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Application of Genome Editing in Pineapple Disease Resistance Breeding: CRISPR/Cas9 Strategies

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Abstract The three main causes of yield loss in pineapple crops include heart rot and black rot and leaf spot. Breeding new types in the usual way takes many years. The crop maintains a restricted genetic diversity because it cannot produce self-pollination and requires extended periods for growth and testing. The research evaluates CRISPR/Cas9 as a fast method to introduce disease resistance. We show the main defense routes, such as SA, JA/ET, and MAPK. We implement editing approaches which have proven effective in different plant species. The main ways are: (i) change promoters or switch on defense genes; (ii) remove susceptibility genes and genes that decrease defense; (iii) modify control sites such as miRNA binding sites; and (iv) edit multiple genes simultaneously to build resistance. Some problems remain. The regeneration rate is low and edited plants can be mosaic and pineapple has high heterozygosity and off-target hits can occur. The solutions for this problem include tissue culture improvement and morphogenic regulator addition and nuclease precision enhancement and RNP delivery without DNA. Our approach consists of three phases which begin with omics-based target selection followed by DNA-free multiplex editing and end with field testing and compliance with transgene-free plant regulations. The described methods enable pineapples to develop robust and extensive resistance that can be used by breeders to create new plant lines.
Keywords Pineapple (*Ananas comosus*); CRISPR/Cas9; Disease resistance; Susceptibility genes; Promoter editing; Multiplex editing; DNA-free delivery

1 Introduction

The tropical fruit pineapple (*Ananas comosus*) gains its popularity from its enjoyable taste and nutritious value and its essential role in global trade because of its multiple industrial uses. Bananas and citrus fruits join mangoes as the world's leading tropical fruits according to global production statistics (Tripathy, 2024). The FAO reports that worldwide production exceeds 28 million tonnes each year according to Gunawardena and Lokupitiya (2024). The cultivation of this plant species occurs in approximately 90 nations which span across Asia and Africa and the Americas. The majority of these products originate from Costa Rica and the Philippines and Brazil and Thailand and Indonesia because these countries produce more than 70% of the world supply (Li et al., 2022). Costa Rica alone produces close to 2.9 million tonnes annually. The pineapple trade is valued at several billion US dollars (Ming et al., 2015) and people use it both as a fresh product and in processed items including juice and canned fruit and confectioneries and bromelain. Given its high economic and nutritional importance, considerable efforts have been devoted to cultivar improvement. The continuous emergence of diseases results in decreased agricultural output and interrupted market operations (Sapak et al., 2021). The creation of resistant crop varieties has become essential because fungal diseases now endanger agricultural production at an unprecedented level. The protection of harvests and farmer incomes and export markets requires immediate development of resistant crop varieties.

Pineapple farming faces its most critical challenge from disease pressure which includes heart rot as one of its most damaging diseases. The disease starts with small water-soaked spots in plant cores which then quickly lead to decay of inner leaf tissue. It is typically caused by *Phytophthora* spp. The disease results from fungal infections by *Phytophthora infestans* and bacterial infections by *Dickeya zeae* which can lead to total plantation destruction

during major outbreaks (Sapak et al., 2021). Leaf spot which also goes by the names black spot or yellow spot represents a significant issue that usually results from *Penicillium funiculosum* fungal infections. The pathogen causes damage to photosynthesis and creates harm to fruits while simultaneously degrading their quality (Serrato-Diaz et al., 2023). The postharvest black rot disease which *Ceratocystis paradoxa* causes damages crops at the same level by entering through fruit injuries to create a soft decay with foul odor (Hubert et al., 2014). The diseases affect pineapple cultivation throughout every pineapple-producing region worldwide. *Phytophthora nicotianae*-induced heart rot has been documented in Latin America Asia and Africa after heavy rainfall events (Ratti et al., 2018). Black rot has spread so widely that in some regions it has been designated a quarantine concern. Brazilian agricultural production faces major losses because fusariosis and black spot diseases continue to be major agricultural threats. The control strategies for disease management include disease-free seedlings and strict field hygiene and fungicides or antibiotics and quick removal of infected plants but these methods are not always effective (Sapak et al., 2021). The continued presence of these pathogens shows that breeding programs need to establish long-term resistance as their primary goal.

Traditional breeding approaches, including hybridization and mutation breeding, have achieved only limited progress in improving pineapple resistance. The main challenge arises from the limited genetic diversity of commercial cultivars including 'Smooth Cayenne' and 'MD2' (Li et al., 2022). The limited availability of useful resistance genes exists because plant breeding operations face additional challenges due to the biological characteristics of the crop. Pineapple requires 2~3 years from planting to fruiting and because it is both self-incompatible and highly heterozygous, progeny populations show broad genetic variation that makes selection challenging. The execution of long-term field tests which extend across multiple years results in both high expenses and prolonged testing periods. The advancement of new crop varieties through chance seedlings and somaclonal variants has led to some progress yet the rate of improvement continues to be sluggish. Mutation breeding produces new traits but the process of screening big populations is time-consuming and unwanted genetic changes frequently emerge (Serrato-Diaz et al., 2023). The development of new hybrid cultivars requires 15–20 years but pathogens can adapt their resistance in less than this timeframe which creates a continuous challenge for plant breeders.

In this context, CRISPR/Cas9 genome-editing technology represents a promising alternative. The system employs guide RNA together with Cas9 protein to generate precise double-strand breaks at predetermined positions in the genome. The plant cell fixes these breaks through its built-in cellular mechanisms which lead to minor genetic changes that result in gene inactivation (Guo et al., 2023). Scientists employ this method to disable disease-producing genes which makes plants more vulnerable to disease while maintaining their important agricultural characteristics (Wan et al., 2020). CRISPR technology enables scientists to evaluate edited lines within a single breeding cycle which results in faster breeding processes. The method allows scientists to turn on defense genes according to Han et al. (2025) and Rivera-Toro et al. (2025) or to edit multiple targets at once by using multiplexing approaches (Li et al., 2025; Oliva et al., 2019). Research studies show that CRISPR technology demonstrates potential for creating long-term disease resistance according to Langner et al. (2018), and its successful application in other crops strongly suggests that pineapple may also benefit from this technology.

This study explores how CRISPR/Cas9 can help build disease-resistant pineapple. It reviews the pineapple genome and key defense routes, takes lessons from other crops, and puts forward editing plans for heart rot, black rot, and leaf spot. It also points out problems such as low transformation efficiency and off-target edits, and suggests possible fixes. The aim is to give clear scientific guidance for accurate breeding and to support lasting disease control and higher yields in tropical fruit crops.

2 Pineapple Genome and Disease-Related Gene Resources

2.1 Pineapple genome sequencing and annotation progress

In the past ten years, research has made big progress in understanding the pineapple genome. This provides a base for using genome editing. The first draft genome of pineapple (‘F153’) was published in 2015 (Ming et al., 2015).

It showed a genome size of about 526–563 Mb, with 25 chromosomes (2n = 50) (Figure 1) (Yow et al., 2021). Pineapple had fewer whole-genome duplication events than crops like grasses, so it has a smaller gene set. Around 27,000 protein-coding genes were found. The first genome gave useful data on traits such as CAM photosynthesis, but the sequence continuity was low (contig N50 < 100 kb).

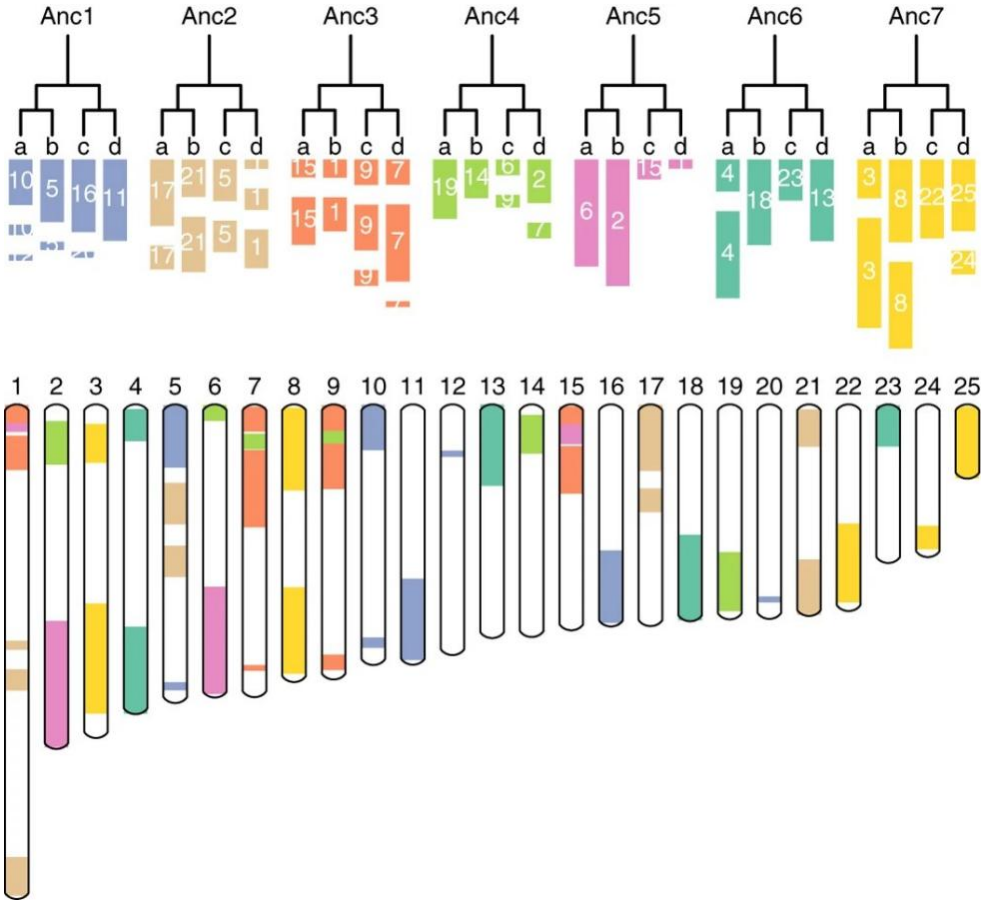

Figure 1 Karyotype structure and chromosome composition of the pineapple genome (Adapted from Ming et al., 2015)

In 2022, researchers built a better genome assembly for the MD2 cultivar using PacBio long-read sequencing and Hi-C scaffolding. In the MD2 v2 version, 99.7% of the sequence was placed on 25 pseudochromosomes. The contig N50 was over 1 Mb. This work confirmed that the haploid genome size is about 563 Mb and that MD2 has high allelic heterozygosity (Yow et al., 2021).

Genome annotation has also improved. Many genes now have predicted roles, and big gene families, such as transcription factors and enzymes, have been listed. Studies show that pineapple has kept many single-copy genes even though it did not have recent whole-genome duplications (Ming et al., 2015).

RNA sequencing from different plant tissues has helped scientists predict gene models and spot genes linked to disease. At present, pineapple has a solid reference genome and detailed annotation data (Yow et al., 2021). These resources make it easier to find resistance genes and plan CRISPR strategies based on its genetic map.

2.2 Identified disease resistance genes in pineapple

Research on pineapple disease-related genes is still limited, but some have been found. Pineapple carries NBS-LRR resistance (R) genes, which help recognize pathogen effectors. However, it has far fewer of these genes than many other crops. One study showed that pineapple has far fewer NBS-LRR genes than grasses, likely because it missed the whole-genome duplication events that expanded these genes in cereals (Chen et al., 2019). Pineapple may have only a few dozen, while rice and maize have hundreds (Zhou et al., 2024). Some pineapple R genes belong to known groups, such as coiled-coil NBS-LRRs, and are close to those in resistant monocots.

Another group is pathogenesis-related (PR) genes, which produce antimicrobial proteins. Transcriptome data show that pineapple can make chitinases and glucanases when attacked by pathogens (Sapak et al., 2021). The exact number of *PR* genes is not well known, but pineapple probably has several *PR1*, *PR2*, and *PR5* genes, which work in the salicylic acid defense pathway.

Regulatory genes also play roles. Pineapple has 54 WRKY transcription factor genes, fewer than Arabidopsis (72) or rice (105) (Chen et al., 2019). This may also be largely due to its genome history without recent duplications. Some *WRKY* genes, such as *AcWRKY28*, have been studied and can improve stress tolerance when overexpressed (Zhou et al., 2024).

Pineapple also carries genes for hormone pathways, such as salicylic acid and jasmonate, and for secondary metabolism, such as phenylpropanoid enzymes. For instance, phenylalanine ammonia-lyase (PAL) genes may help build stronger cell walls and improve resistance to pathogens (Rivera-Toro et al., 2025).

In short, pineapple has fewer resistance genes overall, but important groups like NBS-LRR, PR, WRKY, and hormone regulators are present. These genes can be targets for CRISPR/Cas9, either to remove susceptibility genes or to boost positive defense genes.

2.3 Disease-related defense pathways in pineapple

When pathogens attack, pineapple may turn on defense systems much like those in other plants. These include hormone signals and kinase chains. The main hormones are salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). In many plants, SA mainly fights biotrophic pathogens, while JA and ET defend against necrotrophs and insects (Li et al., 2019; Hou and Tsuda, 2022).

Pineapple has genes such as *NPR1* for SA signals and *COI1/JAZ* for JA signals. When SA is triggered, for example during *Phytophthora* infection, it can turn on PR genes like *AcPR1* and cause systemic resistance (Sapak et al., 2021; Tian et al., 2025). Wounds or insect feeding can start the JA/ET pathways, leading to proteins such as protease inhibitors.

These hormone pathways can affect each other. High SA may weaken JA defense, and high JA may reduce SA activity (Li et al., 2019). Pineapple also seems to use MAPK cascades to carry danger signals from the cell surface to the nucleus. In other plants, sensing pathogens turns on MAPKs, which then trigger defense gene expression (Hou and Tsuda, 2022; Zhang et al., 2025).

The pineapple genome carries *MAPKKK*, *MAPKK*, and *MAPK* genes. MAPK3/6-like genes may turn on WRKY transcription factors, which can boost defenses such as oxidative bursts or stronger cell walls. *PAL* genes in these pathways may raise lignin content, which in tomato has been linked to better resistance (Rivera-Toro et al., 2025).

In short, pineapple makes use of common plant defense routes—SA, JA, and ET hormone signals, along with MAPK pathways. Genome editing can work on these points to improve resistance, either by removing blockers or by boosting helpers (Tian et al., 2025). Using knowledge of these pathways together with genome data can guide better strategies for stronger immunity.

3 Success Stories of CRISPR/Cas9 Disease Resistance in Other Crops

3.1 Grape (*Vitis vinifera*) – resistance to powdery mildew via *MLO* gene knockout

Powdery mildew, caused by *Erysiphe necator*, is a serious grape disease. CRISPR has been used to give grapes resistance by knocking out *MLO* genes, which are susceptibility genes. In grape, *VvMLO3* and *VvMLO7* help the fungus infect plants. When these genes lose function, plants become resistant, as seen before in barley.

Wan et al. (2020) used CRISPR/Cas9 to edit *VvMLO3* and *VvMLO4* in ‘Thompson Seedless’ grapes. They used Agrobacterium to deliver Cas9 and sgRNA into embryogenic callus, creating small indels. The edited plants had much better mildew resistance in greenhouse tests, with fewer lesions and spores than wild type (Figure 2). There were no major growth problems, apart from improved resistance.

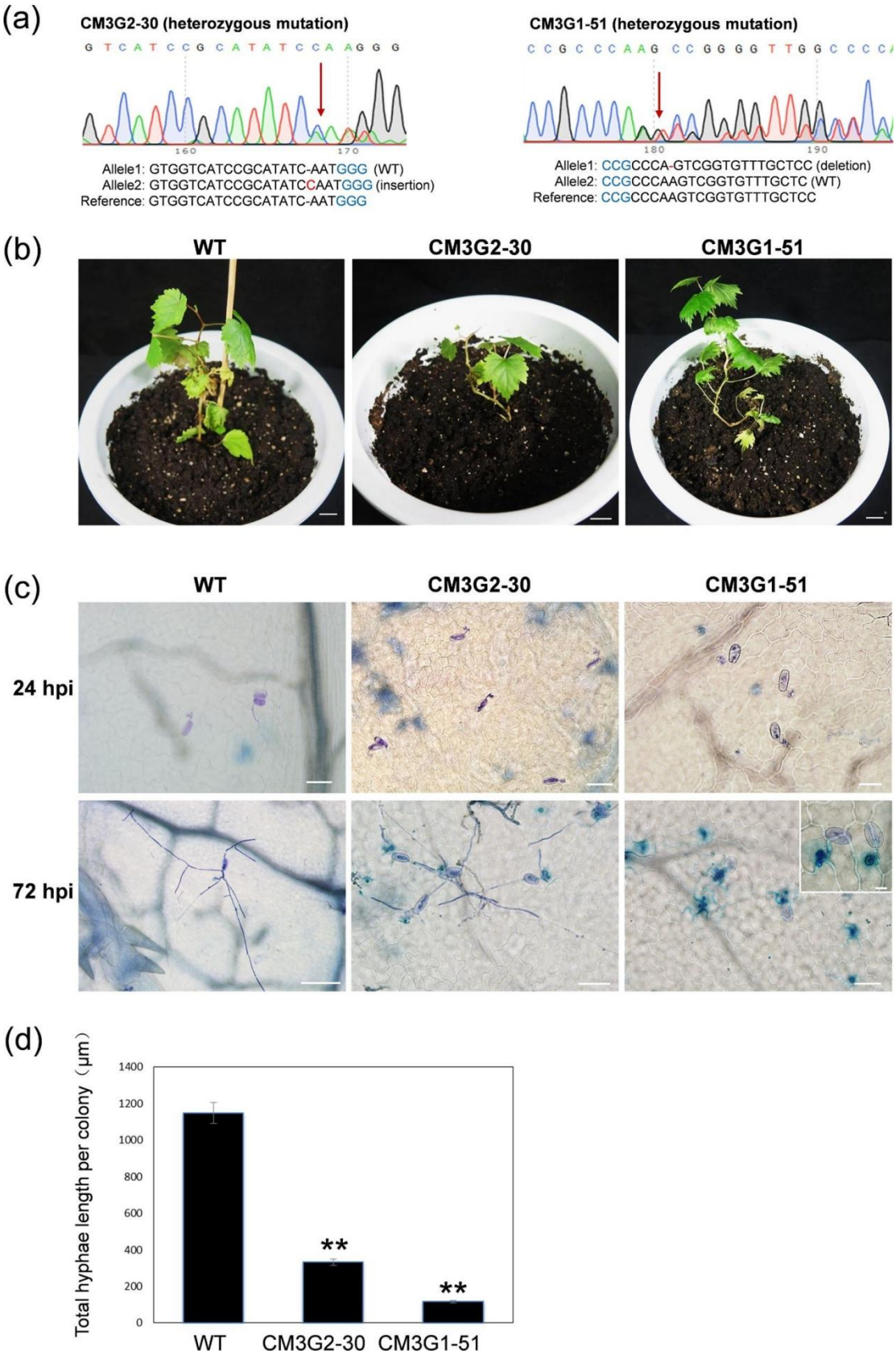

Figure 2 Reduced early fungal growth in CRISPR-edited grape plants lacking functional *VvMLO3* (Adapted from Wan et al., 2020)

Moffa et al. (2025) edited *VvMLO6* and *VvMLO7* at the same time. The double mutants were almost immune, with only very small fungal growth. Growth and fruiting stayed normal. They also used a Cre/lox system to remove transgenic DNA, producing mildew-resistant, transgene-free plants.

These findings show that CRISPR can copy known resistance changes in perennial fruit crops without bringing in outside genes. For pineapple, if matching *MLO* genes (*AcMLO*) are identified, this method may work. They also suggest that CRISPR fits clonal fruit crops, using tissue culture and DNA-free methods to follow rules and current regulations.

3.2 Banana (*Musa* spp.) – resistance to banana streak virus via eBSV sequence editing

Banana suffers from Banana streak virus (BSV). In some cultivars, the virus comes from "endogenous" viral DNA (eBSV) inside the genome. Under stress, these sequences can activate and cause disease. Commercial bananas are sterile, so breeding to remove eBSV is not possible.

Tripathi et al. (2019) used CRISPR/Cas9 to disrupt the eBSV DNA in plantain (*Musa* AAB genome). They designed sgRNAs to target three conserved regions of the viral genome inside the plant's chromosomes. Embryogenic cells from the cultivar 'Gonja Manjaya' were transformed with Cas9 and sgRNAs. The edited plants had indels in the eBSV sites and did not develop BSV symptoms, even under stress. Control plants still showed disease.

The edited bananas grew normally, with no off-target problems. This was one of the first CRISPR uses in a clonally propagated polyploid crop, solving a problem breeding could not. The method shows how precise editing can remove harmful sequences in the plant's own DNA. It also suggests similar strategies could be used in pineapple for viruses or other genetic disease risks.

3.3 Citrus (*Citrus sinensis*) – resistance to citrus canker via *CsLOB1* promoter editing

Citrus canker, caused by *Xanthomonas citri*, is a major disease in sweet orange. CRISPR was used to give resistance by editing the promoter of *CsLOB1*, a susceptibility gene. The pathogen uses TAL effectors to bind to the *CsLOB1* promoter and activate it, leading to disease.

Jia and Wang (2020) used CRISPR/Cas9 to change the effector-binding elements (EBEs) in the *CsLOB1* promoter. They delivered Cas9 and sgRNAs into citrus cells with Agrobacterium. The edited plants had small indels at the binding site and showed no canker symptoms after infection, while controls did.

Peng et al. (2017) edited *CsLOB1* in Duncan grapefruit, changing both the promoter and coding regions. Some plants had both alleles edited in the first generation and showed strong resistance. Editing the promoter kept the normal function of *CsLOB1* for growth but blocked pathogen attack.

In some cases, the editing was transgene-free, which is important for consumer and regulatory acceptance. This case shows CRISPR can protect crops by removing a pathogen's "entry point" in the genome. For pineapple, a similar promoter-editing strategy could help protect against bacterial diseases like heart rot.

4 Potential of CRISPR/Cas9 in Pineapple Disease Resistance Breeding

4.1 Enhancing defense gene function via promoter editing or activation

One way to improve pineapple's disease resistance is to make its own defense genes work harder. CRISPR/Cas9 can do this by changing the promoter of a gene to make it more active, or by using CRISPR systems that turn on a gene without changing its DNA. In traditional breeding, overexpression of genes like those in the phenylpropanoid pathway or PR proteins can give stronger resistance, but adding extra copies by transgenics often causes regulation problems.

With CRISPR, promoters can be adjusted so a defense gene is active more often or responds faster when the plant is infected. In tomato, Rivera-Toro et al. (2025) used a dCas12a activator to boost *SlPAL2*, a gene in the lignin pathway. The treated plants showed thicker cell walls, smaller lesions, and fewer bacteria, while still keeping normal growth.

In pineapple, good targets may include *AcPAL*, *AcWRKY* transcription factors, and *AcPR1*. Editing promoters to cut repressor sites or add stronger motifs could raise PR proteins or lignin enzymes in tissues. CRISPR activation (CRISPRa) is another option. This method uses a dead Cas9 linked to an activator to raise gene expression

without cutting DNA (Ding et al., 2022; Clark et al., 2024). CRISPRa can also be timed, for example after flowering or during fruit growth, to protect fruit from black rot when needed. This method has worked in *Arabidopsis* and may also be applied to pineapple.

Overall, increasing the strength of existing defenses by promoter edits or CRISPRa is a practical approach. It uses the plant's own genes, works precisely, and can avoid big growth penalties.

4.2 Knockout of susceptibility genes to block pathogen entry

A basic CRISPR way to add disease resistance is to delete susceptibility (S) genes. These are plant genes that pathogens use to get in. When these genes stop working, plants may gain resistance (Wan et al., 2020; Moffa et al., 2025).

One prime candidate is pineapple's ortholog of MLO. Powdery mildew is not a known pineapple disease (likely due to environmental differences), but *MLO* genes also can influence other pathogens or developmental processes. If pineapple *MLO* contributes to any fungal infection (perhaps leaf spot fungi), knocking it out might be beneficial.

More directly, consider S genes for bacterial pathogens: many bacterial diseases require host susceptibility factors for infection. In citrus, *CsLOB1* was an S gene for canker that CRISPR was used to disable (Jia and Wang, 2020). Pineapple's bacterial heart rot (caused by *Dickeya* or related Erwinia) may similarly hinge on a host factor.

Research in related bromeliads or monocots could point to such factors – for instance, *Erwinia chrysanthemi* (which causes soft rot in many plants) often secretes pectate lyases and cell wall-degrading enzymes; plants with altered cell wall components can show resistance. A hypothetical S gene might be a pineapple pectin acetylesterase gene required for the bacterium to break down pectin. If so, a CRISPR knockout of that gene could reduce tissue maceration and confer partial resistance to heart rot.

Another category of S genes are those involved in negative immune regulation. For example, *EDR1* is a kinase acting as a negative regulator of defense; wheat plants edited for all three homeologs of *TaEDR1* showed enhanced broad resistance to powdery mildew (Zhang et al., 2017; Zhou et al., 2023). Pineapple has its own *EDR1* homolog – knocking it out via CRISPR might hyperactivate the plant's basal defenses, making it less susceptible to a range of pathogens (albeit with vigilance for any trade-offs like stunted growth).

Given pineapple's relatively small R gene arsenal, targeting S genes is attractive because it removes the weak links that pathogens universally rely on. CRISPR's precision is key: undesirable alleles can be cleanly knocked out without disrupting other genes. In tomato, CRISPR deletion of a few amino acids in the *SlMlo1* gene conferred mildew resistance and was accomplished within 10 months, producing a variety termed "Tomelo" (Nekrasov et al., 2017; Schenke and Cai, 2020). Pineapple's longer generation time means the process would be slower, but still far faster than conventional breeding and without altering fruit traits.

Importantly, resistance via S gene loss is often broad-spectrum and durable because the pathogen cannot easily overcome the absence of a host factor (as opposed to single R gene resistance which pathogens can defeat by mutating an effector). Hence, creating pineapple knockouts for key S genes – once identified – could yield varieties with stable resistance to heart rot, black rot, or other diseases.

The challenge is to pinpoint the right targets: candidate gene discovery might involve transcriptomics (seeing which host genes are upregulated by infection) or ortholog knowledge from other crops. Nonetheless, the concept is clear: if the pathogen needs a host gene, take that gene away using CRISPR, and the plant becomes an unwelcome host.

4.3 Editing regulatory networks: targeting defense regulators and microRNAs

CRISPR can do more than change single genes. It can also adjust pineapple's immune control system to react faster or stay stronger against pathogens. This means editing key regulator genes, such as transcription factors or microRNAs, that control many resistance genes at once.

One way is to remove negative regulators so the plant's defense stays more active. In grapevine, scientists deleted *VvNPR3*, a repressor in the salicylic acid (SA) pathway, and the plants became more resistant to mildew (Moffa et al., 2025). Pineapple also has *NPR1*, *NPR3*, and *NPR4*. If *AcNPR3* is edited, SA defenses could be stronger, leading to more PR proteins and more antimicrobial compounds.

MicroRNAs also control resistance genes. In tomato, miR482 family members lower the activity of NBS-LRR genes. Removing *miR482b* and *miR482c* by CRISPR increased resistance to *Phytophthora infestans* (Zhu et al., 2022; Li, 2024). Pineapple likely has similar miRNAs. Editing them, or their binding sites on target genes, could release defense genes from suppression.

By using CRISPR/Cas9 to mutate the miRNA genes or their binding sites on target genes, pineapple's immune regulators could escape post-transcriptional suppression. For example, if pineapple has a miRNA that downregulates a key defense gene under stress, disrupting that miRNA (or its target site on the gene's mRNA) would result in higher expression of the defense gene and a more robust response to infection.

Regulatory network editing can therefore achieve polygenic resistance effects: altering one regulator can upregulate a battery of defense genes. However, care is needed to avoid autoimmune or growth penalty phenotypes that sometimes result from constitutive defense activation. Fine-tuning is possible – for instance, editing a promoter to slightly reduce a repressor's expression rather than a full knockout might yield a balanced outcome. Nonetheless, the precision of CRISPR allows testing of various allele modifications.

Other possible targets in pineapple include *AcJAZ* genes (repressors in the JA pathway), WRKY repressors, or MAPK phosphatases that turn off defense signals. Editing these could make the immune response last longer or react faster.

Instead of adding new resistance genes, this strategy maximizes use of pineapple's existing defense arsenal by lifting internal brakes. Successful demonstrations in other crops provide a roadmap – e.g. the miRNA editing in tomato and *EDR1* knockout in wheat (Zhang et al., 2017) – that can be followed in pineapple once comparable regulatory elements are identified.

4.4 Multiplex gene editing for pyramiding resistance traits

The wide range of pathogens makes single-gene resistance ineffective for protecting against all diseases. CRISPR/Cas9 technology enables multiplexed editing which allows scientists to modify multiple gene targets within one organism at the same time thus making it possible to stack resistance traits through pyramiding without needing extensive breeding programs. The approach shows great promise for pineapple because it enables the simultaneous disruption of multiple susceptibility genes and defense regulators which results in broad-spectrum disease resistance against major pathogens.

Multiplex editing can be done by giving the plant multiple sgRNAs with Cas9, or by using Cas12a (Cpf1), which can process many guide RNAs together (Schepler-Luu et al., 2023; Li et al., 2025). In rice, Oliva et al. (2019) edited three susceptibility gene promoters (*OsSWEET11*, *OsSWEET13*, *OsSWEET14*) at the same time. They made small changes in each promoter's effector-binding site using one construct. The new rice plants resisted all tested strains of *Xanthomonas*. This result came in one generation, saving years compared with traditional breeding.

A similar method could work for pineapple. One CRISPR/Cas9 plasmid could target *AcMLO*, *AcEDR1*, and *AcNPR3*. A plant edited in all three genes could gain fungal resistance from *mlo*, stronger basic immunity from *edr1*, and more SA signaling from *npr3*. Another set of edits could aim at an S gene for heart rot and another for black rot, giving resistance to both at once.

The slow growth rate of pineapple makes simultaneous editing of multiple genes a highly advantageous approach. The single-step process produces results more quickly than the multi-year process of modifying individual genes through successive alterations. Other fruit crops prove it can be done. The researchers used CRISPR to disable

two *MLO* genes together with one immune suppressor gene in grapevine during a single study. The research produced plants that demonstrated effective resistance against mildew (Moffa et al., 2025). The combination of tRNA-based multiplex guide systems with Cas12a technology enables scientists to edit multiple genes simultaneously which enhances the efficiency of this approach (Schepler-Luu et al., 2023; Li et al., 2025).

Additionally, new variants like Cas12f or Cas12j (ultra-small CRISPR nucleases) may allow delivery of multiple guides even in the size-constrained virus vectors if needed for pineapple (though currently transformation is via tissue culture). One can also use a sequential transformation approach: edit gene set A, then re-transform the edited plant to edit gene set B. While time-consuming, it's faster than classical breeding by orders of magnitude.

It is important to check that adding many edits does not create bad interactions or reduce plant health. If each edit alone has little effect on growth, their combination is more likely to be safe. Careful testing is still needed to choose the best lines.

Scientists can use CRISPR multiplexing technology to create pineapples that protect against all major disease-causing pathogens through successful implementation of this technology. The new method would eliminate the need for decades of conventional breeding because it enables scientists to create resistance gene "pyramids" directly in the genome through one generation of targeted editing.

5 Challenges and Future Prospects

5.1 Technical hurdles in applying CRISPR to pineapple

CRISPR/Cas9 technology faces multiple technical challenges when used for pineapple cultivation. The main problem exists in the steps of plant regeneration and transformation. Pineapple is difficult to modify genetically. The process of delivering DNA into cells followed by normal plant growth of edited plants proves to be challenging. Some progress has been made with Agrobacterium-based transformation of pineapple callus (He et al., 2023), but the efficiency is low and changes with genotype. The cultivation of MD2 and other popular cultivars demands sophisticated tissue culture methods because they prove challenging to grow.

Researchers have worked on methods to produce embryogenic callus and adjust selective agent levels to get shoots. The number of edited plantlets remains limited despite these developments. The extended time it takes for pineapples to grow creates an additional difficulty because field-based plant resistance testing needs multiple years of assessment. The clonal nature of pineapple propagation through tissue culture makes it difficult to distinguish between genetic modifications and somaclonal variations so researchers must include wild-type controls (Wang et al., 2021).

Off-target edits are another concern. The pineapple genome contains multiple repeated sequences which are combined with duplicate gene copies. A guide RNA has the potential to make errors during its cutting process which results in incorrect placement of the cut. Most plant studies show off-target rates are low (Bessoltane et al., 2022), but we need to design guides with care and confirm possible off-target sites by sequencing. The extensive heterozygous genome of pineapple creates additional challenges for this process. For a full knockout, both alleles need to be edited. Plants show mosaic patterns because their cells contain different genetic modifications between affected and unaffected cells. The process requires additional propagation operations or editing iterations according to Guo et al. (2023) and Wang et al. (2021).

Some commercial cultivars are also difficult to grow in tissue culture. The MD2 line proves to be one of the most difficult to grow but laboratory tests show other lines perform better (Cheng et al., 2025). The first step involves working with an easier cultivar before performing backcrossing or using morphogenic regulator genes and optimized media for culture improvement (He et al., 2023). The tissue culture process results in numerous genetic mutations which develop within cultured cells. The grapevine genome contains multiple thousands of single nucleotide variants and indels which developed from tissue culture procedures instead of CRISPR (Wang et al., 2021). The results show that particular regenerant features develop from culture conditions instead of gene editing so proper control systems need to be implemented.

The present circumstances demonstrate progress in the field despite these existing issues. The transformation systems based on organogenesis and somatic embryogenesis have demonstrated better success rates according to He et al. (2023). The use of Cas9 protein or RNA for DNA-free genome editing results in faster processes and prevents stable transformation according to Guo et al. (2023) and Young et al. (2019). This method has worked in other monocots and may make pineapple editing easier to accept by the public. Recent research indicates that pineapple transformants remain stable and scientists have successfully made short-term modifications to a *GFP* marker gene (Cheng et al., 2025) which indicates that the field is advancing.

5.2 Potential solutions and innovations

Several ideas may help solve pineapple's editing problems. One is to improve transformation methods. Researchers are testing physical delivery like particle bombardment to deliver CRISPR into tissues less responsive to *Agrobacterium*. Using biolistic delivery of Cas9–sgRNA complexes (RNPs) could produce edited plants without transgenes if regeneration works (Sturme et al., 2022; Nadakuduti and Enciso-Rodríguez, 2021).

Tissue culture can also be improved. Morphogenic regulator genes such as *Baby Boom* and *WUSCHEL* have boosted transformation in cereals and might work for pineapple. They can be added temporarily to promote embryogenic callus formation, then removed later.

CRISPR tools themselves can be improved. High-fidelity Cas9 types, such as SpCas9-HF1 or eSpCas9, cut off-target DNA far less often (Wu et al., 2022). PAM-relaxed Cas9 types like xCas9, SpG Cas9, or Cas12a can reach more target sites than the normal NGG PAM (Langner et al., 2018). This is useful when the target gene has no NGG nearby. Guide design tools such as CRISPOR, together with genome scans, can also lower off-target risk (Guo et al., 2023).

Transient expression or RNP delivery is another option. Cas9 RNPs could be delivered into pineapple protoplasts or through vascular injection (Modrzejewski et al., 2020). This would create edited plants without foreign DNA. Even if efficiency is low, one good edited plant can be multiplied by vegetative propagation.

New CRISPR types may also help. Cas12b and Cas13 are examples. Cas13 can cut RNA and could be used to fight pineapple RNA viruses during plant propagation.

In general, using better tissue culture, improved CRISPR enzymes, and new delivery methods together may remove the current limits. Sharing methods through networks like the International pineapple Working Group can make progress faster. Other crops once thought hard to edit, such as soybean and sorghum, are now edited regularly. Pineapple will likely follow the same path.

5.3 Regulatory and ethical considerations for genome-edited pineapple

CRISPR-edited pineapples encounter increasing obstacles in commercial markets because of evolving legal frameworks and shifting public opinions. Regulations differ by country. CRISPR plants that contain no foreign DNA elements are not considered GMOs in certain jurisdictions. The United States treats these plants in the same manner as conventional breeding varieties (Sprink et al., 2022). The first commercial CRISPR crop available to consumers was a white mushroom variety. The researchers did not classify this product as a GMO because it contained only a minimal deletion of DNA without any foreign DNA insertion (Smith-Willis and San Martin, 2015; Ahmad et al., 2023).

The European Union uses the classification system of Tomlinson (2018) to categorize most gene-edited crops as GMOs. The approval process for an edited pineapple would require detailed evaluation according to Buchholzer and Frommer (2023). Exporters of pineapple products need to understand that various markets have their own set of regulations. The GMO classification system of Japan and Australia and Brazil does not include DNA-free small edits according to Sprink et al. (2022). The world has seen an increasing number of nations implement precise gene editing technology.

Public views matter a great deal as well. Many people are wary of GMOs, yet polls report higher support for gene-edited foods in many regions. Support rises when the change is small, close to a natural mutation, or gives clear gains, such as lowering pesticide use in many cases (Son and Lim, 2021; Ortega et al., 2022). Work across several countries finds acceptance depends on how much people know and how strongly they trust the information source in practice (Cummings and Peters, 2022). For pineapple, saying that CRISPR helps strengthen natural disease resistance could raise approval.

Ethically, editing crops is less disputed than editing animals or humans, but fairness still matters. Pineapple is mainly grown in developing countries by smallholder farmers. Patents and high costs could limit access if big firms alone control the technology. On the plus side, many CRISPR tools are open source, and groups like CGIAR are working to make them available for public use.

CRISPR pineapples with disease resistance could also reduce the use of chemical fungicides and bactericides, which would help the environment and human health. In short, while regulations vary, the lack of foreign DNA in many CRISPR edits gives a realistic path to approval. Clear communication and fair access could help ensure these benefits reach farmers and consumers alike.

6 Conclusion

This study has described how CRISPR/Cas9 genome editing can help improve pineapple's disease resistance. Pineapple is an important crop with high economic value, but it suffers from serious diseases. These traits make it a good target for precise breeding. The release of a high-quality pineapple genome and the discovery of key immune-related genes now allow targeted genetic improvements.

Work in other crops shows that resistance can be improved by removing susceptibility genes, increasing defense gene activity, changing key regulators, or adding several resistance genes at once. CRISPR/Cas9 can do these changes much faster than traditional breeding. For pineapple, this could produce plants that resist heart rot in the field or avoid black rot after harvest. But CRISPR is only a method. Success will depend on finding the right genes to edit and improving tissue culture and regeneration steps.

Future work should aim to find pineapple genes that drive resistance or susceptibility to key diseases. Researchers can do this with comparative genomics, transcriptome analyses, and functional assay tests. Wild kin may hold useful resistance genes. These genes could be introduced into commercial varieties via CRISPR allele swaps.

On the technical side, work should aim to make transformation and regeneration faster and more reliable. It should also try DNA-free editing, base editing, and prime editing for precise changes. Once promising lines are made, they should be tested in multi-season field trials in areas where disease risk is high.

Using several resistance genes together, or mixing plants with different resistance traits, may help keep protection strong for longer. Social and economic studies should also check how farmers and consumers feel about gene-edited pineapples.

In short, CRISPR/Cas9 is a fast and accurate tool to improve pineapple disease resistance. There are still challenges, but fast progress in plant genome editing suggests these can be solved. In the coming years, the first CRISPR-edited pineapples may be tested in fields, starting a new stage in protecting the crop from disease.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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