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Feature Review **Open Access**

Conifer Genome Sequencing and Functional Study of Disease Resistance Genes

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Abstract This study reviews recent advances in conifer genome sequencing and the functional study of disease resistance genes. Through the construction of high-density genetic maps, such as those developed for limber pine (*Pinus flexilis*), numerous key genes involved in disease resistance have been identified, including nucleotide-binding site leucine-rich repeat genes (NBS-LRRs) and receptor-like protein kinase genes (RLKs). These genetic maps provide essential resources for understanding genetic disease resistance and local adaptation to changing climates in conifers. The research also reveals the detailed organization of resistance gene clusters and the genetic mechanisms involved in generating new resistance specificities. The identification of broad-spectrum quantitative disease resistance (BS-QDR) loci in various plants further underscores the potential for similar discoveries in conifers. Success stories, such as the verification of the *NBS-LRR* gene in pine species for resistance to pine wilt disease, and the *PaPR10* gene in larch species for resistance to larch needle cast, demonstrate the effectiveness of combining laboratory research with field trials. Future research should focus on the functional validation of identified resistance genes and exploring their roles in conifer disease resistance to accelerate the development of disease-resistant conifer varieties. This study aims to advance the development of conifer genomics and promote its application in practical breeding and conservation efforts.

Keywords Conifers; Genome sequencing; Disease resistance genes; High-density genetic maps; Functional genomics

1 Introduction

Conifers play a crucial role in global ecosystems and the forestry industry. They are dominant in many forest ecosystems, particularly in the Northern Hemisphere, and provide essential ecological services such as carbon sequestration, habitat for wildlife, and soil stabilization. Economically, conifers are vital for the forestry industry, supplying timber, paper, and other wood products. Their resilience and adaptability to various environmental conditions make them indispensable for both natural and managed forests (Michelmore, 2000; Liu et al., 2019).

Despite their importance, understanding conifer genomics and disease resistance poses significant challenges. Conifer genomes are typically large and complex, with high levels of repetitive DNA, making sequencing and assembly difficult. Additionally, the genetic mechanisms underlying disease resistance in conifers are not well understood. This complexity is compounded by the evolutionary pressures exerted by pathogens, which drive the diversification of resistance genes. For instance, the genetic mapping of limber pine has revealed numerous resistance genes, but the functional characterization of these genes remains incomplete (Andolfo et al., 2013; Liu et al., 2019; Kale et al., 2022). Moreover, the identification and functional analysis of resistance genes in other plant species, such as tomato and rice, highlight the intricate regulatory networks and diverse proteins involved in plant immunity, which are likely to be similarly complex in conifers (Wisser et al., 2005; Andolfo et al., 2013).

This study is to advance our understanding of conifer genomics and disease resistance through genome sequencing and functional genomics. By leveraging high-throughput sequencing technologies and comprehensive genomic analyses, we aim to identify and characterize resistance genes in conifers. This includes constructing high-density genetic maps, performing genome-wide association studies (GWAS), and elucidating the functional roles of candidate resistance genes. Such efforts will provide valuable genomic resources for breeding and conservation programs, ultimately enhancing the resilience of conifer species to diseases and environmental changes.

2 Advances in Conifer Genome Sequencing

2.1 Overview of recent genome sequencing projects in conifers

Recent advancements in conifer genome sequencing have significantly enhanced our understanding of the genetic basis of disease resistance and adaptation in these species. For instance, the construction of a high-density genetic map for limber pine (*Pinus flexilis*) using exome-seq has provided valuable insights into the evolution of disease resistance genes and local adaptation mechanisms. This project mapped 9 612 unigenes across 12 linkage groups and identified numerous genes involved in defense responses, including 639 nucleotide-binding site leucine-rich repeat genes (NBS-LRRs) and 290 receptor-like protein kinase genes (RLKs) (Liu et al., 2019). Similarly, the identification and annotation of 3816 expressed NLR sequences across seven conifer species have revealed the extensive diversity and evolutionary dynamics of resistance genes in conifers (Ghelder et al., 2019).

2.2 Technological innovations facilitating conifer genome analysis

Technological innovations have played a crucial role in advancing conifer genome analysis. The use of exome-seq, as demonstrated in the Limber pine study, allows for the efficient construction of high-density genetic maps, facilitating the identification of genes associated with disease resistance and other adaptive traits (Liu et al., 2019). Additionally, the development of transcriptome assemblies and the application of RNA sequencing have enabled the detailed characterization of gene expression profiles in response to biotic and abiotic stresses. For example, RNA sequencing of Norway spruce (*Picea abies*) clones has identified key genes involved in terpene biosynthesis and flavonoid pathways, which are associated with resistance to the conifer stem rot pathogen *Heterobasidion parviporum* (Liu et al., 2021). These technological advancements have significantly improved our ability to dissect the complex genetic architecture of conifer disease resistance.

2.3 Comparative genomics: insights gained from sequenced conifer genomes

Comparative genomics has provided valuable insights into the evolutionary conservation and divergence of disease resistance genes in conifers. Syntenic analysis of genome structures across different conifer species has revealed that the majority of orthologs are positional orthologous genes (POGs), indicating a high level of conservation in basic cellular functions and maintenance (Liu et al., 2019). Furthermore, the study of NLR sequences across land plants has shown that conifers possess some of the most diverse and numerous RNLs, with unique evolutionary patterns compared to angiosperms (Ghelder et al., 2019). The identification of conserved motifs in the NBS domains of resistance gene analogs (RGAs) from western white pine (*Pinus monticola*) further supports the hypothesis that conifer RGAs share a common origin with angiosperm R genes, highlighting the evolutionary pressures shaping these gene families (Elfstrand et al., 2020). These comparative genomic studies have deepened our understanding of the genetic basis of disease resistance and the evolutionary dynamics of resistance genes in conifers.

3 Identification of Disease Resistance Genes in Conifers

3.1 Genetic basis of disease resistance in conifers

The genetic basis of disease resistance in conifers is complex and involves multiple gene families and pathways. Conifers, such as limber pine (*Pinus flexilis*), have been shown to possess a variety of genes that contribute to their defense mechanisms against pathogens. For instance, a high-density genetic map of limber pine identified 639 nucleotide-binding site leucine-rich repeat genes (NBS-LRRs), 290 receptor-like protein kinase genes (RLKs), and 1014 genes with potential roles in defense response and induced systemic resistance (Liu et al., 2019). Similarly, the large repertoire of conifer NLR (nucleotide-binding, leucine-rich-repeat) resistance genes includes highly diversified RNLs (Resistance to Powdery Mildew 8-like NLRs), which play a central role in resilience to stress (Ghelder et al., 2019). These genes are crucial for the plant's ability to recognize and respond to pathogen attacks.

3.2 Methodologies for identifying and characterizing resistance genes

Several methodologies have been employed to identify and characterize disease resistance genes in conifers. Exome sequencing (exome-seq) has been used to construct high-density genetic maps, which are essential for understanding genetic disease resistance and local adaptation (Liu et al., 2019). Additionally, association genetics has been utilized to identify quantitative trait loci (QTLs) linked to disease resistance. For example, in Norway spruce (*Picea abies*), eleven QTLs were identified that correlate with variation in resistance to Heterobasidion parviporum, a major pathogen causing stem and root-rot (Elfstrand et al., 2020). Transcript profiling and RNA sequencing are also common techniques used to analyze gene expression in response to pathogen infection. For instance, the differential expression of terpene synthase genes in Sitka spruce (*Picea sitchensis*) was analyzed to understand the biosynthesis of (+)-3-carene, a monoterpene associated with resistance to white pine weevil (Hall et al., 2011).

3.3 Key disease resistance genes discovered in conifers

Several key disease resistance genes have been discovered in conifers through various studies. In limber pine, orthologous loci for resistance to rust pathogens were identified and co-positioned with multiple members of the R gene family, revealing the evolutionary pressure acting upon them (Liu et al., 2019). In Sitka spruce, the *PsTPS-3car2* gene was found to be specifically expressed in resistant genotypes and was associated with higher levels of (+)-3-carene, contributing to resistance against white pine weevil (Hall et al., 2011). Another significant discovery is the Norway spruce laccase gene, *PaLAC5*, which is linked to resistance against *Heterobasidion parviporum*. This gene is specifically and strongly expressed in response to pathogen inoculation, suggesting its role in the formation of lignosuberized boundary zones in bark (Elfstrand et al., 2020). These case studies highlight the diverse genetic mechanisms that conifers employ to resist pathogen attacks.

4 Functional Genomics Approaches

4.1 Techniques for studying gene function in conifers

Functional genomics in conifers employs a variety of techniques to elucidate gene function. High-density genetic mapping, such as exome-seq, has been used to construct genetic maps and identify genes involved in disease resistance, as demonstrated in limber pine (*Pinus flexilis*) (Liu et al., 2019). Additionally, cDNA microarrays have been utilized to monitor gene expression in response to biotic stress, providing insights into the complex defense mechanisms of conifers (Ralph et al., 2006). Genomic, proteomic, and biochemical approaches have also been combined to analyze specific phenotypes, such as the (+)-3-carene biosynthesis in Sitka spruce, revealing the role of specific terpene synthase genes in resistance to white pine weevil (Hall et al., 2011).

4.2 Transcriptomic and proteomic insights into gene function

Transcriptomic and proteomic analyses offer deep insights into gene function by examining gene expression and protein profiles. For instance, microarray gene expression profiling has revealed large-scale changes in the host transcriptome of Sitka spruce in response to mechanical wounding and insect feeding, highlighting genes involved in defense signaling and secondary metabolism (Ralph et al., 2006). These approaches help in understanding the regulatory networks and protein diversity involved in conifer disease resistance (Michelmore, 2000).

4.3 Integrating functional data to validate gene roles in disease resistance

Integrating data from various functional genomics approaches is crucial for validating the roles of genes in disease resistance. For example, the integration of genomic, transcriptomic, and proteomic data in Sitka spruce has provided a comprehensive understanding of the (+)-3-carene biosynthesis pathway and its role in resistance to white pine weevil (Hall et al., 2011). Such integrative approaches are essential for characterizing functional genes underlying complex traits and enhancing our understanding of conifer disease resistance mechanisms (Ralph et al., 2006; Hall et al., 2011; Liu et al., 2019).

5 Case Studies: Functional Verification of Resistance Genes

5.1 Experimental design and implementation for gene function studies

The verification of resistance gene function in conifers involves a series of meticulous experimental steps to ensure the accuracy and reliability of the results. Key elements of the experimental design include: the first step involves identifying candidate resistance genes through genome sequencing and bioinformatics analyses. Once identified, these genes are cloned into suitable vectors for further study. The cloned genes are introduced into model systems or conifer tissue using methods such as Agrobacterium-mediated transformation or biolistic particle delivery. This step is crucial for analyzing the expression patterns and confirming that the genes are active in the host (Liu et al., 2020). Transgenic plants or tissues expressing the candidate genes are subjected to pathogen challenge assays. These involve exposing the plants to specific pathogens to evaluate the effectiveness of the resistance genes in conferring protection.

The response of the transformed plants is assessed through molecular techniques like qPCR, RNA-Seq, and Western blotting to measure gene expression levels. Phenotypic analyses, including disease symptom scoring and growth assessments, provide additional validation of gene function (Rellstab et al., 2019). To confirm laboratory findings, field trials are conducted where transgenic conifers are planted in natural settings and monitored for resistance to diseases under real-world conditions. These trials help determine the practical applicability of the resistance genes in forestry management (Xie et al., 2023).

5.2 Success stories of gene function verification in laboratory and field conditions

Case study 1: *NBS-LRR* gene in pine species

A notable example is the verification of the *NBS-LRR* gene in various pine species. Researchers cloned and expressed the *NBS-LRR* gene, which was identified as a key player in resistance against pine wilt disease (PWN) caused by the nematode *Bursaphelenchus xylophilus*. Xie et al. (2023) demonstrated that *PmNBS-LRR97* can significantly activate the expression of reactive oxygen species (ROS)-related genes in *Pinus massoniana*, participating in the defense response against pine wood nematode (PWN). Due to the lack of a successful genetic transformation system for *Pinus massoniana*, Xie et al. (2023) transformed *PmNBS-LRR97* into *Nicotiana benthamiana* to verify its function (Figure 1).

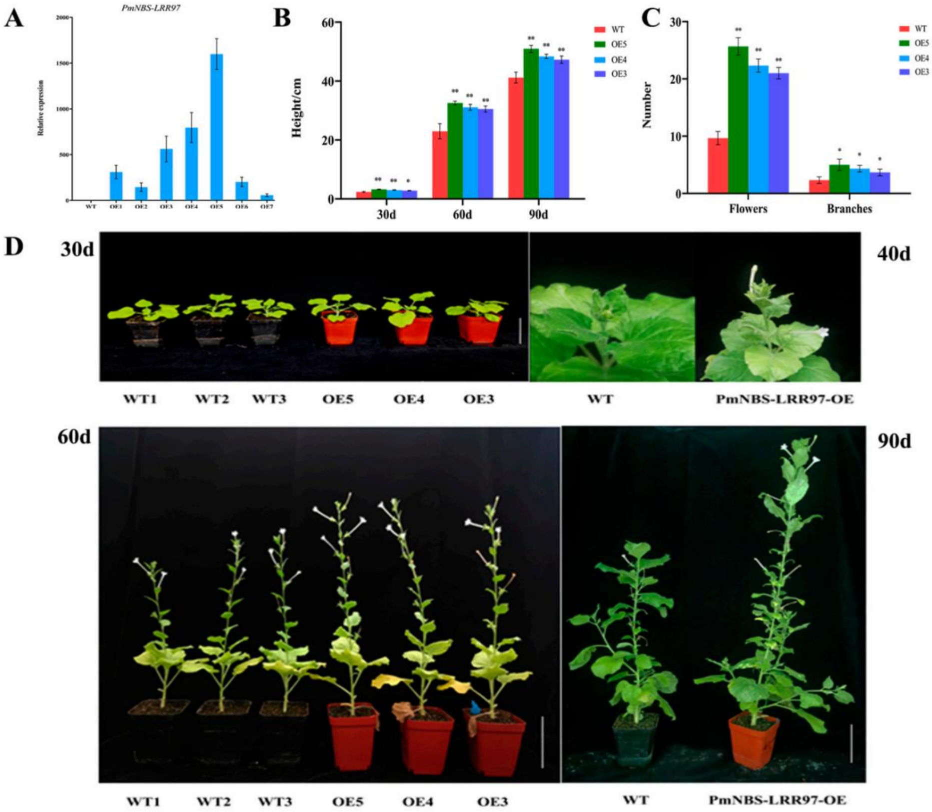

Figure 1 Quantitative verification and phenotypic difference analysis of *PmNBS-LRR97*-overexpressing tobacco (Adopted from Xie et al., 2023)

Image caption: (A) Relative expression of *PmNBS-LRR97* in WT and transgenic tobacco; error bars represent three biological replicates ± SD. (B) Plant height analysis of WT and *PmNBS-LRR97*-overexpressing tobacco at 30, 60, and 90 d; error bars represent three biological replicates ± SD. Student's t-test was used to test the significance (*, $p < 0.05$; **, $p < 0.01$). (C) Differences in the flowering number and branch number between WT and *PmNBS-LRR97*-overexpressing tobacco at 90 d; error bars represent three biological replicates ± SD. (D) Different phenotypes of WT and *PmNBS-LRR97*-overexpressing tobacco. Scale bar = 7 cm (Adopted from Xie et al., 2023)

Case study 2: *PmPR10* gene in larch species

The *PmPR10* gene in larch species has shown promise in fighting larch needle cast caused by a wide spectrum of pathogens. Functional analyses of *PmPR10* genes, which play roles in plant defense, have indicated their involvement in resistance mechanisms across various conifer species. Liu et al. (2021) selected *PmPR10-3.1* from 10 *PmPR10* genes, and expressed in *Escherichia coli*. The purified recombinant protein exhibited inhibitory effects on spore hyphal growth of fungal pathogens *Cronartium ribicola*, *Phoma exigua*, and *Phoma argillacea* by in-vitro anti-fungal analysis (Figure 2).

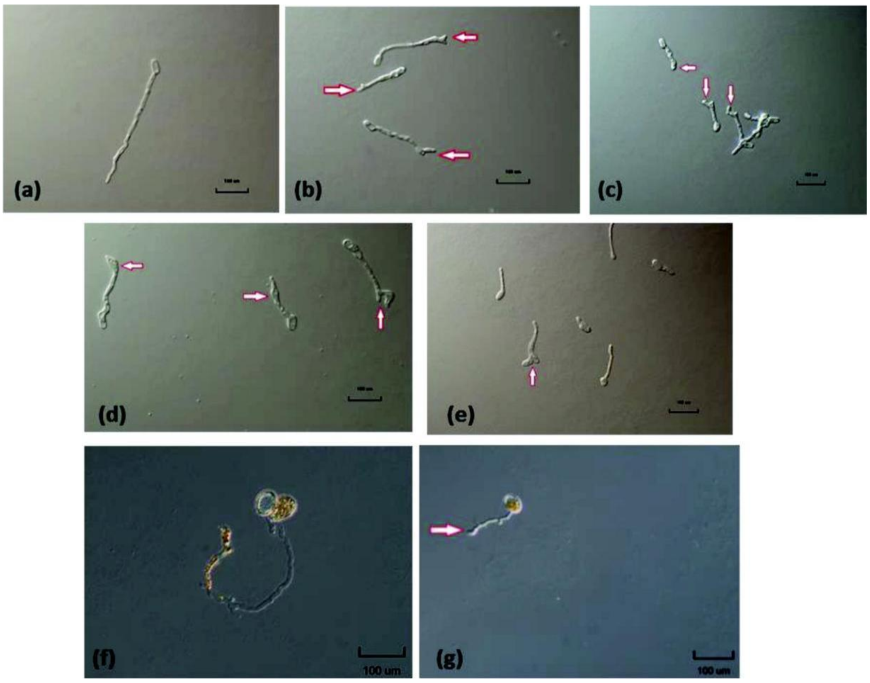

Figure 2 Micrographs showing the effects of *PmPR10-3.1* on spore germination of fungal pathogens (Adopted from Liu et al., 2021)
Image caption: Conidiospores of *Phoma exigua* (isolate PFC 2705) were treated by pure PmPR10-3.1 protein for 18 h and photographs were taken at 63× magnification DIC. (a) Desalt buffer control; (b) 10 μg/mL PR10-3.1; (c) 42 μg/mL PR10; (d) 75 μg/mL PR10; (e) 100 μg/mL PR10-3.1. Urediniospores of *Cronartium ribicola* were treated with pure PmPR10-3.1 protein for 24 h and photographs were taken at 200× using Nimarsky filter: (f) desalt buffer control; (g) 100 μg/mL PR10-3.1. Bars in insets represent 100 μm. Arrows indicate reduced hyphal growth and swelling at hyphal tips due to PmPR10-3.1 (Adopted from Liu et al., 2021)

5.3 Comparative analysis with non-coniferous species

Comparative analysis with non-coniferous species reveals both similarities and differences in the mechanisms of disease resistance. In rice, for example, extensive genetic analysis has identified clusters of resistance genes and quantitative trait loci (QTL) associated with broad-spectrum disease resistance (Wisser et al., 2005). These findings are consistent with the clustering of resistance genes observed in conifers, suggesting a common evolutionary strategy for disease resistance across plant species (George et al., 2022). However, the specific genetic mechanisms and regulatory networks involved in generating new resistance specificities may differ, as evidenced by the unique evolutionary pressures and gene structures identified in conifers (Liu et al., 2019). This comparative analysis underscores the importance of cross-species studies in understanding the complex dynamics of disease resistance and the potential for leveraging insights from non-coniferous species to enhance resistance breeding in conifers.

6 Technological Advances and Tools

6.1 Next-generation sequencing and its impact on conifer research

Next-generation sequencing (NGS) technologies have significantly advanced the field of conifer genomics by providing rapid, cost-effective, and high-throughput sequencing capabilities. These technologies have enabled the construction of high-density genetic maps, which are essential for understanding genetic disease resistance and local adaptation in conifers. Additionally, NGS has facilitated the study of antimicrobial resistance (AMR) by enabling the identification and characterization of resistance genes in various environments, thus contributing to global pathogen surveillance and AMR tracking (Hendriksen et al., 2019; Gupta et al., 2020; Florensa et al., 2022). The integration of NGS with other advanced research fields, such as genome editing technologies, has further enhanced the potential for plant health improvement by diagnosing and mitigating novel or unidentified pathogens (Mushtaq et al., 2021).

6.2 Emerging tools in CRISPR and other genome editing technologies

Genome editing technologies, particularly CRISPR-Cas9, have revolutionized plant biology by enabling precise and targeted modifications of genomes. CRISPR-Cas9 has democratized genome editing in plants due to its ease of use, robustness, and cost-effectiveness. This technology has been successfully applied to enhance disease resistance in crops by targeting and modifying susceptibility genes, thereby providing broad-spectrum and durable resistance (Borrelli et al., 2018; Langner et al., 2018; Zaidi et al., 2018). For example, CRISPR-Cas9 has been used to produce plants resistant to single-stranded RNA viruses by targeting critical genes such as *eIF4E* (Mushtaq et al., 2021). The development of transgene-free and durable disease-resistant crop varieties through genome editing holds great promise for future plant breeding programs (Zaidi et al., 2018). Moreover, the integration of CRISPR with NGS technologies has the potential to revolutionize plant virology by enabling rapid engineering of viral resistance and precise modulation of viral genomes (Mushtaq et al., 2021).

6.3 Bioinformatics tools for data analysis and gene prediction

The rapid advancements in sequencing technologies have generated vast amounts of biological data, necessitating the development of sophisticated bioinformatics tools for data analysis and gene prediction. Bioinformatics approaches are crucial for retrieving, annotating, analyzing, and identifying functional aspects of genes and genomes. For instance, tools such as ARG-ANNOT, CARD, and ResFinder have been developed to detect antimicrobial resistance determinants in DNA or amino acid sequence data, providing essential insights into the evolution and emergence of AMR (Hendriksen et al., 2019; Gupta et al., 2020; Florensa et al., 2022). These tools differ in their input data requirements, search approaches, and sensitivity and specificity of detection, highlighting the importance of selecting appropriate tools for specific analyses (Hendriksen et al., 2019; Florensa et al., 2022). Additionally, bioinformatics platforms have been employed to characterize resistance genes in plants, facilitating the identification and isolation of candidate resistance genes and the development of disease-resistant crops (Joshi et al., 2023). The integration of molecular and bioinformatics approaches has thus become a cornerstone of modern plant pathology, enabling the development of novel diagnostic tools and strategies for plant disease management (Joshi et al., 2023).

7 Applications in Breeding and Forestry Management

7.1 Translating genomic insights into breeding strategies for disease resistance

The integration of genomic insights into breeding strategies for disease resistance in conifers has shown significant promise. High-density genetic maps, such as those developed for limber pine (*Pinus flexilis*), provide essential knowledge for understanding genetic disease resistance and local adaptation to changing climates. These maps include numerous genes with potential roles in defense responses and induced systemic resistance to pathogens, making them practical tools for breeding and genetic conservation programs (Liu et al., 2019). Similarly, the development of high-density exome capture genotype-by-sequencing panels for radiata pine (*Pinus radiata*) has enabled the capture of a vast number of single nucleotide polymorphism (SNP) markers, facilitating marker-based breeding value predictions and accelerating selection processes (Telfer et al., 2019). The application of genomic selection and genetic engineering to enhance oleoresin production in loblolly pine (*Pinus taeda*) demonstrates the potential to increase resistance to bark beetles and improve bioenergy potential, further underscoring the utility of genomic tools in breeding for disease resistance (Westbrook et al., 2013).

7.2 Integrating genomic information into sustainable forestry practices

The integration of genomic information into sustainable forestry practices is crucial for the effective management of genetic resources and the adaptation of forests to environmental changes. Advances in next-generation sequencing (NGS) technologies have significantly accelerated conifer genomics research, providing insights into genome evolution and the genomic basis of adaptation (Prunier et al., 2016). The ProCoGen project exemplifies efforts to promote a functional and comparative understanding of the conifer genome, aiming to develop high-throughput genotyping tools and pre-breeding tools for forest tree breeding programs. This project also emphasizes the importance of international collaboration to enhance the application of genomic tools in diverse conifer species (Díaz-Sala et al., 2011). Additionally, the identification and functional study of transposable elements (TEs) in conifers offer potential applications in breeding and genetic research, contributing to the sustainable management of conifer forests (Wang et al., 2020).

7.3 Challenges and opportunities in applying genetic research

While the application of genetic research in conifer breeding and forestry management presents numerous opportunities, several challenges must be addressed. One major challenge is the complexity of conifer genomes, which are often large and contain high repetitive DNA content. Optimizing genotyping-by-sequencing (GBS) strategies and developing effective SNP calling methods are essential for accurate genomic analysis in conifers (Pan et al., 2015). Another challenge is the need for comprehensive genomic resources, such as reference genomes, to facilitate genome-wide association studies (GWAS) and genomic selection. The draft assembly of the Norway spruce (*Picea abies*) genome represents a significant milestone, providing a valuable resource for comparative genomics and breeding efforts (Nystedt et al., 2013). Despite these challenges, the rapid advancements in genomic technologies and the increasing availability of genomic data offer exciting opportunities to enhance disease resistance, improve forest productivity, and ensure the sustainability of conifer forests (Yin et al., 2019).

8 Environmental and Ecological Impact

8.1 Role of enhanced disease resistance in ecosystem stability

Enhanced disease resistance in conifers plays a crucial role in maintaining ecosystem stability. Conifers, such as limber pine and Sitka spruce, are keystone species in their respective ecosystems, providing essential ecological functions and supporting biodiversity (Ralph et al., 2008; Liu et al., 2019). The development of genetic maps and genomic resources has revealed numerous genes involved in disease resistance, such as nucleotide-binding site leucine-rich repeat genes (NBS-LRRs) and receptor-like protein kinase genes (RLKs), which are critical for the defense response against pathogens (Liu et al., 2019). These genetic advancements enable conifers to better withstand biotic stressors, thereby contributing to the stability and resilience of forest ecosystems (Bonello et al., 2006). The adaptive plasticity of conifers, including inducible resistance mechanisms, allows for a balanced allocation of resources between growth and defense, further enhancing ecosystem stability (Bonello et al., 2006).

8.2 Implications for biodiversity and conservation

The genomic study of disease resistance in conifers has significant implications for biodiversity and conservation. Conifers dominate many temperate and boreal forests, which are vital for global biodiversity (Ralph et al., 2008). By understanding the genetic basis of disease resistance, conservation efforts can be more effectively targeted to protect these critical species from pathogens and other environmental stressors (Baker et al., 2018). For instance, the identification of orthologous loci for resistance to rust pathogens in limber pine provides valuable insights for breeding programs aimed at enhancing disease resistance (Liu et al., 2019). Additionally, the development of genomic resources, such as expressed sequence tags (ESTs) and full-length cDNAs, facilitates the discovery of genes involved in defense mechanisms, aiding in the conservation of genetic diversity within conifer populations (Ralph et al., 2008; Parchman et al., 2010). These efforts are essential for maintaining the ecological roles of conifers and ensuring the long-term health and diversity of forest ecosystems.

8.3 Ethical and regulatory considerations in genomic modifications

The application of genomic modifications in conifers raises several ethical and regulatory considerations. While enhancing disease resistance through genetic modifications can provide significant ecological benefits, it is essential to carefully evaluate the potential risks and unintended consequences. Ethical considerations include the potential impact on non-target species and the broader ecosystem, as well as the long-term effects of introducing genetically modified organisms into natural environments (Prunier et al., 2016). Regulatory frameworks must be established to ensure that genomic modifications are conducted responsibly and transparently, with thorough risk assessments and monitoring protocols in place. Additionally, public engagement and stakeholder involvement are crucial in addressing ethical concerns and building trust in the use of genomic technologies for conservation purposes (Prunier et al., 2016). By balancing the benefits of enhanced disease resistance with ethical and regulatory considerations, we can harness the potential of genomic modifications to support biodiversity and ecosystem stability while minimizing potential risks.

9 Future Research Directions

9.1 Unexplored areas in conifer genomics and functional genomics

Despite significant advancements in conifer genomics, several areas remain underexplored. One such area is the comprehensive understanding of the genetic basis of disease resistance in conifers. For instance, while the high-density genetic map of limber pine has identified numerous genes involved in disease resistance, further functional studies are needed to elucidate the specific roles of these genes in pathogen defense mechanisms. Additionally, the genome of *Casuarina equisetifolia*, a conifer-like angiosperm, has been sequenced, revealing insights into secondary growth and stress tolerance. However, the molecular mechanisms underlying these traits remain largely unexplored and warrant further investigation. Another promising area is the use of conifer needles as passive bioaerosol samplers to monitor antibiotic resistance genes, which could provide valuable data for environmental and public health studies (George et al., 2022).

9.2 Potential for interdisciplinary and international research collaborations

The complexity and scale of conifer genomes necessitate interdisciplinary and international collaborations to advance our understanding of these species. Collaborative efforts can facilitate the sharing of resources, such as high-quality genome sequences and advanced sequencing technologies, which are essential for large-scale genomic studies. For example, the sequencing of the sugar pine megagenome has provided a foundation for future research on disease resistance and genome evolution, highlighting the importance of international cooperation in such large-scale projects (Stevens et al., 2016). Furthermore, integrating molecular biology, bioinformatics, and ecological studies can enhance our understanding of the genetic and environmental factors influencing disease resistance in conifers. Collaborative research on the molecular and chemical basis of disease resistance in Norway spruce clones has already demonstrated the potential of such interdisciplinary approaches (Liu et al., 2021).

9.3 Emerging challenges and technological needs

Several challenges and technological needs must be addressed to advance conifer genomics and functional genomics research. One major challenge is the large and complex nature of conifer genomes, which contain a high proportion of repetitive sequences. Advanced sequencing technologies, such as single-molecule real-time (SMRT) sequencing, have been employed to overcome these challenges, but further improvements in sequencing accuracy and assembly algorithms are needed (Ye et al., 2019). Additionally, the identification and functional characterization of resistance genes in conifers, such as the TIR-NBS-LRR resistance gene analogs in western white pine, require sophisticated bioinformatics tools and functional assays to validate their roles in disease resistance. Another emerging challenge is the need for high-throughput screening methods to identify novel biomarkers for disease resistance, as demonstrated by the molecular and chemical screening of Norway spruce clones (Liu et al., 2021). Addressing these challenges will require continuous technological innovation and the development of new methodologies to enhance the resolution and accuracy of genomic studies in conifers.

10 Concluding Remarks

The research on conifer genome sequencing and the functional study of disease resistance genes has yielded several significant findings. High-density genetic maps constructed for species like limber pine have identified numerous genes involved in disease resistance, including nucleotide-binding site leucine-rich repeat genes (NBS-LRRs) and receptor-like protein kinase genes (RLKs). These genetic maps provide essential resources for understanding genetic disease resistance and local adaptation to changing climates. Additionally, genomic approaches have revealed the detailed organization of resistance-gene clusters and the genetic mechanisms involved in generating new resistance specificities. The identification of broad-spectrum quantitative disease resistance (BS-QDR) loci in rice and other plants further underscores the potential for similar discoveries in conifers, which could lead to improved disease resistance and overall conifer health.

This body of research has significantly advanced the field of tree genetics by providing comprehensive genomic resources and practical tools for breeding and genetic conservation programs. The construction of high-density genetic maps and the identification of orthologous loci for resistance to rust pathogens in limber pine, for instance, offer valuable insights into the evolutionary pressures acting on these genes. The development of resistance gene atlases and chromosome-scale genome sequences in other plant species further illustrates the utility of these genomic resources for breeders and geneticists. These contributions not only enhance our understanding of disease resistance mechanisms but also facilitate the application of genome-wide association studies (GWAS) and the characterization of functional genes underlying complex traits in conifers and other tree species.

Future research should focus on the functional validation of identified resistance genes and the exploration of their roles in conifer disease resistance. This includes the genetic dissection of disease QTL confidence intervals to reduce the number of positional candidate genes for further functional analysis. Additionally, integrating genomic approaches with traditional breeding methods could accelerate the development of disease-resistant conifer varieties. Policymakers should support the establishment of genomic databases and the standardization of analytical pipelines to ensure the clinical relevance of genomic data for disease resistance prediction and management. Collaborative efforts between researchers, breeders, and policymakers will be crucial in leveraging these genomic resources to enhance conifer health and resilience against emerging pathogens.

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Conflict of Interest Disclosure

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

Reference

Andolfo G., Sanseverino W., Aversano R., Frusciante L., and Ercolano M., 2013, Genome-wide identification and analysis of candidate genes for disease resistance in tomato, Molecular Breeding, 33: 227-233.
https://doi.org/10.1007/s11032-013-9928-7

Baker E., Wegrzyn J., Sezen U., Falk T., Maloney P., Vogler D., Delfino-Mix A., Jensen C., Mitton J., Wright J., Knaus B., Rai H., Cronn R., Gonzalez-Ibeas D., Vasquez-Gross H., Famula R., Liu J., Kueppers L., and Neale D., 2018, Comparative transcriptomics among four white pine species, G3: Genes, Genomes, Genetics, 8: 1461-1474.
https://doi.org/10.1534/g3.118.200257
PMid:29559535 PMCid:PMC5940140

Bonello P., Gordon T., Herms D., Wood D., and Erbilgin N., 2006, Nature and ecological implications of pathogen-induced systemic resistance in conifers: a novel hypothesis, Physiological and Molecular Plant Pathology, 68: 95-104.
https://doi.org/10.1016/j.pmpp.2006.12.002

Borrelli V., Brambilla V., Rogowsky P., Marocco A., and Lanubile A., 2018, The enhancement of plant disease resistance using CRISPR/Cas9 technology, Frontiers in Plant Science, 9: 1245.
https://doi.org/10.3389/fpls.2018.01245
PMid:30197654 PMCid:PMC6117396

Díaz-Sala C., and Cervera T., 2011, Promoting a functional and comparative understanding of the conifer genome- implementing applied aspects for more productive and adapted forests (ProCoGen), BMC Proceedings, 5: P158.
https://doi.org/10.1186/1753-6561-5-S7-P158
PMCid:PMC3240006

Elfstrand M., Baison J., Lundén K., Zhou L., Vos I., Capador H., Åslund M., Chen Z., Chaudhary R., Olson Å., Wu H., Karlsson B., Stenlid J., and García-Gil M., 2020, Association genetics identifies a specifically regulated Norway spruce laccase gene, *PaLAC5*, linked to *Heterobasidion parviporum*- resistance, Plant, Cell & Environment, 43(7): 1779-1791.
https://doi.org/10.1111/pce.13768

Florensa A., Kaas R., Clausen P., Aytan-Aktug D., and Aarestrup F., 2022, ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes, Microbial Genomics, 8(1): 000748.
https://doi.org/10.1099/mgen.0.000748
PMid:35072601 PMCid:PMC8914360

George P., Leclerc S., Turgeon N., Veillette M., and Duchaine C., 2022, Conifer needle phyllosphere as a potential passive monitor of bioaerosolised antibiotic resistance genes, Antibiotics, 11(7): 907.
https://doi.org/10.3390/antibiotics11070907
PMid:35884161 PMCid:PMC9312085

Ghelder C., Parent G., Rigault P., Prunier J., Giguére I., Caron S., Sena J., Deslauriers A., Bousquet J., Esmenjaud D., and MacKay J., 2019, The large repertoire of conifer NLR resistance genes includes drought responsive and highly diversified RNLs, Scientific Reports, 9: 11614.
https://doi.org/10.1038/s41598-019-47950-7
PMid:31406137 PMCid:PMC6691002

Gupta C., Tiwari R., and Cytryn E., 2020, Platforms for elucidating antibiotic resistance in single genomes and complex metagenomes, Environment International, 138: 105667.
https://doi.org/10.1016/j.envint.2020.105667
PMid:32234679

Hall D., Robert J., Keeling C., Domański D., Quesada A., Jancsik S., Kuzyk M., Hamberger B., Borchers C., and Bohlmann J., 2011, An integrated genomic, proteomic and biochemical analysis of (+)-3-carene biosynthesis in Sitka spruce (*Picea sitchensis*) genotypes that are resistant or susceptible to white pine weevil, The Plant Journal, 65(6): 936-948.
https://doi.org/10.1111/j.1365-313X.2010.04478.x
PMid:21323772

Hendriksen R., Bortolaia V., Tate H., Tyson G., Aarestrup F., and McDermott P., 2019, Using genomics to track global antimicrobial resistance, Frontiers in Public Health, 7: 242.
https://doi.org/10.3389/fpubh.2019.00242
PMid:31552211 PMCid:PMC6737581

Joshi A., Song H., Yang S., and Lee J., 2023, Integrated molecular and bioinformatics approaches for disease-related genes in plants, Plants, 12(13): 2454.
https://doi.org/10.3390/plants12132454
PMid:37447014 PMCid:PMC10346619

Kale S., Schulthess A., Padmarasu S., Boeven P., Schacht J., Himmelbach A., Steuernagel B., Wulff B., Reif J., Stein N., and Mascher M., 2022, A catalogue of resistance gene homologs and a chromosome-scale reference sequence support resistance gene mapping in winter wheat, Plant Biotechnology Journal, 20: 1730-1742.
https://doi.org/10.1111/pbi.13843
PMid:35562859 PMCid:PMC9398310

Langner T., Kamoun S., and Belhaj K., 2018, CRISPR crops: plant genome editing toward disease resistance, Annual Review of Phytopathology, 56: 479-512.
https://doi.org/10.1146/annurev-phyto-080417-050158
PMid:29975607

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
PMid:33464999

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, 98(4): 745-758.
https://doi.org/10.1111/tpj.14270
PMid:30729601

Liu M., Wang K., Ghimire R., Haapanen M., Kivimäenpää M., and Asiegbu F., 2021, Molecular and chemical screening for inherent disease resistance factors of Norway spruce (*Picea abies*) clones against conifer stem rot pathogen *Heterobasidion parviporum*, Phytopathology, 112(4): 872-880.
https://doi.org/10.1094/PHYTO-09-21-0379-R
PMid:34698543

Liu S., Ma J., Liu H., Guo Y., Li W., and Niu S., 2020, An efficient system for *Agrobacterium*-mediated transient transformation in *Pinus tabuliformis*, Plant Methods, 16: 52.
https://doi.org/10.1186/s13007-020-00594-5
PMid:32308730 PMCid:PMC7149934

Michelmore R., 2000, Genomic approaches to plant disease resistance, Current Opinion in Plant Biology, 3(2): 125-131.
https://doi.org/10.1016/S1369-5266(99)00050-3
PMid:10712954

Mushtaq M., Dar A., Basu U., Bhat B., Mir R., Vats S., Dar M., Tyagi A., Ali S., Bansal M., Rai G., and Wani S., 2021, Integrating CRISPR-Cas and next generation sequencing in plant virology, Frontiers in Genetics, 12: 735489.
https://doi.org/10.3389/fgene.2021.735489
PMid:34759957 PMCid:PMC8572880

Nystedt B., Street N., Wetterbom A., Zuccolo A., Lin Y., Scofield D., Vezzi F., Delhomme N., Giacomello S., Alexeyenko A., Vicedomini R., Sahlin K., Sherwood E., Elfstrand M., Gramzow L., Holmberg K., Hällman J., Keech O., Klasson L., Koriabine M., Kucukoglu M., Käller M., Luthman J., Lysholm F., Niittylä T., Olson Å., Rilakovic N., Ritland C., Rosselló J., Sena J., Svensson T., Talavera-López C., Theißen G., Tuominen H., Vanneste K., Wu Z., Zhang B., Zerbe P., Arvestad L., Bhalerao R., Bohlmann J., Bousquet J., Gil R., Hvidsten T., Jong P., MacKay J., Morgante M., Ritland K., Sundberg B., Thompson S., Peer Y., Andersson B., Nilsson O., Ingvarsson P., Lundeberg J., and Jansson S., 2013, The Norway spruce genome sequence and conifer genome evolution, Nature, 497: 579-584.
https://doi.org/10.1038/nature12211
PMid:23698360

Pan J., Wang B., Pei Z., Zhao W., Gao J., Mao J., and Wang X., 2015, Optimization of the genotyping-by-sequencing strategy for population genomic analysis in conifers, Molecular Ecology Resources, 15(4): 711-722.
https://doi.org/10.1111/1755-0998.12342
PMid:25367371

Parchman T., Geist K., Grahnen J., Benkman C., and Buerkle C., 2010, Transcriptome sequencing in an ecologically important tree species: assembly, annotation, and marker discovery, BMC Genomics, 11: 180.
https://doi.org/10.1186/1471-2164-11-180
PMid:20233449 PMCid:PMC2851599

Prunier J., Verta J., and MacKay J., 2016, Conifer genomics and adaptation: at the crossroads of genetic diversity and genome function, The New Phytologist, 209(1): 44-62.
https://doi.org/10.1111/nph.13565
PMid:26206592

Ralph S., Chun H., Kolosova N., Cooper D., Oddy C., Ritland C., Kirkpatrick R., Moore R., Barber S., Holt R., Jones S., Marra M., Douglas C., Ritland K., and Bohlmann J., 2008, A conifer genomics resource of 200 000 spruce (*Picea* spp.) ESTs and 6 464 high-quality, sequence-finished full-length cDNAs for Sitka spruce (*Picea sitchensis*), BMC Genomics, 9: 484.
https://doi.org/10.1186/1471-2164-9-484
PMid:18854048 PMCid:PMC2579922

Ralph S., Yueh H., Friedmann M., Aeschliman D., Zeznik J., Nelson C., Butterfield Y., Kirkpatrick R., Liu J., Jones S., Marra M., Douglas C., Ritland K., and Bohlmann J., 2006, Conifer defence against insects: microarray gene expression profiling of Sitka spruce (*Picea sitchensis*) induced by mechanical wounding or feeding by spruce budworms (*Choristoneura occidentalis*) or white pine weevils (*Pissodes strobi*) reveals large-scale changes of the host transcriptome, Plant, Cell & Environment, 29(8): 1545-1570.
https://doi.org/10.1111/j.1365-3040.2006.01532.x
PMid:16898017

Rellstab C., Dauphin B., Zoller S., Brodbeck S., and Gugerli F., 2018, Using transcriptome sequencing and pooled exome capture to study local adaptation in the giga-genome of *Pinus cembra*, Molecular Ecology Resource, 19(2): 536-551.
https://doi.org/10.1111/1755-0998.12986
PMid:30575305

Stevens K., Wegrzyn J., Zimin A., Puiu D., Crepeau M., Cardeño C., Paul R., Gonzalez-Ibeas D., Koriabine M., Holtz-Morris A., Martínez-García P., Sezen U., Marçais G., Jermstad K., McGuire P., Loopstra C., Davis J., Eckert A., Jong P., Yorke J., Salzberg S., Neale D., and Langley C., 2016, Sequence of the sugar pine megagenome, Genetics, 204(4): 1613-1626.
https://doi.org/10.1534/genetics.116.193227
PMid:27794028 PMCid:PMC5161289

Telfer E., Graham N., Macdonald L., Li Y., Klápště J., Resende M., Neves L., Dungey H., and Wilcox P., 2019, A high-density exome capture genotype-by-sequencing panel for forestry breeding in *Pinus radiata*, PLoS One, 14(9): e0222640.
https://doi.org/10.1371/journal.pone.0222640
PMid:31568509 PMCid:PMC6768539

Wang J., Lu N., Yi F., and Xiao Y., 2020, Identification of transposable elements in conifer and their potential application in breeding, Evolutionary Bioinformatics, 16: 1176934320930263.
https://doi.org/10.1177/1176934320930263
PMid:32595272 PMCid:PMC7297469

Westbrook J., Resende Jr M., Muñoz P., Walker A., Wegrzyn J., Nelson D., Neale D., Kirst M., Huber D., Gezan S., Peter G., and Davis J., 2013, Association genetics of oleoresin flow in loblolly pine: discovering genes and predicting phenotype for improved resistance to bark beetles and bioenergy potential, The New Phytologist, 199(1): 89-100.
https://doi.org/10.1111/nph.12240
PMid:23534834

Wisser R., Sun Q., Hulbert S., Kresovich S., and Nelson R., 2005, Identification and characterization of regions of the rice genome associated with broad-spectrum, quantitative disease resistance, Genetics, 169: 2277-2293.
https://doi.org/10.1534/genetics.104.036327
PMid:15716503 PMCid:PMC1449593

Xie H., Zhang J., Cheng J., Zhao S., Wen Q., Kong P., Zhao Y., Xiang X., and Rong J., 2023, Field plus lab experiments help identify freezing tolerance and associated genes in subtropical evergreen broadleaf trees: a case study of *Camellia oleifera*, Frontiers in Plant Science, 14: 1113125.
https://doi.org/10.3389/fpls.2023.1113125
PMid:36909419 PMCid:PMC9994817

Xie Y., Liu B., Gao K., Zhao Y., Li W., Deng L., Zhou Z., and Liu Q., 2023, Comprehensive analysis and functional verification of the *Pinus massoniana* NBS-LRR gene family involved in the resistance to *Bursaphelenchus xylophilus*, International Journal of Molecular Sciences, 24(3): 1812.
https://doi.org/10.3390/ijms24031812
PMid:36768136 PMCid:PMC9915305

Ye G., Zhang H., Chen B., Nie S., Liu H., Gao W., Wang H., Gao Y., and Gu L., 2019, *De novo* genome assembly of the stress tolerant forest species *Casuarina equisetifolia* provides insight into secondary growth, The Plant Journal, 97: 779-794.
https://doi.org/10.1111/tpj.14159
PMid:30427081

Yin K., and Qiu J., 2019, Genome editing for plant disease resistance: applications and perspectives, Philosophical Transactions of the Royal Society B, 374: 20180322.
https://doi.org/10.1098/rstb.2018.0322
PMid:30967029 PMCid:PMC6367152

Zaidi S., Zaidi S., Mukhtar M., and Mansoor S., 2018, Genome editing: targeting susceptibility genes for plant disease resistance, Trends in Biotechnology, 36(9): 898-906.
https://doi.org/10.1016/j.tibtech.2018.04.005
PMid:29752192