant yield.

3.1.3. Examples of crop improvement using ZFN

Disease Resistance in Maize

ZFNs use molecular scissors that create a double-stranded break at the desired site in DNA, inhibiting gene expression when ZNF is applied to

maize it was conferred with resistance against northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* through targeted disruption of *IPK1*. This change paved the way for ZFN edited maize lines with enhanced NCLB resistance to becoming commercially viable.

Herbicide Tolerance

Maize: The *acetolactate synthase (ALS)* gene was targeted using ZFN-mediated genome editing and conferred imidazolinone herbicide tolerance in maize by generating the ALSIR1-739 allele, which had an amino acid change at position 194 compared with wild-type plants[2]. This keeps weeds better at bay while also causing less damage to the crop (maize).

Soybean: ZFNs were used to create herbicide-tolerant soybeans by disruption of the AHAS gene (which encodes ALS)[3].

Nutritional Enhancement in Soybean

ZFN has been applied to improve oil quality of soybeans by targeting the FAD2 gene that increased oleic acid content. High-oleic soybean oil has enhanced oxidative stability and provides a healthier option for intake, containing reduced amounts of trans fat[3].

Yield Improvement in Maize

ZFN-mediated gene editing has been employed to modify the Wx1 locus, which encodes a key enzyme involved in starch biosynthesis and is responsible for amylose synthesis in maize kernels[2]. This change may result in better grain quality and possibly higher yields.

3.2. TALEN (Transcription Activator-Like Effector Nucleases)

TALENs (Transcription Activator-Like Effector Nucleases) are also restriction enzymes which have been reengineered to cleave at user-defined locations. Those are generated by injecting TAL effector binding domain with a DNA cleavage domain (nuclease) The proteins in this system, TAL effectors (TALEs), are secreted by Xanthomonas bacteria when they infect a wide range of plant species. These proteins selectively bind to specific DNA sequences in the host plant genome that modifies gene expression and consequently facilitates bacterial infection[4].

TALENs are constituted of two parts

TALE domain: A region of the TALE proteins from plant pathogenic bacteria that is changed to bind specific DNA sequences. It is the modularity of TALEs that presents an opportunity to target any desired DNA sequence.

Nuclease domain: The DNA-binding domain is usually fused to the FokI endonuclease. When two TALENs bind to target sites on opposite DNA strands, the FokI domains dimerize and induce a double-stranded break (DSB) at that site.

The cell itself reseals the DSB using DNA repair mechanisms that can employ small insertions or deletions (indels) to disrupt gene function, or by providing a firing pattern be used for sequence-specific gene insertion/replacement.

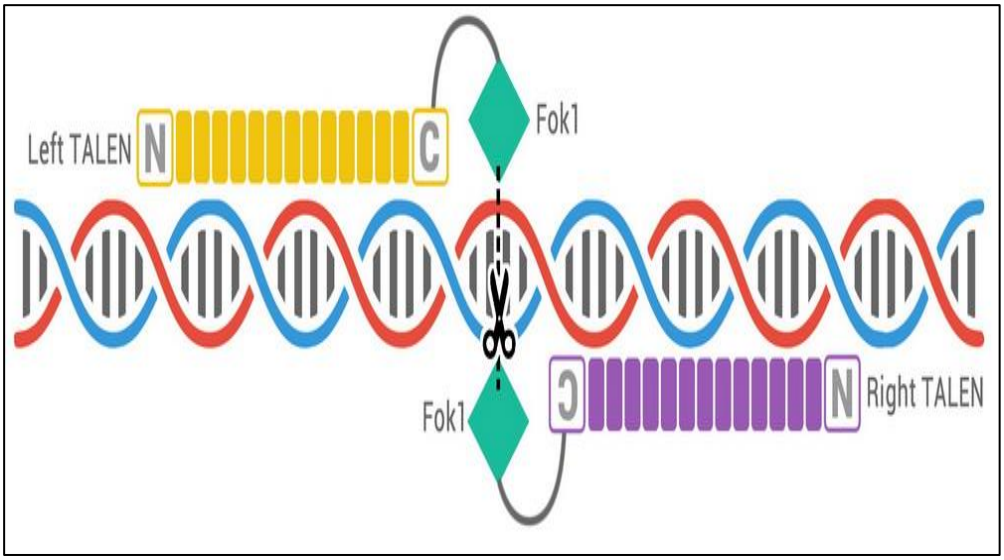

Figure 2: Diagrammatic representation of TALEN functions[5]

3.2.1. Applications of TALEN in Agriculture

***Targeted Gene Knockout*:** TALENs can be used to eliminate genes that are deleterious for crop traits. One example of it is where the researchers could make a knockout mutant of MLO gene in wheat and they developed wheats showing resistance to powdery mildew disease[6].

Development or trait enhancement: TALENs can be used to introduce traits beneficial for agronomic output in crops. These goals may be to increase yield, improve nutritional value or abiotic stress tolerance and strengthen disease resistance. As an example, TALENs editing was used to alter the FAD2 soybean gene[7], producing varieties with improved oil quality and higher oleic acid content.

***Faster Breeding*:** Conventional breeding of new varieties can be very time and labor intensive. TALENs provide a more targeted and efficient approach to breed beneficial traits into crops, thereby shortening the breeding cycle of new improved varieties.

***Creating New Traits*:** TALENs for creating new traits not existing in plant germplasm of crops. It paves the way to develop crops with higher photosynthetic efficiency or altered flowering time.

3.2.2. Examples of crop improvement using TALEN

Disease Resistance

Rice: Knockout of the *OsSWEET14* gene in rice, leading to enhanced resistance against bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*[8]. This is a significant breakthrough as bacterial blight is a major threat to rice production worldwide.

Wheat: Simultaneous target and disruption of three homeo-alleles of the *TaMLO* gene in wheat, resulting in heritable resistance to powdery mildew disease[9].

Nutritional Enhancement in Soybean

High oleic acid oil through FAD2 suppression in soybeans therefore, these oils and fats are more stable for human consumption which explains why hydrogenation of soybean oil is developed to give the desired properties like long shelf life and improved oxidative stability[7].

Quality Traits enhancement in Potato

Knock out of the vacuolar invertase gene (*VInv*) in potato, leading to lowered accumulation of reducing sugars and consequently lower acrylamide formation during frying has made potato safer for human consumption[10].

Yield Improvement in Rice

Genes like OsWx involved in starch biosynthesis could be altered to have better grain quality along with, perhaps, increased yield[11].

3.3. CRISPR Technique

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a technology of genetic engineering that has transformed the field from being quite authentic to addressing different scientific concerns straight up. It was first found in bacterial immunity and is now used with applications amongst various organisms like plants. It has been found that the gene-editing technique works like a pair of molecular scissors to alter an organism's DNA in nearly any way[12].

Usually, CRISPR system comprises of two components:

gRNA (guide RNA): A short complementary sequence of the target DNA.

Cas9 enzyme: An endonuclease acts as molecular scissors to break the DNA at a specific site.

3.3.1. How does CRISPR-Cas9 work?

***Targeting*:** A guide RNA (gRNA) molecule is designed to match a specific DNA sequence in the target organism.

***Cutting*:** The Cas9 enzyme, bound to the gRNA, locates and cuts the target DNA sequence.

***Repair*:** The cell's natural repair mechanisms try to fix the break, introducing changes (insertions, deletions, or replacements) to the DNA sequence.

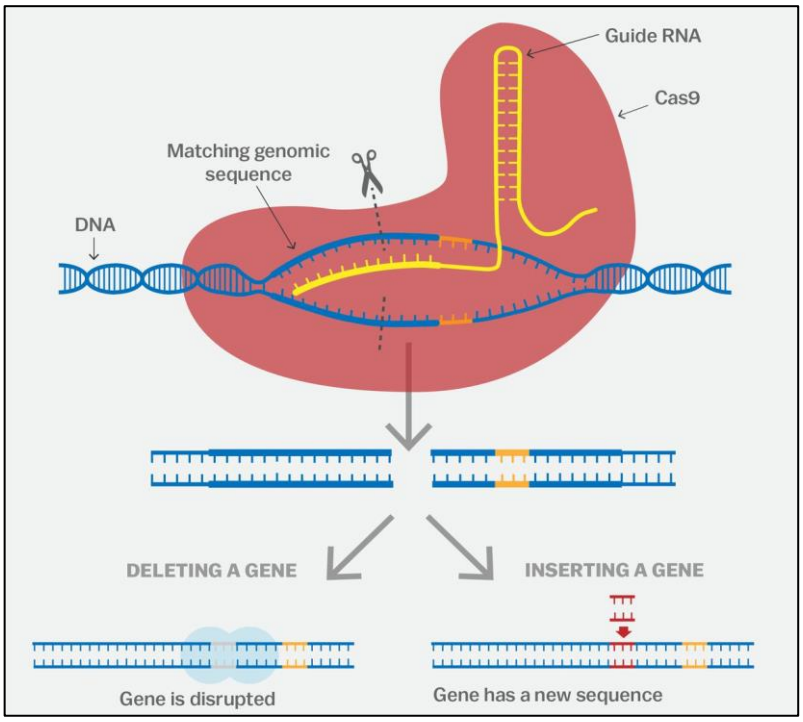

Fig 3: Basic mechanism of CRISPR Cas9 technique[13] (Source: Genetic Literacy Project)

3.3.2. Applications of CRISPR in Agriculture

CRISPR technology has numerous applications in agriculture, aimed at improving crop yield, quality, and resistance to various stresses. Some key applications include,

Disease Resistance: CRISPR can be used to enhance plant immunity against various pathogens. Researchers have used CRISPR to develop wheat varieties resistant to powdery mildew by modifying genes that make the plant susceptible to the disease.

Pest Resistance: By producing crops that are inherently resistant to insect pests, gene editing can lessen the need for chemical pesticides. For example, cotton plants resistant to cotton bollworm have been created using CRISPR.

Drought Tolerance: By altering genes related to plant water consumption and stress reactions, CRISPR can produce crops that are more resilient to drought. Given the effects of climate change and water scarcity, this is especially crucial.

Mushrooms: By using CRISPR to produce mushrooms that aren't brown, their shelf life has been increased.

Nutritional Enhancement: This is a simple instance of gene editing for food security and is only one area where we have witnessed purely eco-sustainable applications carried out in conjunction with enhancing the amount, caliber, or nutritional content of crops. Because higher levels of gamma-aminobutyric acid (GABA) are believed to have health benefits, CRISPR has also been used to engineer tomatoes with more GABA.

Yield Improvement: By altering genes that regulate fruit size, flowering time, and plant architecture, CRISPR may be able to boost crop yields.

Herbicide Resistance: Although debatable, CRISPR can be used to create crops that are resistant to particular herbicides, enabling more efficient weed control.

Accelerated Breeding: By directly introducing desired traits into elite crop varieties, CRISPR can expedite the conventional breeding process.

3.3.3. Examples of CRISPR-Edited Crops

Tomatoes: Using CRISPR, scientists have created tomatoes with enhanced flavor and extended shelf life.

Rice: CRISPR-edited rice cultivars that are more productive and resistant to bacterial blight have been created.

Wheat: To combat celiac disease, CRISPR has been used to develop gluten-free wheat varieties.

3.4. Prime Editing

Prime editing is a flexible and accurate genome editing technique that can achieve targeted insertions, deletions, and all 12 base-to-base conversions without the need for donor DNA templates or double-stranded DNA breaks. It does this by combining a fusion protein consisting of a Cas9 nickase connected to an engineered reverse transcriptase enzyme17 with a primary editing guide RNA (pegRNA), which specifies the target location and provides the new genetic material[14]. This versatile tool can be used to change DNA in a number of ways, including deletions, insertions, and substitutions. This innovative method marks a significant advancement in the field of genome editing and has potential uses in a number of sectors, including agriculture and health.

A modified Cas9 enzyme coupled to a reverse transcriptase enzyme and a prime editing guide RNA (pegRNA) are required for prime editing. The pegRNA directs the Cas9 enzyme to the precise spot in the genome where it nicks a single DNA strand. The reverse transcriptase enzyme then uses the pegRNA as a template to create the new DNA sequence, which is then inserted into the genome at the specified location[15].

3.4.1. Process of Prime Editing

A prime editor consists of A Cas9 nickase (nCas9) fused to a reverse transcriptase A prime editing guide RNA (pegRNA)

While the pegRNA guides the prime editor to the target DNA sequence and contains, A spacer sequence complementary to the target DNA, A primer binding site (PBS), A desired edit sequence

.

The prime editing process involves:

i. nCas9 nicks one strand of the target DNA
ii. The reverse transcriptase uses the pegRNA as a template to synthesize the edited DNA sequence
iii. Cellular repair mechanisms replace the original DNA with the edited sequence.

3.4.2. Applications of Prime Editing in Agriculture

Prime editing has a wide range of potential uses in agriculture, including,

Disease Resistance: By allowing particular mutations in the plant genome, prime editing would shield the plant from viral or fungal infections. Another example is the use of prime editing19 to introduce genes resistant to disease into rice plants[17].

Abiotic Stress Tolerance: Prime editing can assist in creating crops that are resilient to environmental stressors such as salinity, drought, and extremely high or low temperatures. By altering genes related to water-use efficiency20, researchers have used prime editing to increase drought tolerance in wheat[17].

Increased Nutritional Value: By altering the genetic composition of crops, prime editing can raise their nutritional value. For instance, prime editing[21] raised the provitamin A levels in tomatoes[8].

Quicker Breeding: By precisely introducing desired traits into already-existing varieties, prime editing can quicken the crop breeding process. The time needed to create new crop varieties with enhanced traits can be greatly shortened as a result. Novel Traits: Previously unattainable through conventional breeding techniques, prime editing can be used to introduce completely new traits into crops. To create crops with improved photosynthetic efficiency or altered flowering times, prime editing is being investigated[18].

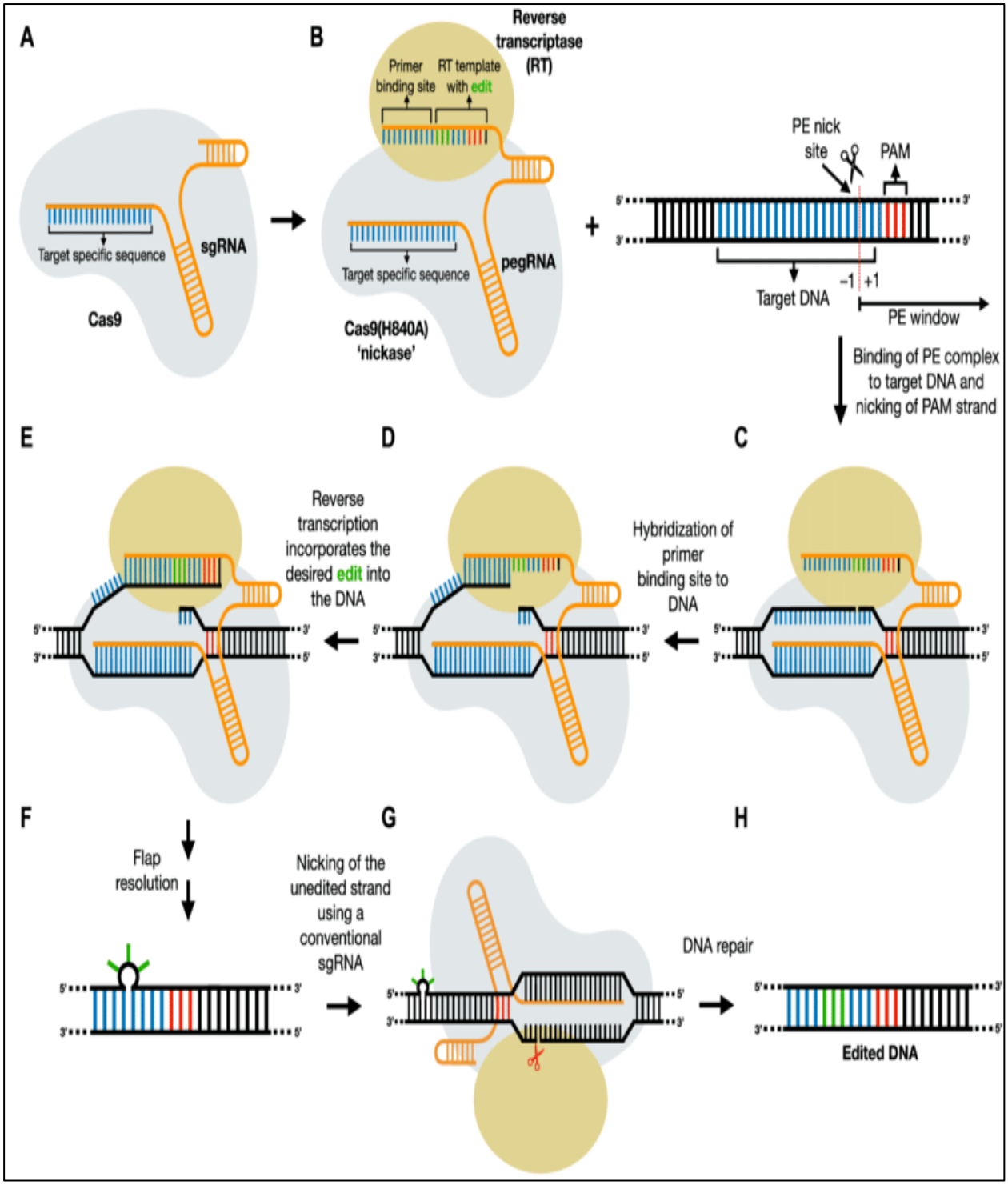

Figure 4: Schematic representation of prime editing[16]

***Less Pesticide*:** Apply Instead of using harsher chemical pesticides, prime editing could be used to breed crops that are naturally resistant to insects and diseases. This can lessen the impact on the environment and support more sustainable farming methods.

***Herbicide Resistance*:** Weeds can be effectively controlled by editing the genome of crops for introducing genes which confer resistance to herbicides while reducing crop damage. It is beneficial for increasing crop yield and reducing the number of herbicide applications owing to weed resistance[18,19].

Table 1. Comparison between CRISPR-Cas9 technique and Prime Editing Technique

Character	CRISPR-Cas9	Prime Editing
Mechanism	Uses a guide RNA to direct Cas9 to cut both strands of DNA, relying on the cell's repair mechanisms to make edits.	Uses a modified Cas9 to nick one DNA strand and a reverse transcriptase to write new genetic information directly into the genome.
Precision	Can sometimes cause unintended edits or off-target effects.	Generally, more precise with fewer off-target effects.
Types of edits	Primarily used for gene knockouts or insertions.	Can perform a wider range of edits including insertions, deletions, and all types of base-to-base conversions.
Efficiency	Often more efficient for certain types of edits, especially knockouts.	Can be less efficient but offers more control over the editing process.
DNA damage	Creates double-strand breaks, which can lead to unintended consequences.	Only nicks one DNA strand, potentially reducing genomic instability.
Size limitations	Can make larger insertions or deletions more easily.	Generally limited to smaller edits (typically up to 80 base pairs).
Complexity	Simpler system, easier to design and implement.	More complex, requires careful design of prime editing guide RNAs (pegRNAs).
Cellular repair pathways	Relies heavily on the cell's DNA repair mechanisms.	Less dependent on cellular repair pathways, offering more control over the editing outcome.

3.6. Synthetic Biology

Synthetic biology is a multidisciplinary field that applies engineering principles to the design and construction of new biological parts, devices, and systems, or to the redesign of existing natural biological systems for useful purposes. It involves manipulation of genetic material to create novel biological entities or modify existing ones to perform specific functions[19]. Its applications in agriculture span a wide range, from developing crops with enhanced traits to creating sustainable agricultural practices.

Disease Resistance: One of the major threats to crop production is plant diseases caused by various pathogens. Synthetic biology offers the potential to engineer crops with enhanced resistance by introducing genes encoding for natural defense mechanisms or entirely new resistance pathways.

Citrus Greening-Resistant Citrus: Genes encoding for antimicrobial peptides, including attacin E and cecropin B genes have been transformed into citrus trees to achieve resistance against the bacterium that is responsible for causing such citrus greening disease[13].

Banana Wilt-Resistant Bananas: Scientists inserted wilting gene RGA2 in banana (*Musa acuminata*) against Banana wilt disease by introgressing the resistance gene (from wild banana *M. acuminata* ssp. *malaccensis*) into susceptible cultivars of edible banana[20].

Late Blight-Resistant Potatoes: By combining the R genes (Resistance genes) from wild potato relatives, late blight-resistant potatoes have been created. In particular, resistance to multiple strains of the late blight pathogen has been conferred by the RB gene, which originated from *Solanum bulbocastanum*[21,22].

Powdery Mildew-Resistant Wheat: According to Wulff and Dhingra[23], the Pm3b gene from wild wheat relatives has been successfully incorporated into cultivated wheat varieties, providing resistance to the powdery mildew disease.

Bacterial Spot-Resistant Tomatoes: genes such as Bs2, Bs3, Bs4, and Pto have been introduced to produce tomatoes that are resistant to the different *Xanthomonas* species that cause bacterial spot disease[24].

II. Abiotic Stress Tolerance: Drought, salinity, and high temperatures are just a few of the environmental stresses that crops frequently

encounter. Table 2 illustrates how synthetic biology can be utilized to alter preexisting genes or add new ones that improve a plant's resistance to these stresses.

Table 2. Engineered genes for enhanced abiotic stress tolerance

Crop	Gene	Trait engineered	Reference
Maize	*DREB1A*	Drought Tolerance	25
Rice	*OsNHX1* (vacuolar Na^+/H^+ antiporter)	Salinity Tolerance	26
Rice	*OsBADH* (betaine aldehyde dehydrogenase)	Salinity Tolerance	27
Tomato	*Aco1* (acetyl-CoA carboxylase)	Cold Tolerance	28
Arabidopsis	*HSP101* (heat shock protein 101)	Heat Tolerance	29
Rice	*Sub1A* (Ethylene Response Factor)	Flood Tolerance	30
Rice	*OsLEA3* (Late Embryogenesis Abundant protein)	Drought Tolerance	31
Soybean	*GmSALT3* (Sodium transporter)	Salinity Tolerance	32
Potato	CBF (C-repeat binding factor)	Cold Tolerance	33
Wheat	*TaHSP16.9* (Heat Shock Protein)	Heat Tolerance	34
Arabidopsis	*AtHMA4* (Heavy Metal ATPase 4)	Heavy Metal Tolerance	35

Table 4. Applications of synthetic biology for biofertilizers and biopesticides

Characters	Genes	Mechanism	References
BIOFERTILIZERS			
Nitrogen-Fixing Cereals	*Nif* genes (nitrogen fixation genes) from nitrogen-fixing bacteria like Rhizobium.	These genes enable cereal crops like rice and wheat to directly fix atmospheric nitrogen, reducing their dependence on synthetic nitrogen fertilizers.	42, 43
Iron-Mobilizing Plants	Genes involved in the production and secretion of phytosiderophores, molecules that bind and solubilize iron in the soil.	This engineering enhances the ability of plants to uptake iron from the soil, especially in iron-deficient conditions.	44, 45
Phosphorus Solubilizing Plants	The introduction of genes encoding for organic acid exudation or phosphatase enzymes.	These genes enable plants to solubilize insoluble phosphorus compounds in the soil, making them available for plant uptake.	6, 46
BIOPESTICIDES			
Bt Crops (*Bacillus thuringiensis*)	*Cry* genes (crystal protein genes)	These genes encode for insecticidal proteins that are toxic to specific pests, providing protection against insect damage.	22, 47
RNA Interference (RNAi)-based Biopesticides	Double-stranded RNA (dsRNA) targeting essential pest genes	The dsRNA triggers RNAi, a gene silencing mechanism in the pest, leading to its death.	48
Cyanogenic Plants	Genes involved in the biosynthesis of cyanogenic glycosides.	When attacked by herbivores, these plants release toxic cyanide, deterring or killing the pests.	49

Improved Nutritional Value: Many staple crops lack essential nutrients, contributing to global malnutrition. Synthetic biology can be used to enrich crops with vitamins, minerals, and amino acids by modifying metabolic pathways. Table 3 enlists some examples of the same.

Table 3. Engineered genes for enhancement of nutritional values in crops

Crop	Gene	Trait engineered	Reference
Tomato	Delila (*Del*) and Rosea1 (*Ros1*)	Increased Anthocyanin	18
Canola	*γ-tocopherol methyltransferase (γ-TMT)*, *homogentisate phytyltransferase* (HPT)	Increased Vitamin E	36
Rice	*Ferritin, nicotianamine synthase* (NAS), *nicotianamine aminotransferase* (NAAT)	Increased Iron and Zinc	37
Potato	*Granule-bound starch synthase* (GBSS)	Increased Resistant Starch	38
Tomato	*GTP cyclohydrolase I*	Increased Folate	39
Lettuce	*GDP-L-galactose phosphorylase*	Increased Vitamin C	40
Maize and Barley	*Low phytic acid 1 (lpa1), lpa2-1*	Low-Phytate	41

Biofertilizers and Biopesticides: Synthetic biology can engineer microorganisms to naturally fix nitrogen or produce compounds that act as biopesticides. This can reduce the need for synthetic fertilizers and pesticides, which have negative environmental impacts. A brief account of the same is provided in table 4.

Bioremediation: Synthetic biology can engineer microorganisms to break down pollutants and toxins in soil and water. This bioremediation approach can help clean up contaminated agricultural sites, promoting a healthier environment for crop production as shown in table 5.

Table 5. Engineered microorganisms for environmental protection

Organism	Trait	Genes	Mechanism	Reference
Pseudomonas putida	Toluene Degradation	Toluene dioxygenase (*tod*) operon and Toluene monooxygenase (*tmo*) operon.	These genes enable the bacterium to break down toluene, a common environmental pollutant, into less harmful substances.	50
Deinococcus radiodurans	Mercury Remediation	The *mer* operon, encoding for mercury reductase and other mercury detoxification enzymes.	This operon allows the bacterium to convert toxic mercury ions into less harmful elemental mercury.	51
Escherichia coli	Atrazine Degradation	*atzA*, *atzB*, and *atzC* genes, which encode for enzymes involved in the degradation of atrazine	These genes enable the bacterium to break down atrazine into less toxic compounds.	52
Shewanella oneidensis	Uranium Reduction	The *mtrCAB* operon, encoding for metal-reducing proteins.	This operon enables the bacterium to reduce soluble uranium (U(VI)) to insoluble uranium (U(IV)), preventing its spread in groundwater.	53
Synthetic Microbial Consortia		Combinations of genes from different organisms to create synergistic effects for enhanced bioremediation.	These consortia leverage the strengths of multiple organisms to degrade complex pollutants or thrive in harsh environments.	54

4. Conclusion

One of the main forces addressing the complex issues facing global food security and sustainable agriculture is the advancement of next-generation agricultural biotechnology. Rapid advancements in genome editing tools, such as primary editing, TALENs, ZFNs, and CRISPR-Cas9, have allowed us to precisely alter crop genomes[55, 56]. Farmers can enhance crop qualities such as disease resistance, abiotic stress tolerance, nutritional value, and yield potential in previously unattainable ways by using these tools. Furthermore, the use of synthetic biology techniques has expanded the field, enabling the creation of beneficial microorganisms and novel biological systems in crops.

From creating disease-resistant cultivars to cultivating crops with improved nutritional profiles and stress tolerance, these technologies are opening the way for a more robust and effective agricultural sector[57]. However, the successful implementation of these technologies requires a comprehensive approach. Open public discussion, clear regulatory frameworks, and thorough safety evaluations are necessary to guarantee responsible and moral use. We can fully realize the potential of next-generation agricultural biotechnology to create a more food secure and environmentally sustainable future by encouraging collaboration among researchers, policymakers, farmers, and end users.

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Authors' Contribution

SS conceptualized the research, provided overall guidance, and contributed substantially to the manuscript's structure and analysis. **MKY** led the writing process, offered critical insights throughout the study, and ensured the accuracy and relevance of the scientific content. **PS** was instrumental in revising the manuscript, improving its clarity, and maintaining coherence across sections. All authors reviewed and approved the final version of the manuscript.

Data Availability

Data supporting this study is available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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