Collection and Functional Verification of Pine Disease Resistance Gene Resources

Minghui Zhao, Zonghui Liu, Xiazhen Huang ✉

Animal Science Research Center, Hainan Institute of Tropical Agricultural Resources, Sanya, 572024, Hainan, China

✉ Corresponding author: xiazhen.huang@hitar.org

Abstract This study explores the latest advancements in research on the disease resistance mechanisms of pine trees, emphasizing the significance of genes in pine disease resistance. Pines are susceptible to a variety of diseases caused by fungi and nematodes, such as needle blight and pine wilt disease. This paper presents the pathogens involved in different pine diseases and their infection mechanisms, analyzing the impacts of these diseases on pine forests and ecosystems. By identifying and utilizing resistance genes, the disease resistance of pine trees can be enhanced. The article also discusses the application of molecular biology and genomics techniques in the identification of resistance genes, such as SNP mapping and transcriptomic analysis, and explores methods for gene cloning, expression, and functional verification. Finally, the paper highlights the impact of environmental and ecological factors on transgenic pines, noting the importance of integrated approaches in forest management and breeding programs.

Keywords Pine diseases; Genomics; Transcriptomic analysis; Molecular mechanisms

1 Introduction

Pine species are susceptible to a variety of diseases, many of which are caused by fungal pathogens. Some of the most common diseases affecting pines include Dothistroma needle blight, Brown spot needle blight, Lophodermium needle cast, Scots pine blister rust, Scleroderris canker, and pitch canker. These diseases can lead to defoliation, increased susceptibility to other diseases and pests, and, in severe cases, tree mortality. Another significant disease is pine wilt disease (PWD), caused by the pine wood nematode (*Bursaphelenchus xylophilus*), which is a major concern for pine forests globally (Raitelaitytė et al., 2017).

The identification and utilization of disease resistance genes are crucial for the sustainable management of pine forests. These genes provide a natural defense mechanism against pathogens by triggering immune responses that inhibit pathogen development and spread. Genetic resistance can be categorized into major gene resistance (MGR), which involves single genes conferring high resistance, and quantitative resistance, which involves multiple genes providing moderate resistance. Advances in genomic technologies, such as SNP mapping and transcriptomic analyses, have facilitated the identification of resistance genes in pine species. For instance, the Cr1 gene in sugar pine and the NLR gene family in various pine species have been associated with resistance to WPBR (Weiss et al., 2020; Liu et al., 2021).

This review aims to summarize the current knowledge on pine disease resistance mechanisms and the genetic basis of resistance, highlight recent advances in identifying and characterizing disease resistance genes in pine species, and discuss the implications of these findings for forest management and breeding programs aimed at enhancing disease resistance in pine populations.

2 Overview of Pine Diseases

2.1 Common pine diseases

Pine species are susceptible to various diseases, primarily caused by fungal pathogens and nematodes. Among the most prevalent diseases affecting pines are Dothistroma needle blight, brown spot needle blight, Lophodermium needle cast, Scots pine blister rust, Scleroderris canker, and pitch canker. These diseases often lead to defoliation,

reduced growth, increased vulnerability to other pathogens and pests, and, in severe cases, tree mortality. For instance, Dothistroma needle blight, caused by the fungus *Dothistroma septosporum*, affects the needles, creating lesions that lead to significant defoliation. Similarly, brown spot needle blight, caused by *Lecanosticta acicola*, results in browning and premature needle drop.

Another critical disease is pine wilt disease (PWD), caused by the pine wood nematode (*Bursaphelenchus xylophilus*), which is transmitted by insect vectors such as *Monochamus* species. PWD leads to rapid wilting and death of infected trees, severely impacting forest health and timber resources. Diplodia tip blight, caused by *Sphaeropsis sapinea*, predominantly affects pines under stress, particularly from drought, causing shoot dieback and reduced tree vigor.

These diseases are significant concerns for forest managers and researchers due to their widespread impact on pine forests globally. Effective management and control strategies are crucial to mitigate the damage caused by these pathogens and ensure the health and sustainability of pine ecosystems (Raitelaitytė et al., 2017; Blumenstein et al., 2020).

2.2 Pathogens and infection mechanisms

Fungal pathogens are the primary cause of many pine diseases, employing various mechanisms to infect and damage host trees. Dothistroma needle blight, for example, is caused by the fungus *Dothistroma septosporum*, which produces spores that infect needles, leading to characteristic red bands and premature needle drop. Brown spot needle blight, caused by *Lecanosticta acicola*, follows a similar infection pattern, with spores infecting needles and causing brown lesions that eventually lead to defoliation.

White pine blister rust, caused by *Cronartium ribicola*, has a complex life cycle involving an alternate host, typically a species of Ribes. The rust infects pine needles, spreads to branches, and causes cankers that girdle and kill branches and sometimes the entire tree. The pine wood nematode (*Bursaphelenchus xylophilus*), responsible for pine wilt disease, is transmitted by *Monochamus* beetles. The nematode invades the tree's resin canals, causing water transport disruption, leading to rapid wilting and death. This nematode also has a mycophagous phase, feeding on fungi within the tree, which aids its development and spread (Vicente et al., 2021).

The opportunistic pathogen *Sphaeropsis sapinea* causes Diplodia tip blight, particularly under conditions of drought or injury. This fungus remains latent in healthy tissue until the tree is stressed, at which point it causes significant shoot dieback and reduced growth. These varied infection mechanisms highlight the complexity of managing pine diseases and the necessity for integrated pest management strategies to effectively control these pathogens and mitigate their impact on pine forests (Blumenstein et al., 2020).

2.3 Impact on pine forests and ecosystems

The impact of pine diseases on forests and ecosystems is profound, leading to significant ecological and economic consequences. Pine wilt disease, caused by the pine wood nematode (*Bursaphelenchus xylophilus*), is one of the most destructive diseases, leading to rapid tree death and extensive forest decline. This disease disrupts water transport within the tree, causing rapid wilting and death, which can devastate entire pine stands and significantly reduce timber yields. The economic impact is substantial, with losses in timber value, increased management costs, and reduced ecosystem services.

White pine blister rust, caused by *Cronartium ribicola*, has similarly devastating effects on North American white pine populations. This disease causes cankers on branches and trunks, leading to tree mortality and a decline in forest health. The loss of white pine, a keystone species, disrupts forest ecosystems, affecting biodiversity, wildlife habitat, and forest structure. The economic impact includes loss of valuable timber and increased costs for disease management and forest restoration efforts.

Climate change exacerbates these impacts by creating conditions that favor the spread and severity of these diseases. Warmer temperatures and increased drought stress weaken trees, making them more susceptible to

pathogens like *Sphaeropsis sapinea*, which causes Diplodia tip blight. This disease leads to significant shoot dieback, reducing tree vigor and growth. Managing these impacts requires a comprehensive approach, integrating disease monitoring, genetic resistance breeding, and sustainable forest management practices to maintain forest health and resilience (Pandit et al., 2020).

3 Identification and Collection of Disease Resistance Genes

3.1 Sources of resistance genes

Resistance genes in pine species are primarily sourced from natural populations that exhibit varying levels of disease resistance. These genes often originate from individuals or populations that have survived severe disease outbreaks, suggesting an inherent genetic resistance. For instance, in sugar pine (*Pinus lambertiana*), the Cr1 gene has been identified as providing significant resistance to white pine blister rust (WPBR) caused by *Cronartium ribicola* (Wright et al., 2022). Similarly, in Masson pine (*Pinus massoniana*), genes associated with resistance to pine wilt disease (PWD) caused by the pine wood nematode (*Bursaphelenchus xylophilus*) have been identified through transcriptomic analyses, which highlight differentially expressed genes linked to resistance mechanisms (Liu et al., 2017).

Wild populations of limber pine (*Pinus flexilis*) and southwestern white pine (*Pinus strobiformis*) have also been valuable sources of resistance genes. Studies have shown that these species harbor major resistance genes (Cr3 and Cr4) that confer resistance to WPBR. These genes have been found to be conserved across species, indicating a shared evolutionary response to the pathogen (Liu et al., 2021). Additionally, loblolly pine (*Pinus taeda*) has been a critical source of resistance genes for fusiform rust, with nine identified pathotype-specific resistance genes (Fr genes) mapped to its genome (Amerson et al., 2015).

3.2 Techniques for gene identification

The identification of disease resistance genes in pines involves a combination of classical genetics, molecular biology, and advanced genomic techniques. One common approach is Quantitative Trait Loci (QTL) mapping, which helps identify regions of the genome associated with disease resistance traits. This technique was effectively used to identify SNPs linked to WPBR resistance in sugar pine and southwestern white pine (Weiss et al., 2020). Another powerful method is Genome-Wide Association Studies (GWAS), which analyze the entire genome to find genetic variations linked to resistance traits. This method has been instrumental in identifying NLR genes associated with resistance in limber pine (Liu et al., 2019).

Transcriptomic analyses are also widely used to identify genes differentially expressed in response to pathogen infection. For instance, RNA sequencing (RNA-seq) has been employed to profile gene expression in resistant and susceptible pine species, revealing key regulatory genes and pathways involved in disease resistance. In Masson pine, transcriptomic profiling identified genes involved in oleoresin biosynthesis and reactive oxygen species (ROS) scavenging as crucial for resistance to PWD (Liu et al., 2017).

In addition, targeted sequencing of specific gene families, such as the NLR gene family, has been utilized to pinpoint resistance genes. This approach has led to the identification of multiple NLR genes in limber pine that co-segregate with resistance traits (Liu et al., 2021). Furthermore, bioinformatic mining of transcriptomic data has identified pathogenesis-related (PR) genes in western white pine that play roles in quantitative disease resistance (Liu et al., 2021).

3.3 Gene collection and storage

The collection and storage of resistance genes involve several steps to ensure the preservation and availability of genetic resources for breeding programs. Initially, resistance genes are identified and validated through field trials and molecular assays. Once confirmed, seeds or tissues from resistant individuals are collected. For instance, seeds from sugar pine trees carrying the *Cr1* gene are collected and used in breeding programs to propagate resistant trees (Wright et al., 2022).

Tissue culture techniques, such as somatic embryogenesis, are employed to clone resistant genotypes, ensuring the propagation of trees with desired resistance traits. This method is particularly useful for maintaining genetic fidelity and producing large quantities of planting material. Additionally, DNA and RNA samples from resistant trees are stored in biorepositories for future research and breeding efforts. These samples are often accompanied by detailed phenotypic data and genetic information to facilitate their use in marker-assisted selection (MAS) and other genetic improvement strategies (Liu et al., 2017).

Moreover, genetic resources are often shared through collaborative networks and databases that allow researchers and breeders access to valuable genetic material. This collaborative approach ensures the wide distribution and utilization of resistance genes, contributing to the global effort to enhance disease resistance in pine forests.

4 Molecular Mechanisms of Disease Resistance

4.1 Gene expression and regulation

The molecular mechanisms underlying disease resistance in pine trees involve complex interactions between various genes and regulatory pathways. Gene expression studies have revealed that resistance to pathogens like pine wood nematode (PWN) and white pine blister rust (WPBR) involves the differential expression of numerous genes. In Masson pine (*Pinus massoniana*), transcriptomic profiling identified key differentially expressed genes (DEGs) related to oxidative stress response, terpenoid biosynthesis, and syncytium formation, which are crucial for resistance against PWN (Liu et al., 2017). Additionally, proteomic analysis of resistant Masson pine clones highlighted significant upregulation of proteins involved in salicylic acid metabolism, antioxidant stress reaction, and polysaccharide degradation, which contribute to enhanced resistance (Gao et al., 2022).

Another study on *Pinus thunbergii* demonstrated that resistant plants exhibited higher expression levels of genes associated with lignin synthesis and the oxidative stress pathway. Specifically, cinnamoyl-CoA reductase (CCR)-coding genes were upregulated in resistant phenotypes, indicating a role in reinforcing cell walls against pathogen invasion (Wang et al., 2023). These findings suggest that gene expression regulation in response to pathogen infection is critical for activating defense mechanisms in pine trees.

4.2 Pathogen recognition and defense response

Pathogen recognition and the subsequent activation of defense responses are key aspects of disease resistance in pine trees. Pine species have evolved various receptor proteins that detect pathogen-associated molecular patterns (PAMPs) and initiate immune responses. For instance, nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins play a pivotal role in recognizing specific pathogen effectors and triggering defense mechanisms. Studies have shown that these proteins are involved in both quantitative and qualitative resistance to WPBR in sugar pine (*Pinus lambertiana*) and southwestern white pine (*Pinus strobiformis*) (Weiss et al., 2020; Liu et al., 2021).

Upon pathogen recognition, pine trees activate a cascade of defense responses, including the production of reactive oxygen species (ROS), synthesis of antimicrobial compounds, and reinforcement of cell walls. The role of ROS in pathogen defense is well-documented, with resistant pine varieties exhibiting enhanced ROS scavenging capabilities to mitigate oxidative damage and inhibit pathogen spread (Liu et al., 2017). Additionally, the biosynthesis of terpenoids and phenylpropanoids is upregulated in resistant pines, contributing to the production of antimicrobial compounds that deter pathogen growth (Modesto et al., 2022).

4.3 Signal transduction pathways

Signal transduction pathways mediate the activation and regulation of defense responses in pine trees. The jasmonic acid (JA) and salicylic acid (SA) signaling pathways are particularly important in coordinating the plant's immune response. In resistant *Pinus pinaster*, the JA pathway is prominently induced, leading to the activation of secondary metabolism and lignin synthesis, which fortifies cell walls and enhances resistance to PWN (Modesto et al., 2021).

Comparative transcriptome analysis of pine trees treated with resistance-inducing substances such as acibenzolar-S-methyl (ASM) and methyl salicylic acid (MeSA) revealed that these elicitors enhance the

expression of genes involved in photorespiration, peroxisome function, and glycine metabolism. These pathways are linked to the oxidative stress response, highlighting their role in protecting pine trees from pathogen-induced damage (Park et al., 2020).

In addition to JA and SA pathways, ethylene signaling is also involved in modulating defense responses. Crosstalk between these signaling pathways ensures a coordinated and effective defense strategy against diverse pathogens. For example, ethylene signaling has been implicated in the regulation of sulfur metabolism and flavonoid biosynthesis, which contribute to the plant's defense arsenal (Visser et al., 2022).

5 Functional Verification of Resistance Genes

5.1 Gene cloning and expression

Cloning and expressing resistance genes in pine trees involve isolating the target genes and inserting them into suitable vectors for propagation and study. For example, in western white pine (*Pinus monticola*), researchers identified multiple novel members of the PR10 gene family through bioinformatic mining and subsequently selected PmPR10-3.1 for further study. This gene was cloned and expressed in *Escherichia coli*, and the purified recombinant protein exhibited inhibitory effects on spore hyphal growth of various fungal pathogens, demonstrating its potential role in disease resistance (Liu et al., 2021).

Another example is the cloning of nucleotide-binding site leucine-rich repeat (NBS-LRR) genes in limber pine (*Pinus flexilis*), which are associated with resistance to white pine blister rust (WPBR). These genes were cloned and sequenced to understand their structure and function, and their expression was analyzed in resistant and susceptible pine varieties (Weiss et al., 2020) (Figure 1). Cloning and expressing these genes in model systems or heterologous hosts helps validate their function and provides insights into their role in disease resistance mechanisms.
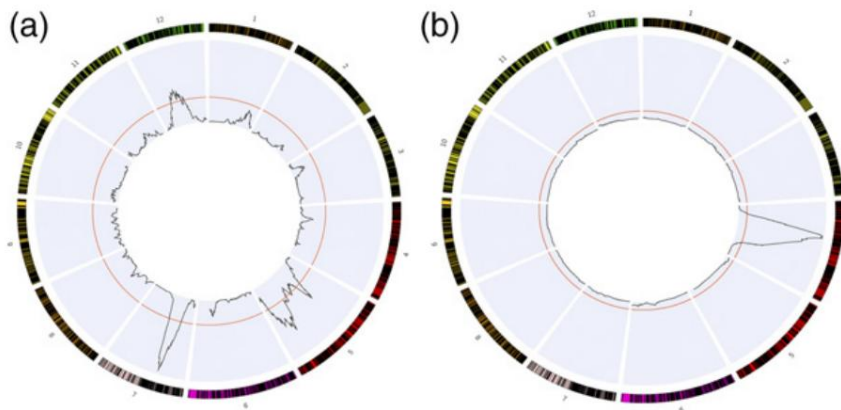

Figure 1 Consensus linkage map for sugar pine showing 12 linkage groups and results of the QTL analysis (Adopted from Weiss et al., 2020)

Image caption: Several significantly associated gene families were identified through Genome-Wide Association Studies (GWAS) and Quantitative Trait Loci (QTL) analysis, including the LRR gene family related to MGR. These genes are involved in various disease resistance mechanisms such as pathogen detection, necrosis of infected cells, ubiquitin-dependent protein catalysis, response to oxidative stress, and immune effector processes. Additionally, some genes are primarily involved in abiotic stress, highlighting the diversity of quantitative disease resistance responses in sugar pine (Adapted from Weiss et al., 2020)

5.2 Functional assays and testing

Functional assays are critical for verifying the role of cloned resistance genes. These assays involve testing the gene's ability to confer resistance when expressed in host plants or model systems. In the case of PmPR10-3.1 from western white pine, in-vitro antifungal assays were conducted where the recombinant protein was tested against various fungal pathogens. The protein exhibited significant antifungal activity, supporting its role in resistance (Liu et al., 2021).

Another approach is the use of genetic association studies combined with functional assays. For instance, SNP markers associated with resistance traits were identified and tested in sugar pine (*Pinus lambertiana*), linking specific genetic variations to resistance phenotypes. These markers were then used in breeding programs to select for resistant individuals, validating their practical application in improving disease resistance (Wright et al., 2022).

5.3 Validation in model systems

Validation of resistance genes in model systems involves introducing the genes into model organisms or alternative hosts to study their function in a controlled environment. This approach helps confirm the gene's role in conferring resistance and elucidates the underlying molecular mechanisms. For example, the NBS-LRR genes from limber pine were introduced into model plants to study their expression and resistance capabilities against WPBR. These experiments demonstrated that the genes conferred resistance to the pathogen, validating their function (Weiss et al., 2020).

In another study, the PR10 gene family from western white pine was analyzed in transgenic Arabidopsis plants. The transgenic plants expressing the *PmPR10-3.1* gene showed enhanced resistance to fungal pathogens, confirming the gene's role in plant defense (Liu et al., 2021). These model system studies are essential for understanding the broader applicability of resistance genes and for developing effective strategies for breeding disease-resistant pine varieties.

6 Genetic Engineering and Breeding Applications

6.1 Development of transgenic pines

The development of transgenic pines involves the introduction of foreign genes into pine genomes to confer resistance to various diseases. This method offers a rapid way to enhance disease resistance compared to traditional breeding. One notable example is the introduction of the *PmPR10-3.1* gene from western white pine (*Pinus monticola*), which has been cloned and expressed in model organisms and transgenic pines to test its efficacy against white pine blister rust (Liu et al., 2021). Transgenic approaches have also been used to insert genes encoding for proteins involved in the synthesis of secondary metabolites, such as terpenoids and phenolics, which play a role in pathogen defense. Recent advances in CRISPR/Cas9 genome editing technology have further accelerated the development of disease-resistant pines by allowing precise modifications of specific genes involved in pathogen resistance (Yin and Qiu, 2019).

Another significant application of genetic engineering is the development of pines resistant to pine wilt disease (PWD). Researchers have successfully introduced genes that enhance resistance to the pine wood nematode (*Bursaphelenchus xylophilus*) in species like *Pinus thunbergii* and *Pinus massoniana*. These transgenic pines exhibit increased expression of genes involved in oxidative stress response and cell wall fortification, which are critical for combating nematode infection (Gao et al., 2022).

6.2 Marker-assisted selection

Marker-assisted selection (MAS) is a powerful tool in the breeding of disease-resistant pines. MAS uses molecular markers linked to disease resistance genes to select and propagate resistant individuals more efficiently. For example, the Cr2 locus in western white pine, which confers resistance to white pine blister rust, has been successfully utilized in MAS programs. Genetic markers associated with Cr2 have been identified and used to screen for resistance in breeding populations, significantly reducing the time required to develop resistant trees (Liu et al., 2020).

In maritime pine (*Pinus pinaster*), MAS has been employed to select for resistance to pine wood nematode. Studies have identified specific genetic markers linked to resistance traits, allowing breeders to screen and select individuals with enhanced resistance more accurately. This method has proven effective in improving the resistance of maritime pine to PWD and is being integrated into breeding programs across Europe (Carrasquinho et al., 2018).

6.3 Breeding strategies for disease resistance

Breeding strategies for disease resistance in pine trees involve both traditional and advanced methods to enhance the genetic resistance of pine populations. Traditional breeding relies on selecting and cross-breeding individuals with desirable traits, but this process is time-consuming. Advanced breeding strategies, such as genomic selection and genomic-wide association studies (GWAS), have revolutionized pine breeding by providing insights into the genetic basis of disease resistance and allowing for more targeted breeding efforts (Sniezko and Koch, 2017).

Genomic selection involves using genome-wide markers to predict the breeding value of individuals for disease resistance traits. This approach has been successfully applied in Norway spruce to select for resistance against the pine weevil (*Hylobius abietis*), demonstrating the potential for similar strategies in pine species (Lenz et al., 2019). GWAS has been used to identify SNPs associated with resistance to WPBR in sugar pine, providing valuable information for breeding programs (Weiss et al., 2020).

In addition to these techniques, breeding programs are incorporating knowledge from proteomic and transcriptomic studies to understand the molecular mechanisms underlying disease resistance. This comprehensive approach allows for the development of pines with enhanced resistance to multiple pathogens, ensuring the sustainability and health of pine forests in the face of increasing biotic stressors (Mukrimin et al., 2019).

7 Case Studies of Disease Resistance in Pines

7.1 Successful gene integration and expression

A significant case study involves the integration and expression of the *Cr1* resistance gene in sugar pine (*Pinus lambertiana*), which confers resistance to white pine blister rust (WPBR) caused by *Cronartium ribicola*. This gene was successfully mapped and linked to specific SNP markers, which facilitated the development of a PCR-based genotyping assay to identify and propagate resistant individuals. Field trials have shown that trees carrying the *Cr1* gene exhibit strong resistance to WPBR, demonstrating the successful integration and functional expression of this gene in natural populations (Wright et al., 2022).

In another study, the PR10 gene family from western white pine (*Pinus monticola*) was investigated for its role in resistance to WPBR. The gene *PmPR10-3.1* was cloned and expressed in *Escherichia coli*, and the recombinant protein exhibited antifungal activity, indicating its functional role in disease resistance. Subsequent expression in transgenic pines confirmed its contribution to enhanced resistance, showcasing a successful case of gene integration and expression (Figure 2) (Liu et al., 2021).

7.2 Field trials and disease resistance

Field trials play a critical role in validating the efficacy of resistance genes under natural conditions. For example, southwestern white pine (*Pinus strobiformis*) has been evaluated for resistance to WPBR through extensive field trials. Progeny from families with known resistance genes were inoculated with *Cronartium ribicola* and monitored for disease symptoms. The trials revealed significant variation in resistance levels, with some families exhibiting high survival rates and reduced disease severity, demonstrating the practical benefits of selecting for resistance genes in breeding programs (Johnson and Sniezko, 2021).

Another notable example is the resistance of maritime pine (*Pinus pinaster*) to pine wilt disease (PWD) caused by *Bursaphelenchus xylophilus*. Field trials conducted on half-sib families identified several resistant lines. These trials included inoculation with the nematode and monitoring for survival and growth. The resistant families showed significantly higher survival rates and lower nematode populations, confirming the effectiveness of selecting for genetic resistance in field conditions (Carrasquinho et al., 2018).

7.3 Lessons learned and challenges

Several key lessons have been learned from these case studies. The importance of integrating molecular and field data to select and propagate disease-resistant pines is evident. Molecular markers linked to resistance genes, when validated through field trials, can significantly enhance the efficiency and accuracy of breeding programs.

However, challenges remain. One major challenge is the genetic complexity of resistance traits, which often involve multiple genes and gene-environment interactions. This complexity can complicate the selection process and requires comprehensive genomic studies to fully understand the resistance mechanisms. The long generation times of trees pose a challenge for breeding programs, necessitating the use of advanced techniques like marker-assisted selection (MAS) and genomic selection to accelerate progress.

Moreover, the variability in pathogen strains and environmental conditions can affect the consistency of resistance expression. For instance, some resistance genes may be effective against certain pathogen strains but not others, highlighting the need for continuous monitoring and evaluation of resistance traits across different regions and conditions.
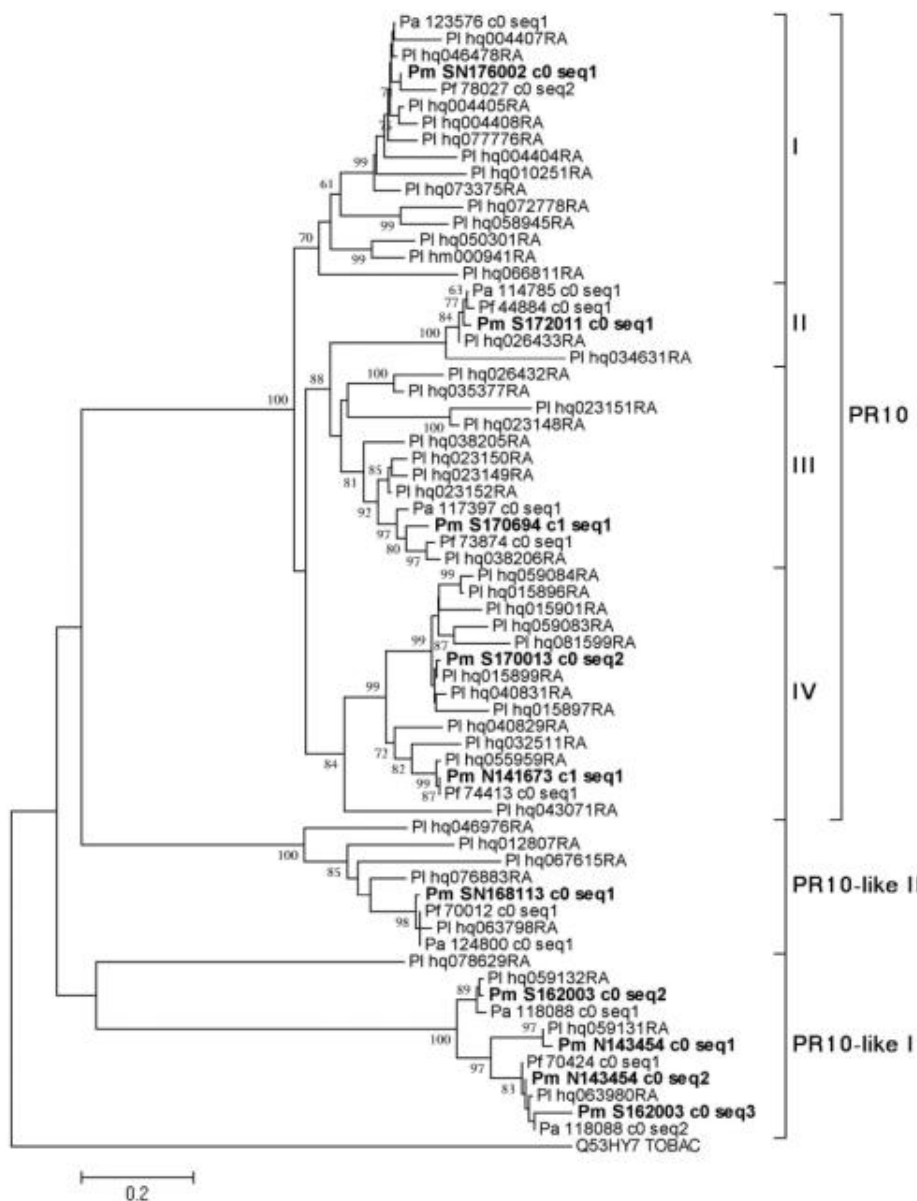

Figure 2 Phylogenetic analysis of putative PR10 and PR10-like proteins from four five-needle pines (Adopted from Liu et al., 2021)
Image caption: Through genome-wide association studies (GWAS) and quantitative trait loci (QTL) analysis, these *PR10* genes were found to be differentially regulated under biotic and abiotic stress conditions. The figure further emphasizes the multifunctionality of PR10 proteins in plant growth, development, and defense responses. Particularly, their significant role in combating fungal and other pathogen infections is highlighted. The study indicates that these gene family members play a crucial role in the quantitative disease resistance of five-needle pines to white pine blister rust (WPBR) (Adopted from Liu et al., 2021)

7.3 Lessons learned and challenges

Several key lessons have been learned from these case studies. The importance of integrating molecular and field data to select and propagate disease-resistant pines is evident. Molecular markers linked to resistance genes, when validated through field trials, can significantly enhance the efficiency and accuracy of breeding programs.

However, challenges remain. One major challenge is the genetic complexity of resistance traits, which often involve multiple genes and gene-environment interactions. This complexity can complicate the selection process and requires comprehensive genomic studies to fully understand the resistance mechanisms. The long generation times of trees pose a challenge for breeding programs, necessitating the use of advanced techniques like marker-assisted selection (MAS) and genomic selection to accelerate progress.

Moreover, the variability in pathogen strains and environmental conditions can affect the consistency of resistance expression. For instance, some resistance genes may be effective against certain pathogen strains but not others, highlighting the need for continuous monitoring and evaluation of resistance traits across different regions and conditions.

8 Environmental and Ecological Considerations

8.1 Impact of transgenic pines on ecosystems

The introduction of transgenic pines into ecosystems has the potential to impact both target and non-target organisms, influencing biodiversity and ecological interactions. For example, transgenic pines engineered for resistance to specific pathogens or pests may affect herbivorous insects and their predators. A study on transgenic poplar expressing pine genes demonstrated that these trees exhibited altered interactions with herbivores, with changes in insect density and diversity observed in field trials (Robischon, 2016). Moreover, the pleiotropic effects of transgenes, such as unintended changes in tree physiology or stress responses, can further influence ecological dynamics.

Transgenic trees may also impact soil microbial communities. A study on Wollemi pine translocation highlighted that translocated trees recruited species-specific fungal communities, which played a critical role in their establishment and growth in new environments (Rigg et al., 2017). This finding suggests that transgenic pines, through altered root exudates or other factors, could similarly influence soil microbiota, potentially altering nutrient cycling and soil health.

Transgenic pines can also affect soil microbial communities. Research in South Africa and Argentina has demonstrated that alien pines can significantly alter arbuscular mycorrhizal (AM) fungal communities, leading to reduced fungal richness and altered community composition. These changes can influence soil health and nutrient cycling, with potential long-term impacts on forest productivity and sustainability (Gazol et al., 2016) (Figure 3).

8.2 Biosafety and regulatory issues

The deployment of transgenic pines is subject to stringent biosafety and regulatory frameworks to ensure environmental and ecological safety. Regulatory considerations include assessing the potential for gene flow to wild relatives, the persistence of transgenes in the environment, and the impacts on non-target species. For instance, the case study on transgenic poplar in China, which involved monitoring gene flow from transgenic Bt poplar, found low probabilities of transgene drift and negligible ecological impacts, supporting the controlled use of such technologies (Zhang and Hu, 2021).

Biosafety assessments often involve field trials to evaluate the environmental risks associated with transgenic trees. These trials are designed to detect unintended effects, such as changes in growth, reproduction, or interactions with other organisms. Regulatory bodies also require comprehensive risk assessments, including studies on gene stability, expression levels, and potential ecological impacts.

The development and use of transgenic trees must comply with international biosafety protocols, such as the Cartagena Protocol on Biosafety, which governs the transboundary movement of genetically modified organisms.

Adhering to these regulatory frameworks helps mitigate potential risks and ensures that the deployment of transgenic pines is conducted responsibly.
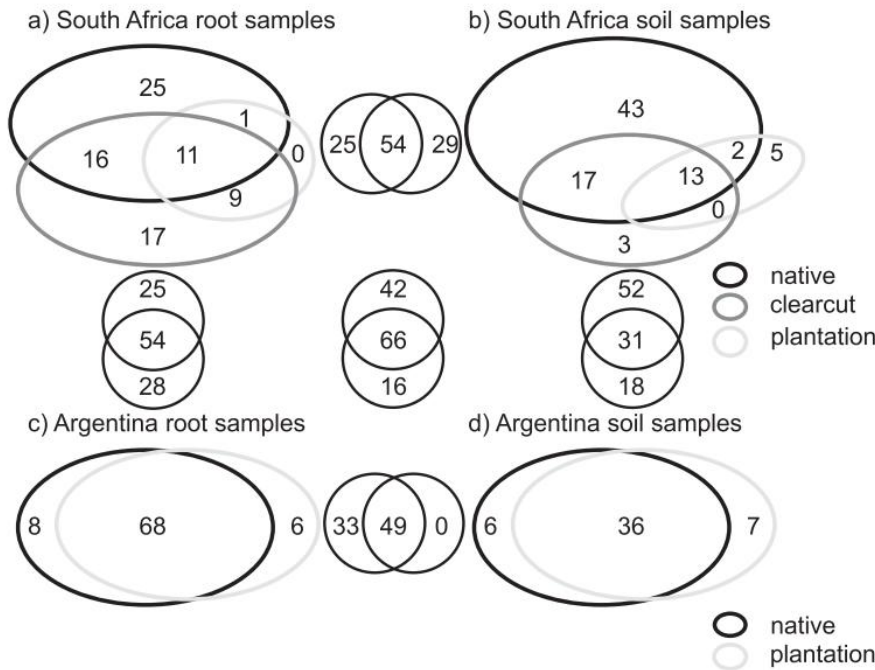

Figure 3 Illustrates the impact of alien pine trees on local arbuscular mycorrhizal (AM) fungal communities (Adapted from Gazol et al., 2016)

Image caption: The study was conducted in two regions with different biogeographic histories: South Africa and Argentina. In South Africa, where no native ectomycorrhizal (EcM) plant species are present, the introduction of alien pines resulted in a decrease in AM fungal richness and significantly altered the community composition. In contrast, in Argentina, where EcM trees occur naturally, the planting of alien pines had a smaller effect on the richness and composition of AM fungal communities. Overall, the presence of alien pines led to subtle changes in the phylogenetic structure of AM fungal communities in both regions (Adapted from Gazol et al., 2016)

8.3 Long-term sustainability

The long-term sustainability of using transgenic pines for disease resistance hinges on several factors, including the durability of resistance, the maintenance of genetic diversity, and the adaptability of trees to changing environmental conditions. One concern is the potential for pathogens to evolve and overcome resistance conferred by transgenes. To address this, strategies such as pyramiding multiple resistance genes and using gene editing technologies to enhance resistance durability are being explored (Voronova et al., 2020).

Maintaining genetic diversity within pine populations is critical for resilience against future environmental changes. Genetic conservation efforts, such as ex situ conservation of seeds and in situ protection of diverse genetic resources, play a vital role in preserving the adaptive potential of pine species (Sniezko et al., 2017). These conservation strategies ensure that valuable genetic traits are not lost and can be utilized in future breeding programs.

Moreover, the integration of transgenic pines into forest management practices must consider long-term ecological impacts. Sustainable forestry practices, combined with continuous monitoring and adaptive management, are essential to balance the benefits of transgenic technologies with the preservation of ecosystem health and biodiversity.

9 Concluding Remarks

The research on pine disease resistance genes has advanced significantly, particularly in understanding the genetic basis and molecular mechanisms of resistance. Key findings include the identification of crucial resistance genes

such as Cr1 in sugar pine (*Pinus lambertiana*), which confers resistance to white pine blister rust (WPBR). Transcriptomic studies have revealed differentially expressed genes associated with resistance to pine wilt disease (PWD) in Masson pine (*Pinus massoniana*), highlighting the role of oxidative stress response and terpenoid biosynthesis in disease resistance. Additionally, advancements in marker-assisted selection (MAS) and genomic selection have significantly enhanced breeding programs by enabling the efficient propagation of disease-resistant pine varieties.

Continued research is crucial for several reasons. The evolving nature of pathogens necessitates ongoing studies to identify and characterize new resistance genes and understand their mechanisms. This helps in developing durable resistance strategies that can withstand pathogen evolution. Secondly, understanding the ecological and environmental impacts of deploying transgenic pines is vital to ensure sustainable forest management. Research in this area can inform best practices and regulatory frameworks to mitigate potential risks. The integration of advanced technologies such as CRISPR/Cas9 for precise gene editing holds promise for developing highly resistant pine varieties, warranting further exploration and development.

Future studies should expand genomic and transcriptomic research to include more pine species and populations, enhancing understanding of resistance mechanisms across different environments. Conducting extensive field trials and long-term monitoring of transgenic and MAS-selected pines will assess performance and ecological impacts, refining breeding programs for sustainability. Utilizing advanced technologies like CRISPR/Cas9 for targeted gene editing and exploring synthetic biology can develop enhanced resistance traits. Investigating the broader ecological effects of transgenic pines will ensure balanced forest health. Finally, fostering international collaboration among researchers and policymakers will accelerate the global development and deployment of disease-resistant pines.

Acknowledgments

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.

References

Amerson H., Nelson C., Kubisiak T., Kuhlman E., and Garcia S.A., 2015, Identification of nine pathotype-specific genes conferring resistance to fusiform rust in loblolly pine *(Pinus taeda* L.), Forests, 6(8): 2739-2761.
https://doi.org/10.3390/F6082739

Blumenstein K., Langer G., Busskamp J., Langer E., and Terhonen E., 2020, The opportunistic pathogen sphaeropsis sapinea is found to be one of the most abundant fungi in symptomless and diseased scots pine in central-Europe, Journal of Fungi, 7.
https://doi.org/10.21203/rs.3.rs-48366/v1

Carrasquinho I., Lisboa A., Inácio M., and Gonçalves E., 2018, Genetic variation in susceptibility to pine wilt disease of maritime pine (Pinus pinaster Aiton) half-sib families, Annals of Forest Science, 75: 1-11.
https://doi.org/10.1007/s13595-018-0759-x

Gao J.B., Pan T., Chen X.L., Wei Q., and Xu L.Y., 2022, Proteomic analysis of Masson pine with high resistance to pine wood nematodes, PLoS ONE, 17(8): e0273010.
https://doi.org/10.1371/journal.pone.0273010

Gazol A., Zobel M., Cantero J., Davison J., Esler K., Jairus T., Öpik M., Vasar M., and Moora M., 2016, Impact of alien pines on local arbuscular mycorrhizal fungal communities-evidence from two continents, FEMS microbiology ecology, 92(6): fiw073.
https://doi.org/10.1093/femsec/fiw073

Johnson J.S., and Sniezko R., 2021, Quantitative disease resistance to white pine blister rust at southwestern white pine's (*Pinus strobiformis)* northern range, Frontiers in Forests and Global Change, 4: 765871.
https://doi.org/10.3389/ffgc.2021.765871

Lenz P.R.N., Nadeau S., Mottet M., Perron M., Isabel N., Beaulieu J., and Bousquet J., 2019, Multi-trait genomic selection for weevil resistance growth and wood quality in Norway spruce, Evolutionary Applications, 13: 76-94.
https://doi.org/10.1111/eva.12823

Liu J., Fernandes H., Zamany A., Sikorski M., Jaskólski M., and Sniezko R., 2021, In-vitro anti-fungal assay and association analysis reveal a role for the *Pinus monticola PR10* gene (*PmPR10-3.1*) in quantitative disease resistance to white pine blister rust, Genome, 64(7): 693-704.
https://doi.org/10.1139/gen-2020-0080

Liu J.J., Schoettle A.W., Sniezko R.A., Waring K., Williams H., Zamany A., Johnson J.S., and Kegley A., 2021, Comparative association mapping reveals conservation of major gene resistance to white pine blister rust in southwestern white pine (*Pinus strobiformis*) and limber pine, 112(5): 1093-1102.
https://doi.org/10.1094/PHYTO-09-21-0382-R

Liu J., Schoettle A., Sniezko R., Yao F., Zamany A., Williams H., and Rancourt B., 2019, Limber pine (*Pinus flexilis James*) genetic map constructed by exome-seq provides insight into the evolution of disease resistance and a genomic resource for genomics-based breeding, The Plant Journal: For Cell and Molecular Biology, 98(4): 745-758.
https://doi.org/10.1111/tpj.14270

Liu J., Williams H., Zamany A., Li X.R., Gellner S., and Sniezko R., 2020, Development and application of marker-assisted selection (MAS) tools for breeding of western white pine (*Pinus monticola Douglas* ex D. Don) resistance to blister rust (*Cronartium ribicola* JC Fisch.) in British Columbia, Canadian Journal of Plant Pathology, 42(2): 250-259.
https://doi.org/10.1080/07060661.2019.1638454

Liu Q., Wei Y., Xu L., Hao Y., Chen X., and Zhou Z., 2017, Transcriptomic profiling reveals differentially expressed genes associated with pine wood nematode resistance in masson pine (*Pinus massoniana Lamb*), Scientific Reports, 7(1): 4693.
https://doi.org/10.1038/s41598-017-04944-7

Modesto I., Mendes A., Carrasquinho I., and Miguel C., 2022, Molecular defense response of pine trees (*Pinus* spp.) to the parasitic nematode *bursaphelenchus xylophilus*, Cells, 11(20): 3208.
https://doi.org/10.3390/cells11203208

Modesto I., Sterck L., Arbona V., Gómez-Cadenas A., Carrasquinho I., van de Peer Y., and Miguel C., 2021, Insights into the mechanisms implicated in pinus pinaster resistance to pinewood nematode, Frontiers in Plant Science, 12: 690857.
https://doi.org/10.3389/fpls.2021.690857

Mukrimin M., Kovalchuk A., Ghimire R., Kivimäenpää M., Sun H., Holopainen J., and Asiegbu F., 2019, Evaluation of potential genetic and chemical markers for Scots pine tolerance against *Heterobasidion annosum* infection, Planta, 250: 1881-1895.
https://doi.org/10.1007/s00425-019-03270-8

Pandit K., Smith J., Quesada T., Villari C., and Johnson D.J., 2020, Association of recent incidence of foliar disease in pine species in the southeastern united states with tree and climate variables, Forests, 11(11): 1155.
https://doi.org/10.3390/f11111155

Park J., Jeon H.W., Jung H., Lee H.H., Kim J., Park A., Kim N., Han G., Kim J.C., and Seo Y., 2020, Comparative transcriptome analysis of pine trees treated with resistance-inducing substances against the nematode *bursaphelenchus xylophilu*s, Genes, 11(9): 1000.
https://doi.org/10.3390/genes11091000

Raitelaitytė K., Rutkauskas A., Radzijevskaja J., Žukauskienė J., Markovskaja S., and Paulauskas A., 2017, The fungal pathogens causing diseases in pines, Biologija, 62: 4.
https://doi.org/10.6001/BIOLOGIJA.V62I4.3414

Rigg J., Offord C., Zimmer H., Anderson I., Singh B., and Powell J., 2017, Conservation by translocation: establishment of *Wollemi pine* and associated microbial communities in novel environments, Plant and Soil, 411: 209-225.
https://doi.org/10.1007/s11104-016-3010-2

Robischon M., 2016, Potential environmental impact of insect-resistant transgenic trees, In Gene Flow: Monitoring Modeling and Mitigation, 82: 173-194.
https://doi.org/10.1007/978-94-017-7531-1_9

Sniezko R., and Koch J., 2017, Breeding trees resistant to insects and diseases: putting theory into application, Biological Invasions, 19: 3377-3400.
https://doi.org/10.1007/s10530-017-1482-5

Sniezko R., Kegley A., and Savin D., 2017, Ex situ genetic conservation potential of seeds of two high elevation white pines, New Forests, 48: 245-261.
https://doi.org/10.1007/s11056-017-9579-3

Vicente C.S.L., Soares M., Faria J.M.S., Ramos A.P., and Inácio M., 2021, Insights into the role of fungi in pine wilt disease, Journal of Fungi, 7(9): 780.
https://doi.org/10.3390/jof7090780

Visser E., Kampmann T.P., Wegrzyn J., and Naidoo S., 2022, Multispecies comparison of host responses to *Fusarium* circinatum challenge in tropical pines show consistency in resistance mechanisms, Plant Cell and Environment, 46(5): 1705-1725.
https://doi.org/10.1111/pce.14522

Voronova A., Rendón-Anaya M., Ingvarsson P., Kalendar R., and Ruņģis D., 2020, Comparative study of pine reference genomes reveals transposable element interconnected gene networks, Genes, 11(10): 1216.
https://doi.org/10.3390/genes11101216

Wang X.Y., Wu X.Q., Wen T.Y., Feng Y., and Zhang Y., 2023, Transcriptomic analysis reveals differentially expressed genes associated with pine wood nematode resistance in resistant *Pinus thunbergii*, Tree Physiology, 43(6): 995-1008.
https://doi.org/10.1093/treephys/tpad018

Weiss M., Sniezko R.A., Puiu D., Crepeau M., Stevens K., Salzberg S., Langley C., and Neale D., 2020, Genomic basis of white pine blister rust quantitative disease resistance and its relationship with qualitative resistance, The Plant Journal: For Cell and Molecular Biology, 104(2): 365-376.
https://doi.org/10.1111/tpj.14928

Wright J., Stevens K., Hodgskiss P., and Langley C., 2022, SNPs in a large genomic scaffold are strongly associated with Cr1R major gene for resistance to white pine blister rust in range-wide samples of sugar pine (*Pinus lambertiana*), Plant Disease, 106(6): 1639-1644.
https://doi.org/10.1094/PDIS-08-21-1608-RE

Yin K.Q., and Qiu J.L., 2019, Genome editing for plant disease resistance: applications and perspectives, Philosophical Transactions of the Royal Society B, 374(1767): 20180322.
https://doi.org/10.1098/rstb.2018.0322

Zhang L., and Hu J., 2021, Transgenic poplar gene flow monitoring in China, In Gene Flow: Monitoring Modeling and Mitigation, 4: 56.
https://doi.org/10.1079/9781789247480.0004