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Sustainability Spotlight

Ensuring global food security while facing climate change and population growth demands sustainable innovations in agriculture. This review highlights how nucleic acid-based diagnostics and gene-editing technologies—such as PCR, LAMP, CRISPR/Cas, and RNAi—are revolutionizing plant protection and crop improvement. By enabling precise, efficient, and targeted breeding strategies, these tools support resilient, high-yield crops and reduce dependence on chemical inputs. The integration of computational approaches further enhances the rational design of genetic interventions. This work aligns with UN SDG 2 (Zero Hunger) and SDG 13 (Climate Action) by promoting sustainable agricultural practices and developing climate-resilient crops, thus contributing to global efforts toward sustainable food production and improved nutritional outcomes.

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Integrating Nucleic Acid Research and Computational Strategies for Advancing Plant Food Security

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ABSTRACT

The evolution of plant breeding, from traditional techniques to cutting-edge omics-driven approaches (genomics, transcriptomics, proteomics, and metabolomics) has revolutionized crop yield enhancement since the mid-twentieth century. Today, genomics empowers breeders with powerful tools to directly associate genetic variation with phenotypes, accelerating the development of desirable traits. Building on this foundation, nucleic acid-based diagnostic techniques (PCR, LAMP) and gene-editing platforms (ZFNs, TALENs, and CRISPR/Cas), along with RNAi enable precise detection and manipulation of plant gene expression. This review focuses on various genome editing, and diagnostic strategies for plant protection, that aim to improve stress resilience, productivity, and nutritional value. Additionally, we highlight the latest computational strategies and methodologies that support the precise and rational design of such interventions with robust tools for targeted crop improvement. Looking ahead, these breakthroughs are poised to drive innovation across agriculture, biotechnology, and nucleic acid testing, opening new frontiers in sustainable food production and precision breeding.

KEYWORDS: Computational genome editing, Off-target prediction tools, Plant pathogen detection, RNAi delivery, siRNA design

1. INTRODUCTION

Population growth and climate change exert substantial pressure on global food security by inducing physiological and environmental stresses in plants, thereby enhancing their susceptibility to both abiotic stressors (such as drought, salinity, and temperature extremes) and biotic stressors (including pathogens, pests, and weeds)[1,2]. Biotic stressors contribute significantly to global agricultural losses, with estimated yield reductions ranging from 20% to 40%, leading to substantial economic impacts[3]. According to the Food and Agriculture Organization (FAO), plant pests destroy as much as 40% of global crop yields annually, leading

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to economic losses exceeding USD 220 billion from plant diseases and at least USD 70 billion from invasive insect infestations[4]. Plants are pivotal in sustaining life on Earth, as they are primary sources of food and energy, forming the foundation of ecological and biological systems. Ensuring effective crop protection against threats is crucial for sustaining agricultural productivity and meeting the growing population demand[5]. According to World Population Prospects 2022[6] the global population reached 8 billion in November 2022, and the UN's medium-variant projection indicates that it will peak at approximately 10.4 billion in 2080s[7]. To address these challenges and to attain second Sustainable Development Goal, i.e., "zero hunger", substantial efforts are necessary for the transition from conventional high-input agriculture toward more resilient, diversified and technologically supported production approaches[8,9,10]. Over the last decades, plant breeding and other technologies have made significant contributions toward minimizing hunger and extreme poverty[11,2]. Prior to the genomics era, conventional breeding methods rely on controlled hybridisation and selection of desirable traits through natural processes to develop new plant varieties[12]**.** Unlike marker-assisted selection, conventional breeding is slower and less precise, making it insufficient to meet the rising global food demand[13]. Innovative strategies to enhance crop yield and stress resistance have been transformed by genetic engineering[14,2]. Techniques such as Novel Plant Breeding Techniques (NPBTs), Next Generation Sequencing (NGS), collectively accelerate crop improvement by providing genomic insights and enabling targeted manipulation of plant genetic material. These approaches offer relevant, versatile, cost-effective, and time-efficient strategies that enhance precision in modern plant breeding[15,2]. These techniques facilitated the development of crop plants with enhanced agronomic traits, improved nutritional quality, and increased resistance to both biotic and abiotic stresses. These technologies not only address the shortcomings of conventional breeding but also offer flexibility by enabling the use of genomic information from both model and non-model plant species[15]. In addition to genetic improvements, early and accurate detection of pathogens plays a crucial role in managing plant diseases and minimizing crop loss. Nucleic acid-based diagnostic techniques such as Polymerase Chain Reaction[16] (PCR), quantitative Polymerase Chain Reaction[16] (qPCR), digital Polymerase Chain Reaction[17] (dPCR) enable rapid and sensitive detection of plant pathogens even at early infection stages. These techniques detect specific DNA or RNA sequences of pathogens, providing precision and timeliness in field-level diagnostics. This review critically discusses genome editing tools, nucleic acid-based diagnostics techniques, and RNA-based technologies, along with the computational approaches that enhance their

precision and applicability. A critical aspect of these technologies is the integration of computational approaches to design, evaluate, and optimize editing tools. For genome editing techniques such as Zinc Finger Nucleases[18] (ZFNs) and Transcription Activator-Like Effector Nucleases[19] (TALENs), software tools predict target site specificity based on DNA-binding motifs, while for Clustered Regularly Interspaced Short Palindromic Repeats[20] (CRISPR/Cas9), several platforms are available for designing guide RNA (gRNA) such as CRISPR Off-target Sites with Mismatches, Insertions and Deletion[21] (COSMID), CHaracterization and OPtimization of CHOPping tools[22] (CHOPCHOP) assist in identifying suitable target sites and minimizing off-target effects. Likewise, nucleic acid-based diagnostic techniques such as PCR[16], dPCR[17] rely on *in silico* tools for primer/probe design and target validation. These computational approaches enhance the accuracy, efficiency, and scalability of both genome editing and diagnostics, facilitating precision in plant protection and crop improvement as shown in **Figure 1**. Together, these pipelines demonstrate how computational approaches have been developed to support the design and optimization of nucleic acid-based tools, including genome editing nucleases, by enabling the prediction and reduction of off-target effects. These advancements contribute significantly to crop improvement, enhance resistance to biotic and abiotic stresses, improve nutritional quality of plants.

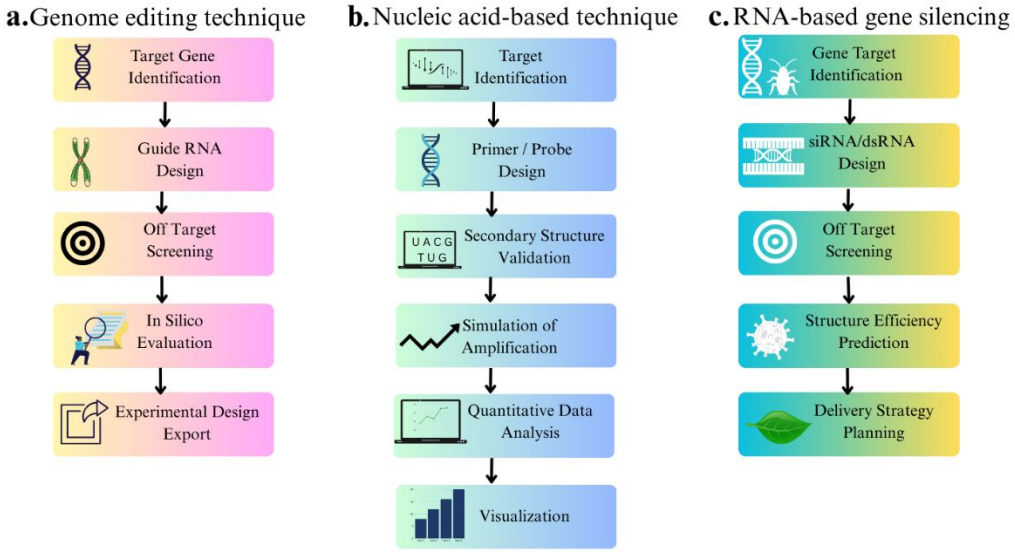

Figure 1. a. Genome editing workflow, starting with target gene identification, followed by gRNA or DNA-binding module design using computational tools, off-target prediction and minimization, and editing efficiency simulation. **b.** Nucleic acid-based pipeline including target sequence selection, primer/probe design, structural validation, thermodynamic analysis, and in silico amplification for specificity and sensitivity. **c.** RNA silencing process, featuring siRNA/dsRNA design via tools like Si-Fi, off-target screening, efficiency prediction, and delivery optimization.

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2. LITERATURE SEARCH STRATEGY

A comprehensive literature search was undertaken to identify studies relevant to plant genome-editing platforms, RNA-based technologies, and nucleic acid–based diagnostic approaches. The search was performed across major scientific databases, including PubMed, SciFinder and Google Scholar. Boolean operators and targeted keywords were applied, such as "CRISPR/Cas9 and plant genome editing," "ZFNs," "TALENs," "RNA interference in plants," "nucleic acid-based diagnostics," "LAMP," "NASBA," "plant pathogen detection," and "computational genome-editing tools." Additional terms such as "RNAi delivery," "siRNA design," "omics-driven crop improvement," and "biosensing technologies" were also used. Literature published between 2000 and 2024 was prioritized, with earlier foundational studies incorporated when scientifically justified.

3. GENOME EDITING TECHNIQUES

Genetic engineering has been a central area of research for several years in elucidating gene functions. This field encompasses the application of physical and biological mutagenesis, along with the identification of molecular mechanisms to enhance crop yield[23]. It relies on utilization of engineered nucleases, which consist of sequence-specific DNA-binding domains conjugated to a nonspecific DNA cleavage module[24]. It includes several methods, such as ZFN[18], TALEN[19], and CRISPR/Cas9[25]. These are chimeric nucleases that facilitate highly efficient and precise genome editing by inducing site-specific DNA double-strand breaks (DSBs). These DSBs activate intrinsic cellular DNA repair pathways, including the error-prone Non-Homologous End Joining (NHEJ) and the high-fidelity Homology-Directed Repair (HDR) mechanisms[26].

3.1 Zinc-Finger Nucleases (ZFNs):

ZFNs are site-specific endonucleases engineered for targeted DNA cleavage, facilitating precise genome modification. It consists of two distinct domains: a zinc finger domain that binds to the target DNA, and a nuclease domain derived from the FokI restriction enzyme, which is responsible for generating DSBs. Zinc finger domains are protein motifs that fold around one or more zinc ions and are capable of recognizing specific DNA sequences[18,24] as shown in **Figure 2a**. Zinc Finger Proteins (ZFPs) contain a tandem array of Cys2-His2 zinc fingers each of which binds with approximately 3 base pairs (bp) of targeted DNA[27,28]. It was reported[18,24] that individual ZFNs used three fingers to bind a 9-bp target, which enabled ZFNs dimers to specify 18 bp of DNA per cleavage site. More recent advancements[18,24] have

incorporated more fingers and a variety of strategies have been described in literature for designing ZFPs with new, user-chosen binding specificities[18,24]. By designing multiple zinc finger domains, ZFNs can be engineered to target a particular DNA sequence with high precision.

Nuclease domain of FokI restriction enzyme is crucial for the function of ZFNs, as it facilitates the targeted cleavage of DNA within complex genomes[18,24]. It must dimerize for effective cleavage of DNA[29,30]. The monomer of FokI is catalytically inactive, and its natural dimerization is weak, therefore cleavage can be achieved by constructing two sets directed to DNA sequence and joined to cleavage domain[31] as shown in **Figure 2b**. The nuclease domain introduces DSB in the DNA at the targeted site and this break activates the repair mechanisms, which occur via NHEJ (absence of donor DNA) or HDR (presence of donor DNA) as shown in **Figure 2c.** Targeting or editing of gene at the site of break takes place in the presence of template donor flanking DNA gene[18,24]. Rejoining of the two broken DNA ends takes place in the absence of template donor flanking DNA gene with some insertion or deletion often causing mutations due to frameshifts and effectively knocking out the targeted gene at site of break which leads to disruption of target gene[18,24].

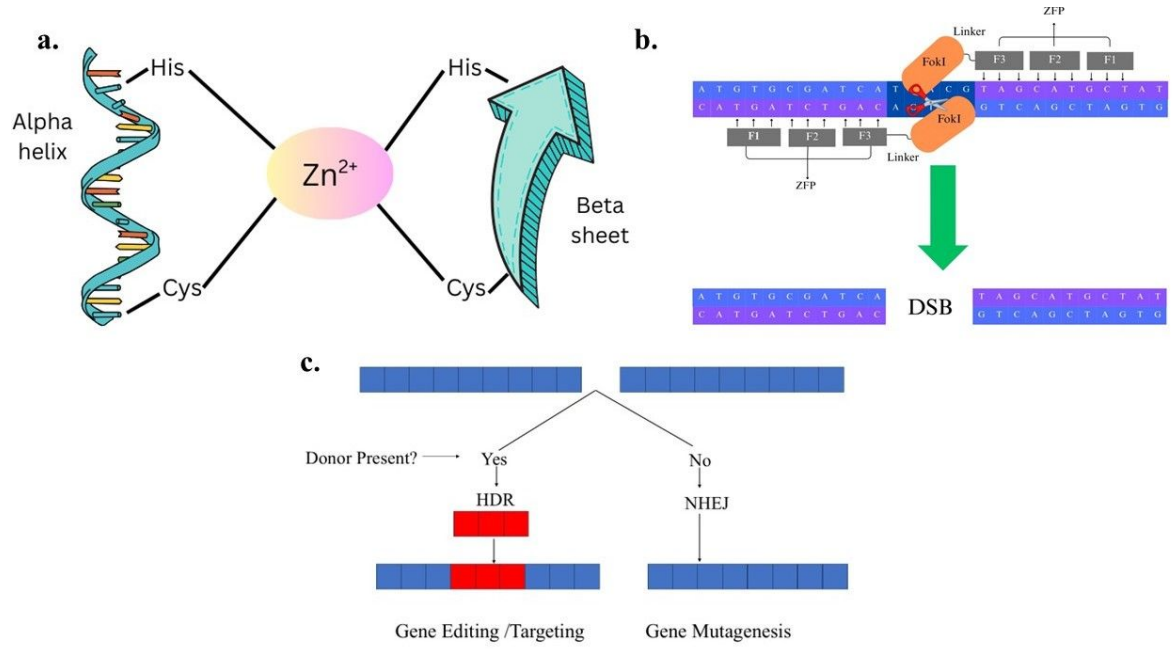

Figure 2. Structure and mechanism of zinc finger nucleases. **a.** Schematic representation of tandem array of Cys2-His2 zinc fingers protein. **b.** Illustration of a pair of zinc finger nuclease binding to target DNA sequence. Zinc finger proteins are shown as grey boxes with vertical arrows indicating base pair contacts. The FokI nuclease domain (orange) is linked to zinc finger

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protein and introduces a double-strand break in DNA. **c.** Different repair mechanisms resulting from the introduction of double-strand breaks in the presence and absence of donor DNA.

Although ZFNs have been proven as effective technology but it also has certain drawbacks. It is an expensive process of designing protein domains for individual genes, risk of off-target DNA cleavage caused by faulty interactions. These challenges prompted the emergence of newer gene-editing tools, such as TALENs and CRISPR/Cas9, which offer easier construction and greater efficiency[32].

3.2. Transcription Activator-Like Effector Nucleases (TALEN):

In 2011, *Nature Methods* recognized TALENs as the Method of the Year[19,33]. The advancement of TALEN system technique is linked to research on bacteria of the *Xanthomonas* genus, which is pathogenic to crop plants such as rice, pepper, and tomato. These bacteria secrete effector proteins (transcription activator-like effectors, TALEs) into the cytoplasm of plant cells, modulating cellular processes and enhancing host susceptibility to infection[32]. Later, it was found that these effector proteins are also capable of DNA binding and activate the expression of target genus[32].

TALEs proteins consist of a central domain responsible for DNA binding and a nuclear localization signal that facilitates their targeting of gene transcription[34]. The central domain is composed of repeating monomeric units, each specifically recognizing and binding a single nucleotide within the target sequence. These units are tandem repeats of 34 amino acid residues, and the last tandem repeat in the domain has 20 amino acids[35]. Amino acids at positions 12 and 13 are highly variable thus called Repeat Variable Diresidues[30,35] (RVDs). RVDs are responsible for recognition of a specific nucleotide and the four most common RVDs are Histidine–Aspartic acid (HD), Asparagine–Glycine (NG), Asparagine–Isoleucine (NI), and Asparagine–Asparagine (NN) accounting for each of the four nucleotides Cytosine (C), Thymine (T), Adenine (A) and Guanine (G), respectively[35, 36]. The first amino acid residue in RVDs, i.e. H or N, is not directly involved in nucleotide binding but plays a crucial role in stabilizing the spatial conformation of domain. In contrast, the second amino acid residue in RVDs i.e. D, G, I and N interact directly with the target nucleotide: D and N form hydrogen bonds, while I and G engage via van der Waals forces[35,37]. DNA-binding domain is integrated into a genetic construct containing a half-repeat, N-terminal domain, and the catalytic domain of *FokI*[35]. It functions in pairs, binding sites strategically positioned on opposite DNA strands and separated by a short spacer sequence (12–25 bp). Upon nuclear entry, the nucleases

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recognize and bind to their target sites, facilitating the dimerization of *FokI* domains at C-terminal of the chimeric protein. This dimerization induces DSBs within the spacer sequence[32] as shown in **Figure 3**. DSBs activate two repair pathways in the cell i.e. NHEJ and HDR[38] .

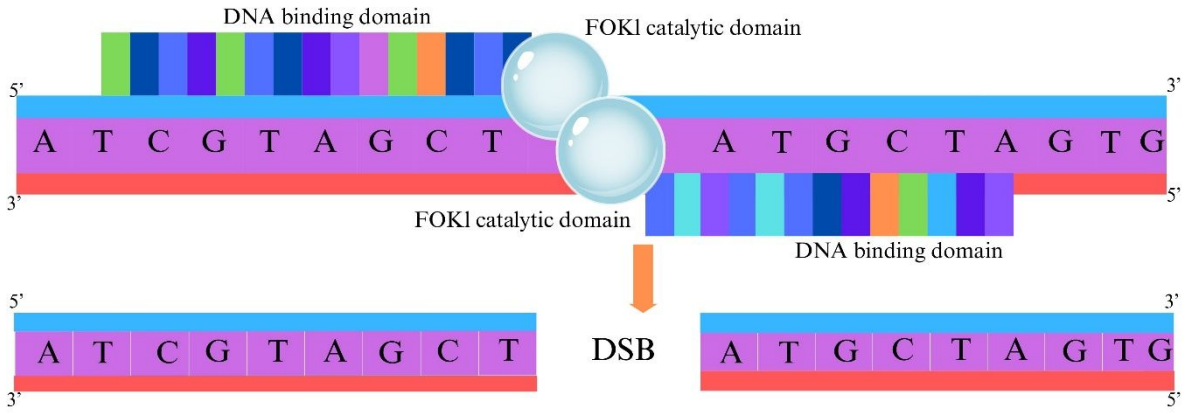

Figure 3. Schematic representation of Transcription Activator-Like Effector Nucleases architecture and double-strand breaks induction.

Despite the success of TALENs, a significant limitation remains as it recognizes the target site based on DNA-protein interactions due to which it leads to some fundamental structural difference which causes its low off target activity, low specificity/effectiveness, and high production cost[39]. These challenges prompted the emergence of newer gene-editing tool i.e, CRISPR/Cas9.

3.3. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9)

In 1987, Yoshizumi Ishino[40] and team first discovered Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) in *Escherichia coli*, characterizing them as unique DNA repeats separated by spacer sequences. In the early 2000s, Barrangou et al.[41] worked on *Streptococcus thermophilus* and identified that bacteria containing spacer sequences homologous to bacteriophage and viral genomes exhibits immunity against infection. During viral infection, CRISPR spacers are transcribed into CRISPR RNAs (crRNAs) with Cas proteins, target and cleave viral DNA or RNA to block infection[20]. Emerging CRISPR-associated nucleases such as Cas12, Cas13, and Cas14 hold significant promise for the development of transgene-free (foreign DNA free) crops[42]. These next-generation genome editors enable precise genetic modifications, offering a regulatory advantage that facilitates easier approval and commercialization across various countries[20]. It is one of the best state-of-the-art genome editing technologies that is gaining popularity because of its broader

applications across different organisms[42,43,44]. The CRISPR-Cas system is mainly classified in two main classes as 1 and 2. Class 1 system includes type I, III and IV multiple effector proteins for the RNA-guided target cleavage, while Class 2 includes types II, V, VI effector proteins required only one RNA-guided endonuclease for the DNA sequence cleavage[20]. Type I utilizes Cas3, which acts like a molecular motor, helping to unwind and cut DNA or RNA structures. Type II employs the well-known Cas9 protein, which accurately cuts double-stranded DNA at targeted locations. Cas9 contains two domains i.e., HNH and RucV-like domain. HNH domain cuts the complementary strand of crRNA whereas RucV-like cleaves the opposite strand of double-stranded DNA[45]. Type III involves Cas10, which can target single-stranded DNA and contains a specialized region called the palm domain that enables its activity. In Type IV, the system includes a protein known as csf1, which functions in a similar way as Cas8. Type V uses Cas12 (also called Cpf1), a versatile protein capable of cleaving both single and double-stranded DNA, and it also plays a role in DNA repair. Lastly, Type VI features Cas13, which is unique because it targets RNA instead of DNA. It contains Higher Eukaryotes and Prokaryotes Nucleotide-binding domain (HEPN) that allows it to cleave single-stranded RNA in both simple and complex organisms[46]. The recent advent of CRISPR derived methodologies, are base editing and prime editing, which has substantially expanded the scope and utility of precision genome editing[47,48]. This review presents an overview of key developments in CRISPR/Cas technology and their applications in modern horticulture and agriculture.

The CRISPR/Cas9 system is a bacterial RNA-guided immune defense, targets and eliminates foreign DNA from plasmids and bacteriophages, functions as a form of bacterial "immune system"[49]. It facilitates precise gene modification by introducing targeted DNA cleavage, followed by endogenous DNA repair mechanisms[43,50]. CRISPR/Cas9 technology, first applied in plant genome editing in 2013, has revolutionized the field by enabling precise, efficient, and versatile genetic modifications[51,52]. CRISPR/Cas9 requires the Cas9 protein and a Protospacer Adjacent Motif (PAM) sequence for efficient DSBs formation in the target DNA. Cas9 utilizes a guide RNA (gRNA) duplex composed of CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) for target recognition, as shown in **Figure 4a**. The crRNA typically 18–20 nucleotides long, is essential for recognizing and binding to the target DNA sequence[53,54]. The tracrRNA, ranging from 50 to 150 nucleotides, plays a vital role in the Cas9-mediated DNA cleavage process[53,54]. In current genetic engineering techniques, the duplex is combined into a single molecule called single guide RNA (sgRNA) as shown in **Figure 4b**. More specifically, sgRNA is generated by linking the 3' end of crRNA with the 5' end of

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tracrRNA using a connector sequence[55]. PAM sequence length varies between two to six nucleotides, such as NGG (any nucleotide(N) followed by two guanine units), NAG (any nucleotide followed by adenine, thymine units), CTT (Cytosine followed by two thymine units), and TTTV (three thymine units followed by V, V is A, C, or G). PAM functions as a key indicator for identifying target sites as it instructs Cas9 to cleave a DNA strand at a precise location[56,57]. The seed region adjacent to PAM consisting of 10–12 base pairs, determines Cas9 specificity, and makes it more essential than other regions in the sgRNA[55]. The interaction allows Cas9 to make precise DSBs in the DNA[58]. DSBs then activate repair processes, which can occur through one of two primary mechanisms NHEJ, HDR that are then utilized to introduce genetic modifications[58,59]. Both NHEJ and HDR provide versatility and precision in plant genome editing and enhance resistance to diseases and insect pests[60,61] however, HDR remains challenging due to its limitations in supplying sufficient repair templates[62].

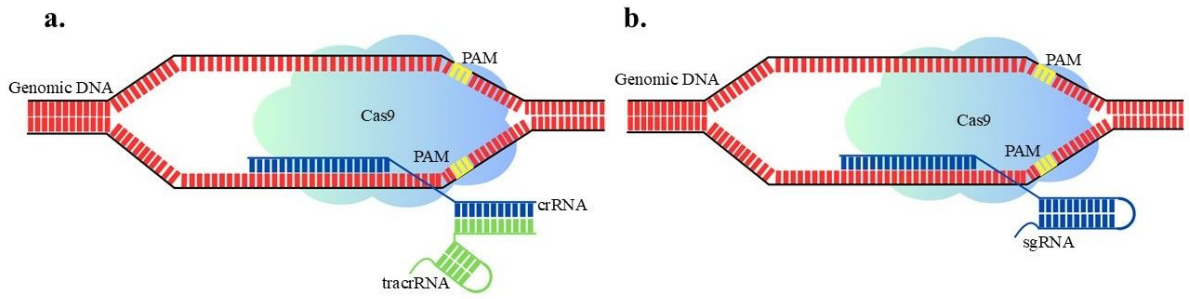

Figure 4. Basics of the Clustered Regularly Interspaced Short Palindromic Repeats technology in genome editing. **a.** Native system with Cas9 guided by CRISPR RNAs (crRNA) and trans-activating CRISPR RNA (tracrRNA). **b.** Modern Clustered Regularly Interspaced Short Palindromic Repeats system using a single guide RNA (sgRNA) that fuses CRISPR RNAs (crRNA) and trans-activating CRISPR RNA (tracrRNA)

3.3.1 BASE EDITING

It is an innovative technique that enables precise, programmable nucleotide changes without breaking the DNA strand or using a donor template[63]. It comprises two main parts: a Cas enzyme that binds to DNA in a programmable way and a single-stranded DNA-modifying enzyme that performs the specific nucleotide change. Two primary classes of DNA base editors i.e., Cytosine Base Editors (CBEs) and Adenine Base Editors (ABEs) have been developed which enable four transition mutations such as Cytosine (C) to Thymine (T), Adenine(A) to Guanine(G) and vice-versa[64]. These advanced editors expand conventional base editing by

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enabling transversion mutations and multiplex nucleotide substitutions beyond the limits of single-base systems.

3.3.1.1 Cytosine Base Editing (CBEs)

First-generation CBE (CBE1) was developed by Liu and co-workers in 2016[65]. The CRISPR/Cas9-mediated base editing platform incorporating cytosine deaminase facilitates highly precise and efficient single-nucleotide substitutions at defined genomic loci without generating double-stranded DNA breaks. This approach holds substantial potential for targeted gene correction and enhancement of genetic diversity across yeast, plant, mammalian, and human cells[66]. It converts C to U (uracil), which is subsequently recognized as T during replication, resulting in a C–G to T–A transition. It has low efficiency because of cellular-mediated repair of the U-G intermediate in DNA by the Base Excision Repair (BER). To overcome this several base-editors such as CBE2, CBE3 were developed to improve editing efficiency. CBE3 shows the highest efficiency by six-fold over CBE2. It was developed by restoring the histidine residue at position 840 (H840) within the HNH catalytic domain of Cas9 to produce a base editor utilizing the Cas9 nickase (nCas9) variant. This introduced a single-strand nick in G containing strand of U-G intermediate, directing the cellular repair machinery to favour conversion of the intermediate to a U–A pair, which is subsequently transformed into a stable T–A base pair during DNA replication[64] as shown in **Figure 5a**.

3.3.1.2 Adenine Base Editing (ABEs)

CBEs are restricted to mediating C–G to T–A transitions, which substantially limits its capacity to correct a wider set of pathogenic nucleotide substitutions. Notably, methylated cytosines exhibit a high susceptibility to spontaneous deamination, and it is estimated that nearly half of all pathogenic point mutations could theoretically be corrected through ABEs by converting an A–T base pair back to its original G–C configuration[67]. ABEs function through a similar mechanism as CBEs. The ABE-dCas9 fusion complex binds to the target DNA sequence in a guide RNA directed manner, where the deoxyadenosine deaminase domain catalyzes the conversion of adenine to inosine. During DNA replication, inosine is recognized as guanine, leading to the substitution of the original A–T base pair with a G–C base pair at the designated genomic site[64] as shown in **Figure 5b**.

In comparison to CBEs, ABEs produce markedly cleaner editing outcomes, characterized by an almost complete absence of indels and no reported instances of significant off-target A-to-G substitutions. Alkyl Adenine DNA Glycosylase (AAG), enzyme responsible for recognizing

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and excising inosine from DNA does not enhance ABEs efficiency or product purity relative to wild-type cells[68]. CBEs and ABEs are limited to four transition mutations, a constraint that prompted the development of prime editing for broader nucleotide modification.

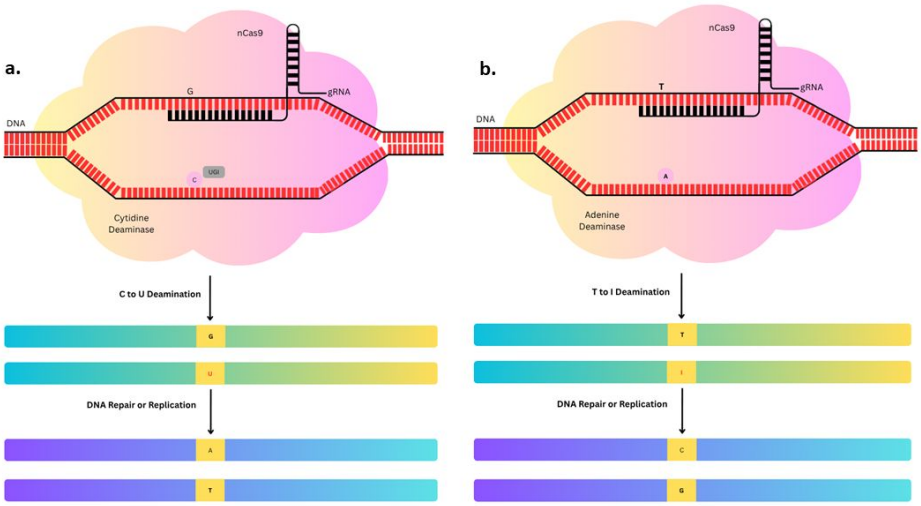

Figure 5. Schematic illustration of primary class of base editing tools. **a.** Cytosine Base Editing (CBEs) **b.** Adenine Base Editing (CBEs).

3.3.2 PRIME EDITING

It is an advanced CRISPR-based genome-editing technique described by Anzalone et al.[69] to overcome the limitations of CBEs and ABEs, which are restricted to mediating transition mutations (C•G→T•A and A•T→G•C). Unlike conventional CRISPR–Cas9 systems that rely on DSBs and donor templates, prime editing employs a fusion of Cas9 nickase (nCas9) and a reverse transcriptase (RT), guided by a prime-editing guide RNA (pegRNA)[70] as shown in **Figure 6**. The pegRNA not only guides the nCas9 to the target DNA site but also carries an additional sequence encoding the desired genetic change. Once the nCas9 makes a single-strand nick near the target site, the RT enzyme uses the pegRNA as a template to synthesize the edited DNA sequence[69]. This process generates two DNA flaps an edited 3′ flap and an unedited 5′ flap. Unedited flap is preferentially degraded by endogenous endonucleases, facilitating incorporation of the edited sequence through cellular repair and replication mechanisms[69]. The first-generation prime editor (PE1) achieved modest efficiency, which was later improved in PE2 through enhanced RT variants and further optimized in PE3 by adding a second gRNA to nick the opposite DNA strand, boosting editing efficiency to around 33%[69]. Prime editing can introduce all twelve types of base substitutions as well as small insertions or deletions, offering higher precision and fewer off-target effects, as shown in **Figure 6**.

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Although its efficiency and specificity are still being refined, prime editing represents a major milestone toward precise, versatile, and potentially therapeutic genome editing.

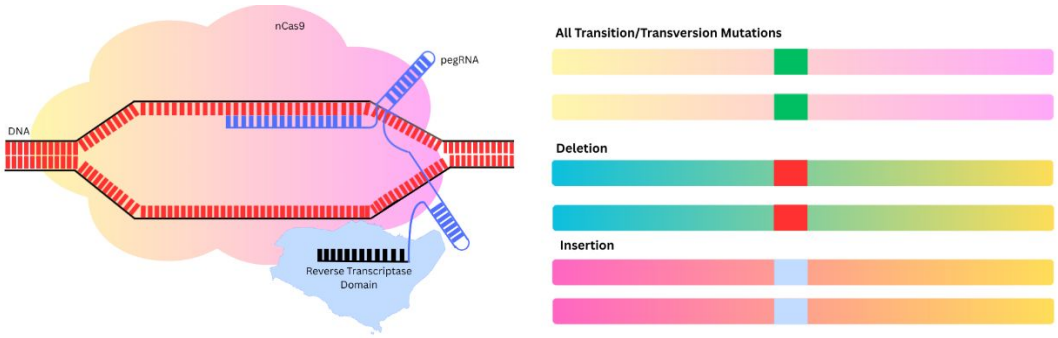

Figure 6. Prime Editing and its derived techniques. Prime-editors expand the scope of DNA editing to not all transition and transversion mutations, as well as small insertion and deletion mutations.

CRISPR/Cas9 leads to these advancements, providing unmatched precision and efficiency in genome editing. This integrative approach will enable the development of crops that are not only resistant to diseases, pests, and weeds but also tailored to thrive in specific environmental conditions. Researchers experienced with CRISPR have helped newcomers by openly sharing methods and resources, unlike the restricted, company-controlled framework of zinc-finger nucleases[45]. Several studies have successfully demonstrated the use of ZFN, TALEN, and CRISPR/Cas9 in various plant species for precise gene editing to improve agronomic traits. These applications are summarized in **Table 1**, highlight the practical outcomes of each technology in enhancing crop resistance, yield, and nutritional quality.

Table 1. Comparative overview of genome editing tools used in diverse plant species for trait improvement.

Tool	Researcher(s)	Plant Species	Target Gene(s) / Region	Outcome	References
ZFNs	Sylvia de Pater	Arabidopsis	PPO	Herbicide-insensitive enzyme due to gene mutation	70
ZFNs	Vipula K. Shukla	Zea Maize	IPK1	Herbicide tolerance; modified inositol phosphate profile	71
TALENs	Qiwei Shan	Brachypodium, Rice	Multiple genes	Gene editing via NHEJ; large deletions with dual TALENs	72
TALENs	Yanpeng Wang	Bread wheat	3 homoeoalleles	Powdery mildew resistance	73
TALENs	William Haun	Soybean	FAD2-1A, FAD2-1B	Improved oil quality and shelf life	74
TALENs	Toni Wendt	Barley	Genome-wide	~20% transformation efficiency; small deletions via NHEJ	75
CRISPR	Andrew S. Fister	Cacao	TcNPR3	Resistance to Phytophthora tropicalis	76

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Tool	Researcher(s)	Plant Species	Target Gene(s) / Region	Outcome	References
CRISPR	Valero Pompili	Apple	MdDIPM4	Potential for improved stress and disease resistance	77
CRISPR	Jun Li	Rice	EPSPS (via NHEJ)	Glyphosate resistance via targeted substitution (TILLING)	78
CRISPR	Anning Zhang	Rice	OsRR22	Enhanced salinity tolerance	79
CRISPR	Xiaohong Sun	Apple (Malus sp.)	MdMKK9	Increased anthocyanin accumulation	80
CRISPR	Akira Endo	Rice	OsOr	Elevated β-carotene levels	81

4. NUCLEIC ACID-BASED DIAGNOSTIC TECHNIQUES

Foodborne illnesses pose a significant threat to public health and the global economy. They impact consumers, food industry, and regulatory systems. Bacterial and viral pathogens are the primary biotic agents compromising food safety[82]. Traditionally, pathogen detection has relied on culture-based methods but these are often time-consuming, costly, and labour-intensive. Therefore, nucleic acid-based techniques are widely adopted due to their higher sensitivity, speed and specificity. These molecular tools are increasingly replacing conventional approaches in routine food testing[84,85].

Conventional detection methods for plant and foodborne pathogens are broadly categorized as direct and indirect techniques[83,85]. Direct methods include Polymerase Chain Reaction[16,86] (PCR), immunoassays[87], and culture-based colony counting[88]. Indirect methods are non-invasive and involve technologies such as thermography, gas chromatography, hyperspectral imaging, and fluorescence imaging[89]. Despite their accuracy, these standard techniques are often time-consuming, costly, and labour-intensive[90]. Initially, nucleic acid-based methods were limited to research laboratories due to their complexity and the requirement for skilled personnel[91]. However, with technological advancements these approaches have become more accessible and are replacing culture-based and immunoassay techniques in routine food safety analysis[85, 92]. Nucleic acid-based technologies also play a critical role in both basic research and applied sciences. They are extensively used in clinical diagnostics, pathogen identification, gene cloning, and industrial quality control[85].

In recent years, various advanced nucleic acid-based diagnostic methods have emerged that overcome the limitations of conventional assays. These include techniques like Quartz Crystal Microbalance[90] (QCM) using DNA amplicons, Recombinase Polymerase Amplification Surface-Enhanced Raman Scattering[93] (RPA-SERS) and gold nanoparticle-based PCR[94]. Additionally, isothermal amplification methods such as Loop-Mediated Isothermal Amplification[95] (LAMP), Reverse Transcription Loop-Mediated Isothermal Amplification[96] (RT-LAMP), and Nucleic Acid Sequence-Based Amplification[97] (NASBA) offer rapid

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detection without the need for thermal cyclers. Multiplexed Localized Surface Plasmon Resonance[98] (LSPR) approaches allow simultaneous detection of multiple DNA targets with high sensitivity. These technologies are not only faster but also suitable for point-of-care and field diagnostics[98]. A schematic overview of these diagnostic techniques is presented in **Figure 7**. Although these techniques differ in their experimental setups, but they largely follow a similar core mechanism[99]. All these methods target and amplify specific nucleic acid sequences (DNA or RNA) of the pathogen to enable sensitive detection. The general mechanism involves four key steps: sample preparation, nucleic acid extraction, amplification, and detection[99,100]. The process begins with sample collection followed by nucleic acid extraction (DNA or RNA). If the target is RNA, it is usually converted to complementary DNA (cDNA) using reverse transcription. This is followed by amplification of the target nucleic acid, which can be achieved through isothermal techniques. Finally, detection is carried out using various methods, including gel electrophoresis, fluorescence, colorimetric changes, nanoparticle-based sensors (AuNPs), or signal-enhancing platforms such as quartz crystal-based biosensors [101,102]. A systematic diagram representing this common mechanism is shown in **Figure 8,** while the methodological variations among these techniques are summarized in **Table 2**. Several studies have successfully demonstrated the use of LAMP[95], QCM[90], NASBA[97] in various plant species for detection of plant pathogen to improve agronomic traits. These applications, summarized in **Table 3**, demonstrate the use of nucleic acid-based biosensing tools for sensitive and rapid detection of plant pathogens and genetic markers using integrated amplification and nanotechnology platforms. Several other technologies are also used to detect plant pathogen such as CRISPR-based diagnostics[101] that represent a transformative approach in the field of molecular diagnostics, offering rapid, accurate, and sensitive detection capabilities across various applications. These diagnostics leverage the CRISPR/Cas system, originally known for its gene-editing capabilities, to identify specific nucleic acid sequences, making them highly versatile tools for detecting pathogens, genetic mutations, and invasive species. While CRISPR-based diagnostics offer significant advantages, challenges remain in terms of scalability and integration into existing diagnostic frameworks. Future developments may focus on automation and modularization to enhance the accessibility and efficiency of these diagnostics in various settings[102].

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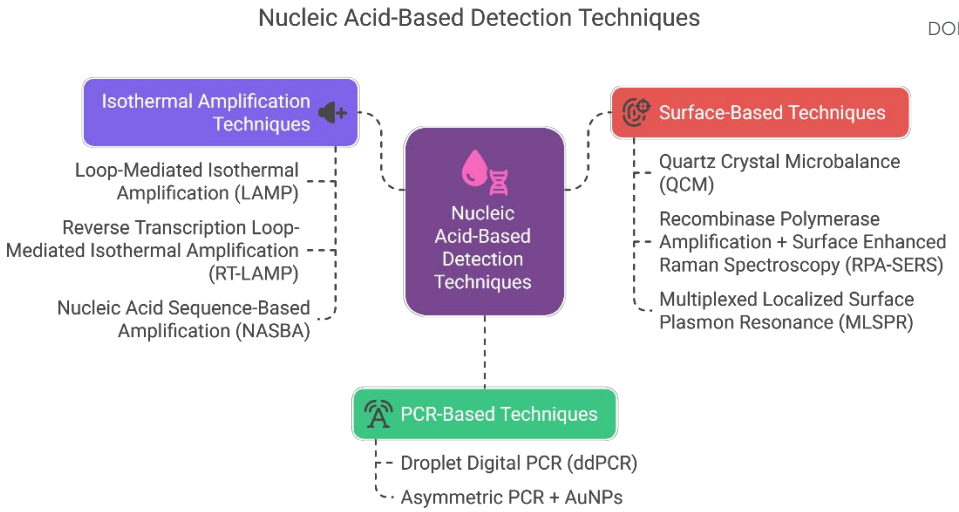

Figure 7. Types of Nucleic Acid-Based Detection Techniques

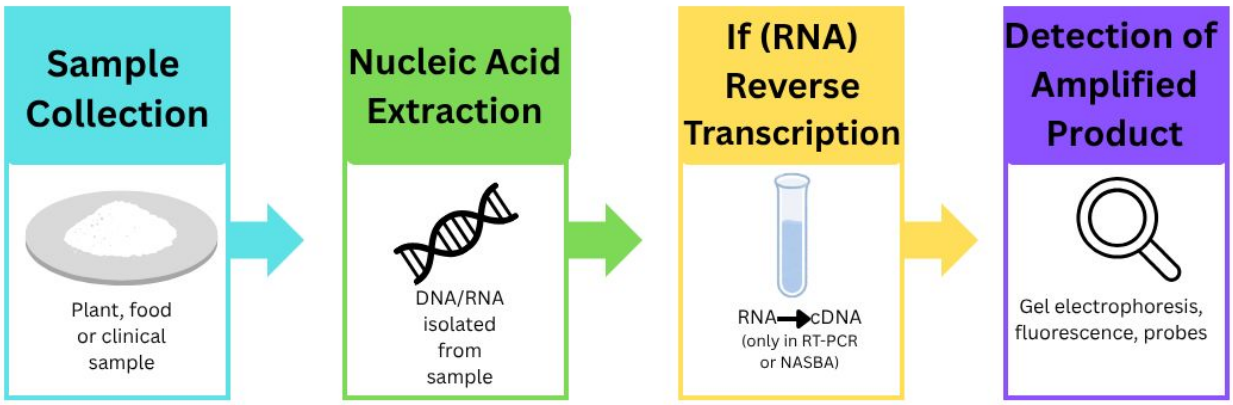

Figure 8. General Workflow of Nucleic Acid-Based Detection Techniques.

Table 2. Comparison of Nucleic Acid-Based Detection Techniques.

Technique	Target	Amplification Type	Isothermal?	Key Enzymes / Features	Detection Method	References
ddPCR	DNA	PCR-based (partitioned)	✗	DNA polymerase; droplet-based quantification	Fluorescence in droplets	103
QCM	DNA	PCR (Multiplex/ Conventional PCR)	✗	PCR-amplified DNA detected	mass-change-based acoustic biosensing using (QCM)	90
RPA–SERS	DNA	Recombinase-based	✓	Recombinase, DNA polymerase, SSB	Raman signal of labeled probes	93
LAMP	DNA	Polymerase-based loop amplification	✓	DNA polymerase with loop primers	Turbidity, color, fluorescence	95
RT-LAMP	RNA (converted to cDNA)	Isothermal	✓	Reverse transcriptase + Bst polymerase	Color change, fluorescence, turbidity	96
NASBA	RNA	Transcription-based	✓	Reverse transcriptase, RNase H, T7 RNA polymerase	Fluorescent or probe-based	97

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Technique	Target	Amplification Type	Isothermal?	Key Enzymes / Features	Detection Method	References
Asymmetric PCR + AuNPs	DNA	PCR (with excess primer)	✕	DNA polymerase; creates more ssDNA	Gold nanoparticle colour shift	94
MISPR	DNA	Not always with amplification	Sometimes	Surface Plasmon Resonance on nanoparticle arrays	Optical signal based on nanoparticle resonance	104

Table 3. Overview of nucleic acid-based biosensing tools for detecting plant pathogens and genetic markers using integrated amplification and nanotech platforms.

Tool / Technique	Researcher(s)	Plant Species / Matrix	Target Gene(s) / Pathogen	Outcome / Finding	References
LAMP–QCM	Reona Takabatake	Papaya	Cauliflower Mosaic Virus 35S promoter and Papaya Endogenous	Detect genetically modified papaya with high specificity and sensitivity (≤0.05%) without requiring PCR instruments.	105
RPA–LFD	Y. Zhou	Tomato	Tomato yellow leaf curl virus (TYLCV)	Rapid DNA detection TYLCV	106
LAMP	M.K. Prasannakumar	Rice	*Sarocladium oryzae, Magnaporthe Oryzae*	Detection of 100 femtogram of pathogen DNA	107
RT-PCR	Sunil B. Kokane	Citrus tristiza	*Citrus tristeza virus* RNA	Real-time RNA detection using RT-PCR with high specificity	108
Multiplex PCR	Nelly Datukishvili	Maize, Soybean	35S, NOS, EPSPS and *cry1Ab* gene	Identification of new DNA markers in GMO	109
LAMP–QCM	Sirirat Wachiralurpan	Bacterial DNA Solution	*Listeria monocytogenes*	LAMP products monitored by QCM in real-time	110
LAMP–AuNP (colorimetric)	Mila Djisalov	Mushroom substrate	*Trichoderma* spp., *tef1*	AuNP-based colorimetric detection for rapid fungal screening	111

5. RNA BASED GENE SILENCING

Sustainable production strategies including the development of safer pesticide alternatives, are critically required to improve the current cropping system. RNA interference (RNAi) mechanism has become a promising approach for effectively managing phytophagous pests and combating pathogenic attacks[112]. With its inherent capability for sequence-specific target, this technology is rapidly becoming a major focus of research as an environmentally sustainable and cost-efficient solution for pest management[113]. It is a conserved eukaryotic mechanism that regulates gene expression at either the posttranscriptional level (posttranscriptional gene silencing (PTGS)) or the transcriptional level (transcriptional gene silencing (TGS))[114]. TGS halts transcription by methylating the 5′-untranslated region (5′ UTR), preventing the binding of transcription factors, while in PTGS, methylation of the coding region marks the transcript for degradation[115]. In fungi, this PTGS mechanism is referred to as quelling[116,117]

In agriculture, RNAi has been widely utilized, especially for developing resistance to biotic stressors, bacteria, nematodes, fungal infections, and viruses[118]. This phenomenon was initially discovered in the free-living nematode *Caenorhabditis elegans*[119]. RNAi mechanism is used to inhibit key growth and developmental genes in targeted phytophagous pests at the post-

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transcriptional level, that helps to mitigate their harmful impact on crop plants. RNAi is activated by double strand RNAs (dsRNA) and multidomain enzymes such as the RNase III classes of enzymes in the Dicer family, which convert dsRNA into small interference RNA (siRNA) a double-strand RNA[120,121]. These siRNA are fused with RNA induced silencing complex (RISC) having Argonaute (AGO) proteins. This RISC complex and siRNA complement with viral RNA and cleave/degrade the viral RNA[122] as shown in **Figure 9**.

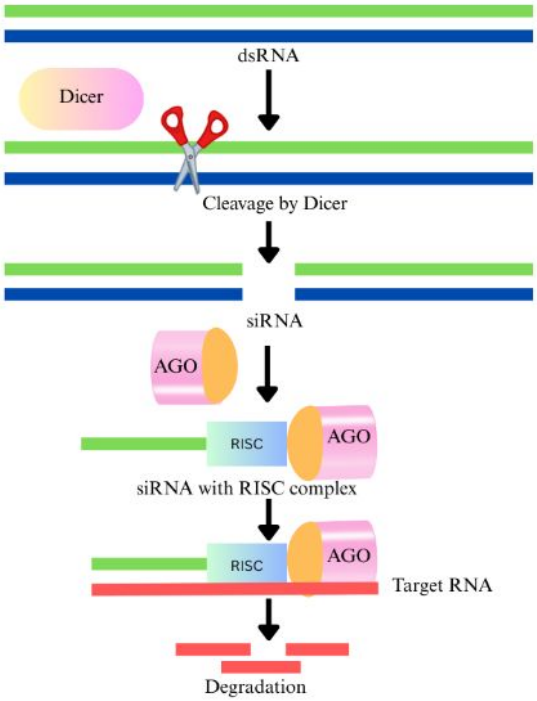

Figure 9. Mechanism of RNA silencing incorporating Dicer family enzyme, RNA-induced silencing complex (RISC) formation and degradation of viral RNA.

There are two sources of dsRNA i.e endogenous dsRNA (inside the cell) and exogenous dsRNA (outside the cell)[123,124]. The introduction of exogenous dsRNA in plants involves various approaches, depending on the specific research objectives and intended applications. These approaches have been widely investigated in plants to examine gene functions via reverse genetics and to attain crop enhancement objectives through genetic modifications[115]. The major strategies of dsRNA introduction in plants to protect from pest are Host Induced Gene Silencing[125] (HIGS), Spray Induced Gene Silencing[125] (SIGS), and a specialized RNAi-based approach Virus Induced Gene Silencing[126] (VIGS).

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In HIGS, transgene plant (wheat, barley, rice, and maize) is genetically modified to produce dsRNA which is taken by pest to silence the insect critical gene and protect plant from degradation [125] as shown in **Figure 10a**.

In SIGS, dsRNA is synthesized *in vitro* which targets the essential gene in pathogen. Synthesized dsRNA is sprayed onto the plant surface (strawberry, lettuce, canola, and barley) by using nanocarriers or protective formulations which is absorbed by the pest or pathogen, either through feeding or direct contact. As a result, silencing of the targeted gene takes place inside the target organism[125] as shown in **Figure 10b**.

Virus Induced Gene Silencing is a specialized RNAi-based approach that uses engineered plant viruses to assess gene functions in plants and control plant pests. Once the virus infects the plant, it triggers the plant defense mechanism. The plant produces dsRNA as a part of its response to the viral infection and as a result, silencing of gene takes place[126] as shown in **Figure 10c**. It offers significant advantages for functional genomics as it is rapid, does not require stable transformation, and also allows high-throughput screening of candidate genes involved in stress responses, defense mechanisms, and developmental processes. This makes VIGS particularly valuable for studying gene function in non-model plants or those with long generation times. Xijun Chen[127] explore the use of composite nanomaterials as delivery systems for dsRNA to enhance plant protection against viral pathogens.

Artificial dsRNA produces a large population of siRNAs, out of which only a limited subset is functionally active in guiding RISC mediated target cleavage. The remaining, non-functional siRNAs can contribute to off-target effects, thereby reducing the overall efficiency and specificity of RNA silencing[128]. However, a major challenge with topical application of naked dsRNA is its instability on the plant surface, leading to limited protection duration. Neena Mitter[129] and her colleagues developed a novel nanocarrier system called BioClay, which uses Layered Double Hydroxide (LDH) nanosheets to deliver dsRNA in a stable form. When dsRNA is loaded onto these clay nanosheets, it becomes protected from environmental degradation, including nucleases and rain. Similarly, Marie Knoblich[130] and colleagues developed an experimental platform termed as 'eNA screen', which utilizes lysates from cultured *Nicotiana tabacum* BY-2 cells (BY-2 lysates). This system enables the identification of '*effective siRNAs'* (esiRNAs) which are capable of directing efficient AGO/RISC-mediated target RNA cleavage. The application of the eNA screen to Cucumber mosaic virus (CMV)

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allowed the identification of esiRNAs with strong antiviral activity, demonstrating their potential in conferring resistance against CMV infection.

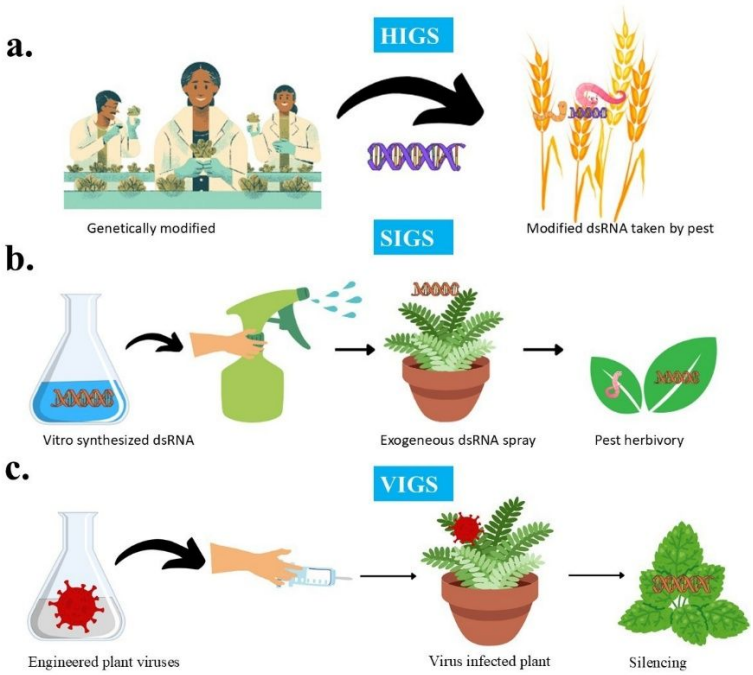

Figure 10. Strategies for dsRNA delivery for pest/pathogen control. a. Host Induced Gene Silencing (HIGS). b. Spray Induced Gene Silencing (SIGS). c. Virus Induced Gene Silencing (VIGS).

RNAi can be effectively used to enhance the yield of crops, fruits and plants by modifying key agronomic traits such as plant height, branching, and size. Kenneth A Feldmann[131] employed RNAi to reduce the activity of a gene OsDWARF4 of rice that resulted in shorter plants with their leaves growing more upright instead of drooping thus, this increases light interception, leading to higher photosynthesis and improved yields are observed under dense planting conditions. Hisano et al[132] uses RNAi in the downregulation of some lignin genes like cinnamate 4-hydroxylase (C3H), shikimate hydroxycinnamoyl transferase (HCT) and 4-coumarate-coA ligase (4CL) in plants that resulted in the reduction of lignin content, improved accessibility of cellulases for cellulose degradation, and increased dry matter degradability. Qiano et al.[133] employed RNAi to reduce the activity of a gene GA 20-oxidase (OsGA20ox2) that resulted in decrease in the length of rice variety named QX1 which helps in increase in panicle length, number of seeds per panicle and test weight (1000 grain). Jiao[134] and Miura[135] observed that OsSPL16 gene is a positive regulator of cell proliferation with an increase in grain width and yield in over-expressing rice plants. Its over expression decreases the grain

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appearance and quality therefore Jiao[134] and Miura[135] decreased the expression of OsSPL16 gene and resulted in slender grains with better quality. Davuluri et al.[136] suppressed DET1 gene in tomato that resulted in an increase in level of flavonoid and carotenoid which are highly beneficial for human health. Yu et al.[137] suppressed the expression of lycopene epsilon cyclase (ε-CYC) gene by using RNAi and resulted in the enhancement of carotenoid content of rapeseed (Brassica napus). Dandekar et al.[138] also utilized RNAi in apple to improve the fruit quality by enhanced self-life. Regina et al.[139] uses RNAi to down-regulate the starch-branching enzyme resulting in high-amylose wheat, which has great potential to improve human health. Gil Humanes et al.[140] silenced the expression of specific γ-gliadins in different wheat cultivars and resulted in the enhancement of protein content of transgenic lines.

6. INTEGRATION OF ARTIFICIAL INTELLIGENCE WITH GENOME EDITING TECHNIQUES FOR ENHANCED PRECISION

Artificial Intelligence (AI) is being integrated into genome-editing pipelines to overcome major bottlenecks in precision and predictability. Deep learning and machine learning (ML) models are widely used to design tools with high efficiency, generate efficient gRNAs with minimal off-target effects by analyzing sequence performance patterns from extensive data. AI platforms such as DeepCRISPR[141], ZFN-Site[143], CCTop[143] and siRNA-Finder [144] enable prediction of off-target sites with far greater accuracy than rule-based tools. In base and prime editing. ML frameworks help in optimizing the sequence context, thereby reduce by products and improve editing fidelity. AI-driven prediction supports the selection of biologically relevant targets before experimental validation. In plants, AI also assists in optimizing delivery constructs, regulatory elements, and tissue specific expression strategies. Collectively, the integration of AI with genome editing substantially enhances edit precision, reduces experimental iterations, and accelerates the development of reliable, high-performance crop-improvement strategies.

7. COMPUTATIONAL RESOURCES FOR PRECISION GENOME EDITING AND NUCLEIC ACID-BASED BIOSENSING

AI models can analyze large genomic datasets to optimize gRNA selection, predict off-target effects, and model protein-DNA/RNA interactions with high accuracy. Platforms such as DeepCRISPR[141] and CNN-based gRNA scoring[145] systems exemplify how AI is transforming genome engineering into a more predictive, efficient, and scalable process. Over the past few years various computational technologies utilizing AI, ML have been designed for target

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identification, predicting/reducing off-target activity of gene editing and gene regulation methodologies. In addition to genome editing, AI/ML approaches are also being employed in nucleic acid-based detection techniques such as droplet digital PCR[103] (ddPCR), LAMP[107] where they assist in designing specific primers and probes, enhance signal analysis from biosensor platforms and improve multiplex detection efficiency. Several dedicated tools are now available for designing primers, like PrimerExplorer[146], NEB LAMP Primer[146] Design Tool and FastPCR[147] etc as listed in **Table 4**. Databases used for providing target sequences in ZFN, TALEN, CRISPR/Cas9 and RNAi are presented in **Table 5**. Off-target activity encompasses mainly in three major categories: software tools and packages, specialized databases, and web-based platforms, each of which exhibits diverse areas of application. Certain resources are tailored for specific genomes, whereas others offer broader compatibility, accommodating any user defined genomic input. While some tools prioritize computational speed and scalability for larger genomes, others are optimized for precise algorithmic control and customization[148]. Common features shown by each tool are off-targets, scoring, ranking and genome specificity. Computational tools and their detailed comparison of off-target feature are presented in **Table 6.**

Table 4. Computational tools and webserver for different nucleic acid-based techniques.

Software/Tool	Purpose	References
PrimerExplorer	A widely used software for designing LAMP primers, offering a user-friendly interface for constructing primers for Loop-mediated Isothermal Amplification.	146
NEB LAMP Primer Design Tool	A tool provided by New England Biolabs for designing LAMP primers, gaining popularity for its ease of use and specificity.	146
FastPCR	An integrated tool for designing primers for various PCR applications, including LAMP, multiplex PCR, and long-distance PCR.	147
ThermoPlex	An automated design tool for target-specific multiplex PCR primers based on DNA thermodynamics, ensuring optimal primer design.	149
PrimerJinn	A tool for designing multiplex PCR primer sets and performing in silico PCR evaluation, particularly for targeted sequencing of pathogens.	150
PMPrimer	A Python-based tool for automated design and evaluation of multiplex PCR primer pairs using diverse templates.	151
PrimerScore2	A high-throughput primer design tool that uses a piecewise logistic model to score primers for multiple PCR variants.	152
SADDLE	A stochastic algorithm for designing highly multiplex PCR primer sets with minimal primer dimer formation.	153
MOPSO-based Primer Design	A multiobjective particle swarm optimization (MOPSO) approach for designing primers based on user-specified parameters.	154
Ultiplex	A web-based multiplex PCR primer design tool that supports up to 100-plex multiplicity and includes compatibility checking for primer groups.	155
NGS-PrimerPlex	A command-line application for designing primers for amplicon-based genome target enrichment in multiplex PCR.	156
MRPrimerW	A web-based tool for designing high-quality primers for multiple target qPCR experiments, including homology testing and TaqMan probes.	157

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GPrimer	A GPU-based pipeline for rapid primer design, significantly improving computational speed compared to traditional methods.	158
PrimerServer	A high-throughput primer design and specificity-checking platform with web and command-line interfaces for large-scale applications.	159

Table 5. Databases used to provide target sequences for different techniques.

Database	Purpose	References
EENdb	Database for ZFNs and TALENs, providing target sequences and efficiency data	160
PICKLES	Provides data from pooled CRISPR knockout screens	161
Cas-Database	Designs genome-wide guide RNA libraries for CRISPR screens	162
CHOPCHOP	Designs CRISPR/Cas9 and TALEN constructs	22
grlD	Guide RNA database with gRNA properties	263
CRISPRz	Validated CRISPR target sites in zebrafish	164
CRISPRdirect	Designs target site-specific gRNA sequences	165

Table 6. Computational tools, database and webserver for different genome editing techniques.

ZFN-based			
ZFN Tools	Type	Off-targets	References
Zinc Finger Tools	Tool	X	166
ZifBASE	Database	X	167
ZiFiT (v4.2)	Tool	√	168
ZFN-Site	Tool	√	142
TALEN-based			
TALEN Tools	Type	Off-targets	References
TALE-NT 2.0	Tool	√	166
TALENoffer	Package	√	170
E-TALEN	Tool	√	171
SAPTA	Tool	√	172
CRISPR-based			
CRISPR Tools	Type	Off-targets	References
Cas9 Design	Tool and Database	√	173
CasOT	Tool	√	174
Cas-OFFinder	Tool	√	175
CRISPR Optimal Target finder	Tool	√	176
E-CRISP	Tool	√	177
CRISPR-P	Tool	√	178
GT-SCAN	Tool	√	179
CRISPy	Tool	√	180
sgRNAcas9	Package	√	181
CRISPRseek	Package	√	182
COSMID	Tool	√	21
CRISPRdirect	Tool	√	183
Off-Spotter	Tool	√	184
CRISPR multitargeter	Tool	X	185
CCTop	Tool	√	143
CrisprGE	Database	√	186
WGE	Package	√	187
ZFN/TALEN/CRISPR-Cas-based			
Tools	Type	Off-targets	References
EENdb	Database	X	160
Mojo Hand	Tool	√	188
CHOPCHOP	Tool	√	22
PROGNOS	Tool	√	189
RNAi-based			
RNAi Tools	Type	Applications	References

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pssRNAit	Tool	Designing siRNAs	190
siRNA-Finder	Tool	Designing siRNAs	144
sPARTA	Tool	Analyzing miRNA	191
AttSiOff	Tool	Off-targets	192
MIRZA-G	Tool	Off-targets	193
siRNADesign	Tool	Off-targets	194
dsRNAEngineer	Tool	Designing dsRNAs	195
kmerPMTF	Tool	Predicting miRNA	196
PAREameters	Tool	Identifying miRNA	197
RNA Degradome	Tool	Predicting sRNA	198

8. CONCLUSIONS and FUTURE PROSPECTS

Gene editing and nucleic acid-based technologies are opening new possibilities in plant biotechnology. These tools offer accurate and sustainable methods to boost crop yield, improve disease resistance, and ensure food security. Among them, genome editing tools like ZFNs, TALENs, and CRISPR/Cas9 have transformed plant breeding. They allow targeted changes in the plant genome by creating DSBs, which are repaired by the plant's natural mechanisms. On the other hand the Base editing and Prime editing tools enable precise, programmable nucleotide changes without DSBs. While ZFNs and TALENs provided the early framework for gene editing, CRISPR/Cas9 is now most widely used. Its popularity comes from its simplicity, high precision, and ease of design. The capacity to induce targeted genetic modifications enables the precise introduction or alteration of desirable traits in elite crop varieties utilized for breeding and agricultural production. Moreover, the potential to stack multiple genetic modifications offers opportunities for the development of multi-trait resistance and the controlled expression of pharmaceutically important proteins. However, challenges such as off-target mutations, low editing accuracy, and inefficient delivery methods still need to be addressed. While no system has yet been established as most efficient and safe, ongoing efforts, particularly computational approaches aimed at minimizing off-target effects, hold promise for enhancing the precision and overall efficacy of therapeutic applications.

Alongside gene editing, nucleic acid-based diagnostic methods are essential for plant health monitoring. Techniques such as PCR[16], LAMP[95] offer fast and sensitive detection of pathogens, even at early stages. These diagnostics are being improved through integration with nanotechnology. These enhancements make them suitable for field-level use and point-of-care testing. This helps in reducing yield loss caused by undetected plant diseases.

In recent years, the global scientific and regulatory communities have been engaged in an ongoing debate regarding whether the application of these genome editing techniques falls within the framework of existing genetically modified organism legislation[199]. Regulatory clarity for New Genomic Techniques (NGTs) is still evolving. The European Food Safety

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Authority (EFSA) concludes that cisgenic and intragenic plants developed through NGTs do not pose new or additional risks compared with conventionally bred plants, and recommends risk-proportionate, science-based assessment criteria[200]. The US department of Agriculture decides in a case-by-case manner and stated on requests that small mutations in corn induced by ZFNs fall outside their scope of regulation[201].

RNA interference (RNAi) technologies, including siRNA and miRNA, have become valuable for plant researchers. These techniques are employed not only to explore plant functions but also to engineer plants with enhanced or novel traits through the manipulation of both beneficial and detrimental genes. This technology has been effectively applied in crops to enhance not only food productivity (such as biomass and grain yield) but also their nutritional value, with cereals, fruits, and vegetables being enriched with essential minerals, vitamins, fatty acids, and amino acids. RNAi technology has also been exploited to develop plants with improved resistance against various environmental stresses (especially drought). Although RNAi technology can serve as a potential tool for crop improvement but certain limitations are also associated with it. Altering the expression of a target gene might lead to undesirable changes in plant morphology and development therefore, transgenic strategies should be designed only after completely understanding the mechanism of its regulation. siRNA-based RNAi strategies might not be suitable for some applications requiring tissue-specific silencing of genes[202].

In coming years, ongoing advancements and innovations are expected to yield more robust computational frameworks for genome editing. Computational tools and AI play key role in advancing these technologies. They help in improving the accuracy, speed, and scalability of genome editing, diagnostics, and RNAi technologies. Tools including grlD[163], CHOPCHOP[22], PrimerExplorer[146], siFi[144] and sPARTA[172] assist in designing gRNAs, primers, and siRNAs. These tools reduce off-target effects and improve targeting efficiency. Specialized databases also support gene selection, off-target prediction, and RNA structure analysis. Together, they make molecular tools more reliable and user-friendly. Notably, recent computational studies demonstrated the potential of modified siRNA molecules to effectively inhibit viral RNA-silencing suppressor proteins, such as p19, thereby restoring the RNAi mechanism and improving resistance against plant viruses[203]. These emerging tools and algorithms hold the potential to significantly enhance genome editing and nucleic acid-based technologies by contributing to both pre-editing design and post-editing analysis, thereby expanding their applicability.

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Looking ahead, combining genome editing, RNA-based methods, diagnostics, and AI could transform agriculture. These integrated systems will help develop crops that are more resilient, high-yielding, and rich in nutrients. These approaches will also allow precision breeding tailored to local environment and nutritional needs. The integration of genome editing with synthetic biology could pave the way for designing new metabolic pathways and creating biofortified crops to tackle malnutrition. However, the widespread application of these technologies will also require robust regulatory frameworks, ethical considerations, and increased public awareness to ensure their safe and acceptable use. Promoting farmer education and consumer acceptance will be essential for realizing the full potential of nucleic acid research in ensuring sustainable food security for future generations. In future, tools that exhibit substantial potential in terms of their applicability may consequently be envisioned as among the most widely adopted next-generation technologies for both scientific and therapeutic applications.

In conclusion, Multiple proof-of-concept studies have already demonstrated the practical success of various technologies. CRISPR-edited rice lines with OsRR22 mutations show enhanced salinity tolerance[79], TALEN-mediated *MLO* knockouts in wheat confer powdery mildew resistance[73] and ZFN-engineered IPK1 maize exhibits herbicide tolerance[71]. Likewise, LAMP-QCM assays detect GMO papaya with 0.05% sensitivity[102]. Such validated examples collectively confirm that nucleic-acid-based editing, diagnostics, and RNAi technologies are not merely theoretical innovations but proven tools with strong translational potential in agriculture. They offer precise, efficient, and eco-friendly solutions to address global challenges such as food insecurity and climate change. Continued innovation, supportive policies, and responsible use will ensure these technologies become foundational for resilient food systems in the future.

9. LIMITATIONS and BIOSAFETY CONSIDRATIONS

Notwithstanding the accelerating developments in genome editing and RNA-based methodologies for enhancing crop traits, a number of considerable challenges remain unresolved. The efficacy of introducing editing tools is severely compromised in plant species characterized by complex genomic architectures or strong inherent biological barriers. Off-target effects and variable editing efficiency restrict the reproducibility and stability of edited traits. The stability and successful cellular uptake of dsRNA in RNAi-based applications are highly susceptible to ambient environmental parameters.

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From a biosafety standpoint, the comprehensive assessment of ecological and regulatory implications is imperative. Elevated risks require analysis, specifically concerning unintended genetic dispersal (gene flow), non-specific gene silencing in organisms outside the target scope, and the environmental persistence of the introduced nucleic acids or editing machinery. Risk assessment frameworks and public acceptance also play critical roles in translating laboratory advances into sustainable agricultural solutions.

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