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Review Article

CRISPR/Cas9 Genome Editing for Enhancing Lentil Resistance to Fungal Diseases

Md. Kawsar Alam Nadim✉, Md. Monirul Islam and Reza Mohammad Emon

Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh

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Correspondence
Md. Kawsar Alam Nadim
✉: nadim.kawsaralam@gmail.com

ABSTRACT

Lentil (*Lens culinaris* Medik.) is a nutritionally and economically vital legume crop cultivated globally. However, its productivity is severely threatened by fungal diseases such as Stemphylium blight, Fusarium wilt, and Ascochyta blight. Traditional breeding and chemical control strategies have shown limited success in providing long-term resistance against these pathogens. The advent of CRISPR/Cas9 genome editing offers a revolutionary and targeted approach to enhance disease resistance in lentils by enabling precise modification of key genes. This review highlights the mechanism of CRISPR/Cas9 and its application in lentil improvement, with particular focus on editing susceptibility (S) genes such as *Lc07593*, *LcMLO*, and *LcSWEET13*, which facilitate pathogen infection, and modulating genes in defense signaling pathways like LcNPR1, LcEDS1, and LcMYC2. Furthermore, the potential of engineering pattern recognition receptors (PRRs) like FLS2, EFR, and CERK1 for improved pathogen detection is discussed. The integration of CRISPR with speed breeding and marker-assisted selection further accelerates the development of resistant cultivars. Despite current challenges in transformation efficiency and regulatory barriers, CRISPR/Cas9 presents a promising tool for sustainable lentil disease management. Future directions include multiplex editing, base/prime editing, and epigenome modulation for fine-tuned resistance, all contributing to a resilient and high-yielding lentil crop.

Introduction

Lentil (*Lens culinaris* Medik 2n=2x=14), belonging to the Fabaceae family, is an important pulse crop cultivated globally for its high nutritional value, rich protein content, and contribution to sustainable agriculture through nitrogen fixation (Dhull et al., 2022; Hill, 2022; Marette and Roosen, 2022; Joshi et al., 2017; Matny, 2015; Asakereh et al., 2010). However, fungal diseases pose a significant challenge to lentil production, leading to severe yield losses and economic consequences. Among these, Stemphylium blight (caused by *Stemphylium botryosum*), Ascochyta blight (caused by *Ascochyta lentis*), and Fusarium wilt (caused by *Fusarium oxysporum* f. sp. *lentis*) are particularly destructive, compromising plant health and productivity (Sharma and Joshi. 2021; Jiskani et al., 2021; Taylor et al., 2007). Traditional breeding approaches have been employed to enhance disease resistance in lentils, but these methods are time-consuming, labor-intensive, and limited by the availability of resistant germplasm (Roy et al., 2023; Yin and Qiu, 2019; Kumar et al., 2016; Podder et al., 2013). In recent years, genome editing technologies such as CRISPR/Cas9 have emerged as a powerful tool for developing disease-resistant crops, offering precision and efficiency in genetic improvement (Erdoğan et al., 2023).

In Bangladesh, lentil is a key Rabi season crop cultivated on over 200,000 hectares of land. However, its average yield remains significantly below potential, primarily due to disease outbreaks, especially Stemphylium blight, which alone can cause up to 70% yield loss under favorable conditions (Podder and Vandenberg, 2013; Roy et al., 2023). Local genotypes such as BARI Masur-4 and BARI Masur-7 have shown partial resistance, yet no commercially grown variety has complete resistance to multiple fungal pathogens (Kumar et al., 2022). Disease pressure is further exacerbated by climate change, erratic rainfall, and limited use of disease-free seeds.

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Traditional breeding approaches have been employed to enhance disease resistance in lentils, but these methods are time-consuming, labor-intensive, and limited by the availability of resistant germplasm and the narrow genetic base of cultivated varieties (Roy et al., 2023; Yin and Qiu, 2019; Kumar et al., 2016; Podder et al., 2013). In recent years, genome editing technologies, particularly CRISPR/Cas9, have emerged as powerful tools for developing disease-resistant crops, offering precision and efficiency in genetic improvement (Erdoğan et al., 2023).

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9) is a revolutionary genome editing tool that allows targeted modification of DNA sequences in a precise and efficient manner. By employing guide RNA (gRNA) sequences that direct the Cas9 nuclease to specific genomic loci, this technology enables targeted gene knockouts, insertions, and modifications with high specificity. In plant biotechnology, CRISPR/Cas9 has been extensively used to enhance traits such as yield, abiotic stress tolerance, and disease resistance (Ambika et al., 2024; Yin and Qiu, 2019; Kato-Inui., 2018). In the context of lentil improvement, CRISPR/Cas9 offers a promising approach to knock out or modify susceptibility (S) genes that facilitate pathogen infection, thereby enhancing resistance to fungal diseases (Singer et al., 2024; Roy et al., 2023).

Recent advances in plant pathology and functional genomics have facilitated the identification of key genes involved in lentil-pathogen interactions. Study has shown that susceptibility genes such as *Lc07593* may play a role in making lentil susceptible to fungal pathogens (Cao et al., 2019). Targeted editing of such genes using CRISPR/Cas9 could lead to the development of lentil genotypes with enhanced resistance without compromising agronomic performance. Furthermore, CRISPR/Cas9 can be utilized to introduce novel resistance (R) genes or activate plant immune responses by modifying key regulatory elements (Roy et al., 2023). This approach offers a rapid and sustainable solution for disease management in lentil cultivation, reducing dependency on chemical fungicides and enhancing crop resilience.

Despite its immense potential, several challenges remain in the application of CRISPR/Cas9 for lentil disease resistance. Efficient delivery of the CRISPR/Cas9 system, off-target effects, and regulatory constraints pose significant hurdles in translating laboratory success to field applications (Uddin et al., 2020). Moreover, integrating genome editing with traditional breeding programs and molecular marker-assisted selection is essential for developing stable and commercially viable disease-resistant lentil varieties (Kumar et al., 2024; Roy et al., 2023). Future research should focus on optimizing transformation protocols, identifying novel resistance targets, and ensuring the biosafety and acceptance of genome-edited crops (Faizal et al., 2024).

CRISPR/Cas9 presents a transformative approach for enhancing fungal disease resistance in lentil by precisely modifying key genetic elements. As global food security concerns continue to rise, the integration of genome editing into lentil breeding programs holds great promise for developing resilient crop varieties that can withstand biotic stresses (Manzoor et al., 2024). Continued advancements in CRISPR/Cas9 technology, coupled with a deeper understanding of plant-pathogen interactions, will play a crucial role in shaping the future of lentil improvement and sustainable agriculture. Strategic targeting of susceptibility genes and defense regulators, coupled with robust transformation protocols and molecular selection tools, will be essential for developing next-generation lentil cultivars with enhanced resistance to fungal diseases. Collaborations among genomics researchers, breeders, policymakers, and farmers will be crucial to ensure successful adoption of genome editing for resilient lentil production.

Mechanism of Crispr/Cas9 in Plant Genome Editing

The CRISPR/Cas9 system operates through a highly specific and efficient mechanism, making it a powerful tool for targeted genome modifications in plants. This mechanism involves a sequence of well-defined steps, beginning with the identification of a target sequence and culminating in precise genome alterations (Aljabali et al., 2024). The fundamental principle underlying this system is the use of a guide RNA (gRNA) to direct the Cas9 nuclease to a specific site within the genome, enabling highly precise genome manipulation. The gRNA contains a sequence complementary to the target DNA region, which allows it to form a complex with the Cas9 protein and guide it to the exact genomic location of interest. Upon recognition of the target site, which must be immediately adjacent to a protospacer adjacent motif (PAM) sequence—typically NGG in most systems—the Cas9 protein induces a site-specific double-strand break (DSB) in the DNA (Asmamaw and Zawdie, 2021; Xue and Greene, 2021; Tian et al., 2017). This break acts as a signal for the plant cell's natural DNA repair mechanisms to become activated, most commonly through either the error-prone non-homologous end joining (NHEJ) pathway, which often results in small insertions or deletions (indels) that disrupt gene function, or the more precise but less efficient homology-directed repair (HDR) pathway if a donor template is provided. This targeted DSB

introduction forms the basis for versatile applications such as gene knockouts, point mutations, and insertions, making CRISPR/Cas9 a powerful tool for functional genomics and crop improvement (Wen and Zhang, 2022; Liao et al., 2024; Bharat et al., 2020).

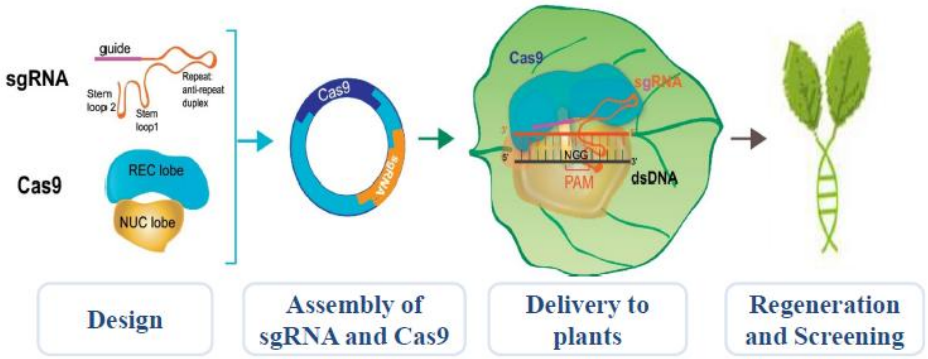

Fig. 1. The basic workflow of CRISPR/Cas9-mediated genome editing in plants.

A key component of this system is the single guide RNA (sgRNA), which is engineered to be complementary to the target DNA sequence. The sgRNA forms a complex with the Cas9 protein, creating a ribonucleoprotein complex capable of recognizing and binding to its target (Lone et al., 2018; Ceasar et al., 2016). For this recognition to occur, a protospacer adjacent motif (PAM) sequence, typically NGG, must be present immediately downstream of the target site. This sequence requirement ensures specificity, preventing off-target effects and enhancing the precision of genome editing (Labun et al., 2021).

Table 1. Key Components of CRISPR/Cas9 System

Component	Function
sgRNA	Guides Cas9 to the target DNA sequence
Cas9 Nuclease	Induces double-strand breaks at target sites
PAM Sequence	Required for Cas9 recognition and binding

Once the CRISPR/Cas9 complex successfully binds to the target sequence, the Cas9 protein induces a double-strand break at the specified location. This break is then repaired by one of two major DNA repair pathways: non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ, the predominant repair pathway in most plants, is error-prone and frequently results in small insertions or deletions (indels) at the break site, effectively disrupting gene function. This makes NHEJ an ideal mechanism for gene knockout studies (Wen and Zhang, 2022; Asmamaw and Zawdie, 2021; Xue and Greene, 2021). On the other hand, homology-directed repair (HDR) offers a highly precise repair mechanism by utilizing a homologous DNA template to accurately restore or replace the target sequence, enabling targeted gene insertions or specific nucleotide corrections (Liao et al., 2024; Smirnikhina et al., 2022). Despite its precision, HDR remains considerably less efficient in plant cells compared to non-homologous end joining (NHEJ), primarily due to the limited availability and incorporation of the donor template during the repair process. This reduced efficiency presents a significant challenge for achieving precise genome modifications in plants (Bhuyan et al., 2023; Bharat et al., 2020).

Table 2. Comparison of DNA Repair Pathways in CRISPR/Cas9

Repair Pathway	Characteristics	Applications
NHEJ	Error-prone, efficient	Gene knockouts
HDR	Precise, requires donor template	Targeted gene insertion

The efficiency and accuracy of CRISPR/Cas9 in plant genome editing have been widely demonstrated across various species. In crops such as rice, wheat, and maize, researchers have successfully used this system to introduce traits such as disease resistance, improved yield, and enhanced stress tolerance (Ndudzo, et al., 2024; Hamdan. and Tan, 2024; Mughair et al., 2022). For instance, targeted mutations in disease susceptibility genes have conferred resistance to fungal and bacterial pathogens, reducing reliance on chemical pesticides (Rani et al., 2024). Similarly, modifications in key regulatory genes controlling drought and salinity responses have led to the development of climate-resilient crop varieties (Shelake et al., 2022).

Table 3. Examples of CRISPR/Cas9 Applications in Plants

Crop	Target Gene	Modification	Outcome
Rice	*OsSWEET13*	Gene knockout	Bacterial blight resistance
Rice	*OsERF922*	Gene knockout	Blast disease resistance
Wheat	*TaMLO*	Gene knockout	Powdery mildew resistance
Maize	*ARGOS8*	Gene modification	Drought tolerance
Tomato	*SlPMR4*	Gene knockout	Enhanced resistance to powdery mildew
Soybean	*GmFAD2-1A/1B*	Gene knockout	Increased oleic acid content
Potato	*StGBSSI*	Gene knockout	Reduced amylose content (waxy starch)
Banana	*MusaPDS*	Gene knockout	Albino phenotype for transformation marker
Arabidopsis	*AtWRKY70*	Gene knockout	Enhanced resistance to pathogens

Despite its transformative potential, CRISPR/Cas9-mediated genome editing faces several challenges. Off-target effects, unintended genetic modifications, and low HDR efficiency remain critical concerns. Advances in base editing and prime editing technologies are addressing some of these limitations by enabling single nucleotide changes without introducing double-strand breaks (Garg et al., 2025; Liu et al., 2021; Uddin et al., 2020). Additionally, the development of high-fidelity Cas9 variants and improved gRNA design algorithms is enhancing the specificity and accuracy of genome editing applications in plants (Aljabali et al., 2024).

In summary, the CRISPR/Cas9 mechanism in plant genome editing represents a breakthrough in biotechnology, offering unparalleled precision in genetic modifications. Its ability to introduce targeted mutations with high efficiency has far-reaching implications for agricultural improvement, crop sustainability, and food security. As the technology continues to evolve, further refinements and innovations will unlock new possibilities for plant genetic engineering, revolutionizing modern agriculture.

CRISPR Applications in Lentil Disease Resistance

Traditional breeding methods for developing disease-resistant cultivars are time-consuming, labor-intensive, and often constrained by the intrinsic limitations of the crop's biology. In lentils, these limitations are further amplified by a relatively long generation cycle, which slows down the process of incorporating desirable traits through successive breeding generations. Additionally, the narrow genetic base and limited availability of naturally resistant germplasm hinder the effective identification and introgression of disease resistance traits (Mitache et al., 2024; Yadav et al., 2023; Roy et al., 2023). These challenges make it difficult to achieve durable and broad-spectrum resistance against multiple fungal pathogens using conventional breeding alone. In contrast, CRISPR/Cas9 genome editing offers a rapid, precise, and efficient alternative by enabling targeted modifications of key genes involved in susceptibility, pathogen recognition, and downstream defense signalling pathways. This technology allows researchers to bypass the lengthy breeding cycles and directly enhance resistance traits at the molecular level, thereby accelerating the development of improved lentil cultivars with enhanced disease resilience.

Targeting Susceptibility Genes

Susceptibility genes (S-genes) encode proteins that facilitate pathogen infection by suppressing defense responses, aiding pathogen entry, or interfering with immune signaling (Talakayala et al., 2022). Disrupting these genes via CRISPR/Cas9 can effectively block pathogen invasion and enhance lentil's resistance (Garcia-Ruiz et al., 2021; Zaidi et al., 2018).

Several S-genes have been identified as potential targets for genome editing in lentil, based on findings from other legume crops (Roy et al., 2023; Kumar et al., 2015; Kumar et al., 2014).

Table 4. CRISPR Knockout Targets for Disease Resistance in Lentil

Target Gene	Function	Disease	CRISPR Strategy	Expected Outcome
Lc07593	Negative regulator of defense	Stemphylium blight	Knockout (NHEJ)	Enhanced resistance to SB
ERF (Ethylene Response Factor)	Suppresses immune response	Fusarium wilt	Knockout (NHEJ)	Increased FW tolerance
LcMLO (*Mildew Locus O*)	Susceptibility factor	Powdery mildew	Knockout (NHEJ)	Loss of PM susceptibility
LcSWEET13	Sugar transporter aiding pathogen entry	Bacterial blight	Knockout (NHEJ)	Reduced pathogen infection
LcDND1	Regulates cell death	Fusarium wilt	Knockout (NHEJ)	Increased FW tolerance

By disrupting these susceptibility genes, lentil plants can exhibit significantly improved resistance to fungal pathogens

Enhancing Pathogen Recognition

Importance of Pattern Recognition Receptors (PRRs)

Plants detect pathogens through Pattern Recognition Receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) and trigger early immune responses. Engineering lentil plants to express modified PRRs can enhance pathogen detection and immune activation (Ranf, 2018; Zipfel, 2014; Postel and Kemmerling, 2009).

Key Pattern Recognition Receptors for Engineering Disease Resistance

Several PRRs identified in model crops can be introduced or modified in lentil using CRISPR to enhance disease resistance (Manzoor et al., 2024; Mushtaq et al., 2019).

Table 5. Pattern Recognition Receptor Engineering for Enhanced Disease Resistance in Lentil

PRR Gene	Function	Pathogen Targeted	CRISPR Strategy	Expected Benefit
FLS2 (Flagellin-Sensing 2)	Recognizes bacterial flagellin	*Pseudomonas* spp.	Overexpression	Faster immune activation
EFR (EF-Tu Receptor)	Detects bacterial elongation factors	*Xanthomonas* spp.	Gene insertion (HDR)	Enhanced bacterial recognition
CERK1 (Chitin Elicitor Receptor Kinase 1)	Detects fungal chitin	*Fusarium*, *Stemphylium*	Promoter activation	Stronger fungal resistance
LYK5 (Lysin Motif Receptor Kinase 5)	Recognizes fungal β-glucan	*Fusarium* spp.	Gain-of-function mutation	Improved fungal recognition

By modifying PRR genes, lentil plants can detect pathogens more efficiently, triggering early immune responses to prevent infection.

Boosting Antifungal Defense Pathways

Salicylic Acid and Jasmonic Acid Pathways in Disease Resistance

Lentil defense mechanisms are primarily governed by two key hormonal signaling pathways: the salicylic acid (SA) pathway and the jasmonic acid (JA)–ethylene (ET) pathway (Kour et al., 2024; Veselova et al., 2024; Stroud et al., 2022). The SA pathway plays a crucial role in activating systemic acquired resistance (SAR), particularly against biotrophic pathogens that rely on living host tissue. In contrast, the JA and ET pathways are predominantly involved in regulating defense responses against necrotrophic pathogens, which kill host cells to derive nutrition. These signaling networks coordinate complex gene expression cascades that contribute to pathogen recognition, defense gene activation, and reinforcement of structural barriers. By using CRISPR/Cas9 to edit genes associated with these pathways—such as transcription factors, receptors, or signal regulators—it is possible to enhance the plant's innate immune responses and develop lentil varieties with robust and broad-spectrum antifungal resistance.

Table 6. CRISPR Editing of Defense Pathway Genes in Lentil

Gene	Pathway	Function	CRISPR Strategy	Expected Effect
LcNPR1	SA	Activates systemic acquired resistance	Promoter activation	Increased SAR response (Zhang et al., 2006)
LcEDS1	SA	Defense regulator	Knock-in mutation	Faster immune response (Cui et al., 2015)
LcMYC2	JA	Regulates JA-mediated defense	Knock-in mutation	Stronger JA signaling (Kazan and Manners, 2013)
LcWRKY22	ET	Transcription factor	Gain-of-function	Enhanced pathogen resistance (Phukan et al., 2016)

By fine-tuning these defense pathways, CRISPR can enhance lentil's natural immune responses against fungal infections.

Improving Lentil Germplasm Via CRISPR

Integration of CRISPR with Speed Breeding

To accelerate the development of disease-resistant lentil varieties, CRISPR can be combined with speed breeding techniques, which reduce generation time by using controlled light and temperature conditions.

Advantages of CRISPR + Speed Breeding

Reduced Breeding Cycles: Traditional breeding methods for lentils can take several years to develop new varieties. The integration of speed breeding can reduce generation length by 2.5 to 5 times compared to conventional methods, thereby accelerating crop improvement (Santos et al., 2020).

Accelerated Screening of CRISPR-Edited Lines: Speed breeding allows for the rapid advancement of generations, enabling quicker evaluation of CRISPR-induced mutations and their phenotypic effects. This facilitates the prompt identification and selection of lines exhibiting desired traits, such as disease resistance (Zaidi et al., 2020).

Enhanced Fixation of Beneficial Traits: The combination of speed breeding and CRISPR technology accelerates the development of improved crop varieties by significantly reducing the time required for breeding cycles while maintaining genetic diversity (Kumari and Singh, 2022).

Marker-Assisted Selection (MAS) and CRISPR

CRISPR can be integrated with Marker-Assisted Selection (MAS) to improve selection efficiency. The integration of CRISPR technology with Marker-Assisted Selection (MAS) and speed breeding can significantly accelerate the development of disease-resistant lentil cultivars. This approach involves identifying CRISPR-edited variants using molecular markers, selecting lines with confirmed disease resistance traits, and backcrossing with elite cultivars for field adaptability. By combining these methodologies, breeding cycles can be reduced, and the efficiency of developing improved lentil varieties can be enhanced (Kumari and Singh, 2024; Kumar et al., 2022; Bhowmik et al., 2019).

Future Research Prospect

The continuous advancements in CRISPR technology offer numerous possibilities for improving lentil disease resistance. Researchers are exploring innovative applications that can enhance genome editing efficiency and precision, allowing for the development of more resilient lentil cultivars.

CRISPR Multiplexing to Edit Multiple Genes Simultaneously

CRISPR multiplexing enables the simultaneous editing of multiple genes within a single transformation event, which is particularly advantageous for developing polygenic disease resistance in lentils. Fungal pathogens such as *Stemphylium botryosum*, *Fusarium oxysporum* f. sp. *lentis*, and *Ascochyta lentis* employ diverse infection strategies, targeting multiple host genes to overcome plant immunity. Therefore, targeting several susceptibility (S) genes, signaling components, and transcriptional regulators in lentils at once using CRISPR multiplexing can disrupt multiple points of fungal invasion and colonization. For instance, concurrent editing of *Lc07593*, *LcMLO*, and *ERF* genes could reduce vulnerability to SB, FW, and AB by blocking pathways exploited by fungi for host entry, suppression of immunity, or nutrient acquisition. This strategy has been successfully demonstrated in crops like rice and wheat, showing its potential in lentils. In wheat, multiplex CRISPR/Cas9 has been used to simultaneously edit multiple *TaMLO* alleles, resulting in enhanced resistance to powdery mildew (Wang et al., 2018). Similarly, in rice, multiplex editing of disease-related genes has achieved high efficiency and heritability (Xie and Yang, 2015). Implementing multiplex editing in lentils could drastically reduce breeding time and minimize the need for sequential transformation cycles, thereby accelerating the development of disease-resistant cultivars with broad-spectrum fungal resistance.

Base Editing and Prime Editing for Precise Genetic Modifications

While traditional CRISPR/Cas9 relies on double-strand breaks (DSBs) to introduce mutations, base editing and prime editing offer more precise and predictable modifications, especially when targeting genes associated with pathogen perception and response. Fungal pathogens often hijack host transcription factors and membrane proteins involved in susceptibility, such as sugar transporters and defense repressors. Base editing uses deaminase enzymes to convert specific bases, such as cytosine to thymine or adenine to guanine, enabling the fine-tuning or deactivation of such S genes without creating DSBs (Li et al., 2023; Tan et al., 2022; Kantor et al., 2020). This is particularly beneficial when subtle mutations in conserved domains of receptor kinases or regulatory elements are sufficient to disrupt pathogen compatibility.

Prime editing, on the other hand, leverages a modified reverse transcriptase fused with a Cas9 nickase to insert, delete, or replace targeted sequences in a highly controlled manner. This approach allows for the precise manipulation of genes involved in the fungal response, such as introducing beneficial allelic variants in resistance-associated kinases, or editing cis-regulatory elements that control inducible defense gene expression (Kantor et al., 2020). These editing methods offer safer and more stable alternatives to conventional genome editing, reducing off-target risks while maintaining the plant's genomic integrity during pathogen challenge.

Epigenome Editing to Fine-Tune Disease Resistance Gene Expression

Epigenome editing provides another powerful strategy to combat fungal infections in lentils by modulating gene expression through targeted changes in DNA methylation or histone modification, without altering the DNA sequence itself. Fungal pathogens often suppress host immunity by inducing epigenetic silencing of defense-related genes or activating S genes. By using CRISPR-based transcriptional repressors or activators, breeders can reverse these epigenetic changes. For instance, silencing *Lc07593*, a putative S gene associated with SB susceptibility, through targeted methylation can reduce lentil sensitivity to *Stemphylium botryosum* (Cao et al., 2019). Conversely, activating positive regulators such as LcNPR1 or WRKY transcription factors using CRISPR activation systems

can enhance antifungal responses and reinforce resistance against a broad range of fungal pathogens (Zaynab et al., 2020). This approach is not only reversible and non-transgenic but also aligns well with regulatory guidelines in many countries, offering a sustainable pathway for improving fungal disease resistance in lentil breeding programs (Mehdi et al., 2025).

Challenges in CRISPR Lentil Improvement

CRISPR-based lentil improvement, while promising, faces several challenges, particularly in transformation efficiency, regulatory hurdles, and limited genomic resources. One of the primary barriers to CRISPR editing in lentils is the low transformation efficiency, as lentil plants are notoriously difficult to transform using Agrobacterium-mediated methods (Nivya and Shah, 2023; Baloglu et al., 2022). Compared to model crops like rice and Arabidopsis, lentil transformation protocols are still inefficient, leading to low success rates in gene editing (Bhowmik et al., 2021). Researchers are addressing this challenge by optimizing tissue culture conditions, developing new transformation protocols, and exploring alternative CRISPR delivery methods such as nanoparticle-mediated and electroporation-based systems. Another challenge lies in the regulatory landscape for genome-edited crops, which varies significantly between countries. While countries like the United States and Japan have more relaxed regulations for CRISPR-edited crops, the European Union classifies them as GMOs, requiring lengthy and costly approval processes (Vora et al., 2023). For lentil breeders, navigating these regulations is essential, with SDN-1 mutations offering a potential avenue for more efficient approval (Kumar et al., 2022). Lastly, the limited genomic resources for lentil, compared to major cereals, constrain the identification of resistance genes. Expanding genomic databases, sequencing diverse lentil germplasm, and integrating omics technologies will be key in unlocking new resistance loci, making CRISPR-based breeding more effective for lentil disease resistance.

Conclusion

CRISPR/Cas9 holds immense potential for improving lentil disease resistance, but its widespread adoption requires overcoming technical, regulatory, and genomic challenges. Advancements in multiplexing, base editing, and epigenome editing offer exciting possibilities for precise genetic modifications. However, efforts must be made to enhance transformation efficiency, streamline regulatory approvals, and expand genomic resources to fully leverage CRISPR for lentil improvement. As research progresses, the integration of CRISPR with traditional breeding and cutting-edge technologies will pave the way for durable and sustainable disease resistance in lentils, contributing to global food security and sustainable agricultural practices.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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