Review Article **Open Access**

Pushing the Limits: Advances and Innovative Applications of Tree Stress Resistance Gene Identification Technology

Kaiwen Liang

Hainan Institute of Tropical Agricultural Resources, Sanya, 572025, Hainan, China

Corresponding email: kaiwen.liang@hitar.org

Tree Genetics and Molecular Breeding, 2024, Vol.14, No.3 doi: 10.5376/tgmb.2024.14.0012

Received: 20 Apr., 2024

Accepted: 23 May, 2024

Published: 31 May, 2024

Preferred citation for this article:

Liang K.W., 2024, Pushing the limits: advances and innovative applications of tree stress resistance gene identification technology, Tree Genetics and Molecular Breeding, 14(3): 119-131 (doi: 10.5376/tgmb.2024.14.0012)

Abstract As the challenges posed by climate change and environmental stress intensify, there is an urgent need for advanced strategies to enhance the resilience of trees. This study comprehensively explores the latest developments in gene identification and their transformative applications in forestry science. The research traces the evolution of gene identification technologies from traditional methods to cutting-edge techniques like CRISPR-Cas9 and multi-omics integration, which have revolutionized our understanding of tree stress resistance. Case studies highlight the successful identification and application of stress-resistance genes, while critically assessing the environmental and ecological impacts of transgenic trees. Additionally, the study discusses the importance of collaboration and interdisciplinary research efforts in advancing tree genomics and outlines strategic directions for future research. This study emphasizes the potential of genetic technologies to contribute to sustainable forestry and ecosystem conservation in the face of global environmental challenges.

Keywords Tree stress resistance; Gene identification; CRISPR-Cas9; Genomic technologies; Sustainable forestry

1 Introduction

The increasing frequency and severity of environmental stressors due to climate change pose significant challenges to forest ecosystems worldwide. Trees, as the backbone of these ecosystems, are essential not only for maintaining biodiversity but also for mitigating climate change through carbon sequestration (Juarez et al., 2023). However, their survival and growth are increasingly threatened by various stress factors such as drought, pests, diseases, and temperature extremes. Enhancing tree stress resistance has therefore become a critical focus in forestry and ecological conservation efforts (Naidoo et al., 2019).

As climate change accelerates, the ability of trees to withstand and adapt to environmental stressors is of paramount importance. Stress-resistant trees contribute to the stability and resilience of forest ecosystems, which in turn supports the overall health of the planet. Enhancing tree stress resistance is not only crucial for preserving existing forests but also for ensuring the success of reforestation and afforestation projects aimed at combating climate change (Guevara-Escudero et al., 2021).

Recent advancements in gene identification technologies have opened new avenues for understanding and enhancing tree stress resistance. Techniques such as next-generation sequencing (NGS), genome-wide association studies (GWAS), and CRISPR-Cas9 gene editing have revolutionized the identification and manipulation of stress resistance genes in trees. These technologies allow for the precise mapping of genetic traits linked to stress resistance, enabling the development of more resilient tree species through targeted breeding and genetic engineering (Younessi-Hamzekhanlu and Gailing, 2022).

This study explores the cutting-edge applications of advanced gene identification technologies in forestry. By examining the latest research and developments, it aims to highlight how these technologies are pushing the boundaries of tree stress resistance. The study will also discuss the potential impacts of these innovations on sustainable forest management and climate change mitigation, offering insights into the future directions of this rapidly evolving field.

2 Advances in Gene Identification Technologies

2.1 Evolution of gene identification methods from traditional to modern techniques

Historically, gene identification in trees relied heavily on traditional methods such as phenotypic selection and classical breeding techniques. These approaches, while effective to some extent, were time-consuming and limited by the complex genetic architecture of trees. The advent of molecular markers, such as RFLP (Restriction Fragment Length Polymorphism) and SSR (Simple Sequence Repeats), marked a significant leap forward, allowing for the identification of specific regions of the genome associated with stress resistance traits.

As technology progressed, marker-assisted selection (MAS) became a critical tool in forestry, enabling more precise and faster breeding decisions. However, the true revolution in gene identification came with the development of high-throughput genomic technologies, which have dramatically accelerated the discovery of stress resistance genes. Genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping have become standard methods for linking genetic variation with stress resistance traits in trees, providing a more comprehensive understanding of the underlying genetics (Younessi-Hamzekhanlu and Gailing, 2022).

2.2 Current state-of-the-art technologies: genomic sequencing, CRISPR, and beyond

Today, the field of gene identification in trees is dominated by state-of-the-art technologies that offer unprecedented precision and efficiency. Next-generation sequencing (NGS) has revolutionized genomics by allowing for the rapid and cost-effective sequencing of entire tree genomes. This has enabled researchers to identify stress resistance genes at a much finer scale, facilitating the development of more resilient tree species.

CRISPR-Cas9 gene editing technology represents another transformative advancement. This technology allows for the precise modification of specific genes, enabling the direct manipulation of genetic pathways involved in stress resistance. CRISPR has been used to enhance traits such as drought tolerance and disease resistance in various tree species, offering a powerful tool for improving forest sustainability (Cao et al., 2022).

In addition to NGS and CRISPR, emerging technologies such as transcriptomics and epigenomics are expanding our understanding of gene expression and regulation in response to environmental stressors. These technologies allow for the exploration of how genes are turned on or off in response to stress, providing insights that are critical for developing trees that can better withstand the challenges posed by climate change (Naidoo et al., 2019).

2.3 Challenges and limitations in current gene identification methods

Despite the significant advances in gene identification technologies, several challenges and limitations remain. One of the primary challenges is the complex and often polygenic nature of stress resistance traits in trees. Unlike annual crops, trees have long lifecycles and large genomes, which complicate the identification of specific genes responsible for stress tolerance. Moreover, environmental factors can greatly influence gene expression, making it difficult to isolate genetic effects from environmental ones (Guevara-Escudero et al., 2021).

Another limitation is the current reliance on model species and limited genetic diversity in gene editing studies. Many CRISPR-based studies focus on a few model tree species, which may not fully capture the genetic diversity found in natural forest populations. This can limit the applicability of findings to other species or environments. Additionally, while CRISPR technology holds great promise, its application in forestry is still in its infancy, and large-scale field studies are needed to validate the effectiveness of edited genes under natural conditions (Polle et al., 2019).

3 Genetic Basis of Stress Resistance in Trees

3.1 Key genes involved in drought, cold, and salt resistance

Several key genes have been identified as playing crucial roles in enabling trees to tolerate drought, cold, and salt stresses. For instance, the ICE (Inducer of CBF Expression) gene family is known for its involvement in cold and drought tolerance. In *Malus baccata*, the gene *MbICE1* has been shown to enhance drought and cold resistance by regulating antioxidant capacity and stress-responsive genes (Duan et al., 2022). Similarly, the *SnRK1* (Sucrose

Non-Fermenting-1-Related Protein Kinase-1) gene in sweet potato (*Ipomoea batatas*) is involved in drought, salt, and cold tolerance through its role in activating stress-responsive pathways (Figure 1) (Ren et al., 2020).

Figure 1 Phenotypic performance and changes in fresh weight (FW) and dry weight (DW) of transgenic sweet potato, wild-type (WT), and empty vector control (VC) plants under different stress conditions (Adapted from Ren et al., 2020)

Image caption: The results indicate that under 86 mmol L^{-1} NaCl or 20% PEG 6000 stress, transgenic plants exhibited significant salt and drought tolerance, continuing to grow and form new leaves and roots, with FW and DW significantly higher than those of WT and VC plants. Under non-stress conditions, there were no significant differences in growth among the groups. This demonstrates that the overexpression of the IbSnRK1 gene significantly enhanced the stress resistance of sweet potato plants, particularly under salt and drought stress (Adapted from Ren et al., 2020)

Another important gene, *WRKY8*, a transcription factor in *Solanum lycopersicum*, plays a significant role in drought and salt stress tolerance by regulating stress-responsive gene expression and improving osmotic balance under stress conditions (Gao et al., 2019). Moreover, the *MaPIP2-7* gene, an aquaporin in banana (*Musa acuminata*), enhances drought, cold, and salt tolerance by improving water transport and maintaining osmotic balance under stress (Xu et al., 2019).

3.2 Mechanisms by which these genes confer stress resistance

The mechanisms by which these genes confer stress resistance in trees often involve complex pathways that regulate gene expression, cellular responses, and physiological adaptations. For example, *MbICE1* enhances stress tolerance by upregulating genes involved in the antioxidant defense system, thereby reducing oxidative damage caused by reactive oxygen species (ROS) during drought and cold stress (Duan et al., 2022).

Similarly, *SnRK1* regulates the ABA (abscisic acid) signaling pathway, which is critical for stomatal closure and osmotic adjustment under drought and salt stress conditions. By modulating these pathways, *SnRK1* helps maintain cellular homeostasis and water balance, which are vital for stress tolerance (Ren et al., 2020). The *WRKY8* transcription factor, on the other hand, activates stress-responsive genes that lead to the accumulation of osmoprotectants like proline and enhanced antioxidant enzyme activities, thereby mitigating the effects of drought and salt stress (Gao et al., 2019).

MaPIP2-7 functions by facilitating water transport across cell membranes, which is crucial for maintaining cell turgor and avoiding dehydration during drought and salt stress. The increased expression of this gene leads to enhanced water retention, reduced membrane injury, and improved overall stress tolerance in the transgenic plants (Xu et al., 2019).

3.3 Comparative analysis of stress resistance genes across different tree species

The comparative analysis of stress resistance genes across different tree species reveals both conserved and unique strategies employed by trees to combat environmental stresses. Genes like *ICE1* and *SnRK1* are found in multiple species, each contributing to stress tolerance through similar yet species-specific mechanisms. For example, while *ICE1* in *Malus baccata* regulates cold and drought tolerance through antioxidant pathways, a similar role is observed in *Cryptomeria fortunei*, where *CfICE1* enhances cold, drought, and salt resistance by regulating the CBF (C-repeat binding factor) gene pathway (Zhu et al., 2022).

Furthermore, the expression of genes such as *MaPIP2-7* and *WRKY8* in different species underscores the shared molecular pathways in stress resistance, albeit with variations in gene expression levels and specific physiological responses. The identification of such genes across diverse tree species offers valuable insights into the universal and adaptive mechanisms that trees have evolved to survive under adverse environmental conditions.

4 Case Studies: Successful Identification and Application of Stress Resistance Genes

4.1 Detailed examination of specific gene identification studies

A notable example of successful gene identification is the work done on the tree species *Dalbergia sissoo*, where researchers identified resistance gene analogs (RGAs) of the NBS-LRR family through transcriptome analysis. This study utilized advanced molecular and bioinformatics techniques to identify and characterize genes involved in resistance to dieback disease, a major threat to this species (Ijaz et al., 2022). Similarly, the identification of candidate genes in *Populus trichocarpa* through genome-wide association studies (GWAS) and QTL mapping has significantly contributed to understanding the genetic basis of disease resistance in this species (Younessi-Hamzekhanlu and Gailing, 2022).

Another critical study focused on *Casuarina equisetifolia*, a stress-tolerant forest species, where researchers assembled the genome to identify genes associated with secondary growth and stress tolerance. This high-quality genome assembly provided valuable insights into the molecular mechanisms underlying the tree's resilience to environmental stressors such as typhoons and drought (Ye et al., 2019).

4.2 Implementation of identified genes in breeding programs

The successful identification of stress resistance genes has paved the way for their implementation in breeding programs aimed at developing more resilient tree species. For instance, the integration of gene markers identified through GWAS and QTL mapping into marker-assisted selection (MAS) has significantly accelerated the breeding of disease-resistant forest trees. This approach allows for the selection of elite genotypes with enhanced resistance, thereby reducing the breeding cycle time and improving the efficiency of breeding programs (Figure 2) (Younessi-Hamzekhanlu and Gailing, 2022).

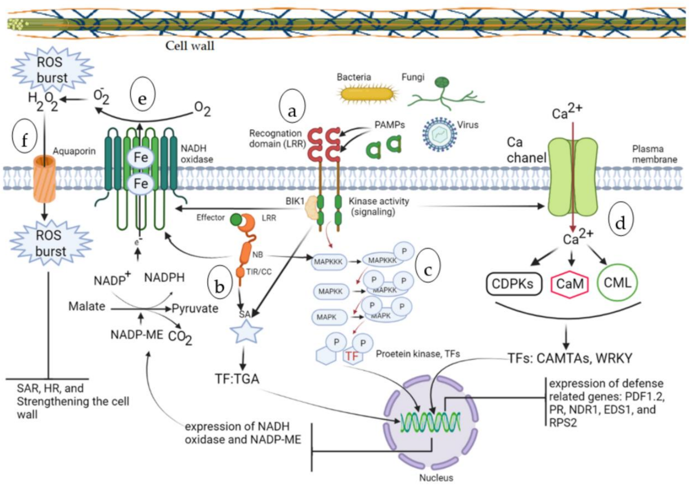

Figure 2 Perception of pathogens by trees, various signaling pathways, and corresponding defense mechanisms (Adapted from Younessi-Hamzekhanlu and Gailing, 2022)

Image caption: The figure illustrates how pattern recognition receptors (PRRs) and nucleotide-binding leucine-rich repeat receptors (NBS-LRRs) recognize pathogen-associated molecular patterns (PAMPs) and effectors, initiating PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). Additionally, the figure shows the downstream responses triggered by these signals, including the production of reactive oxygen species (ROS), activation of calcium signaling pathways, kinase cascade reactions, and the roles of plant hormones such as jasmonic acid and salicylic acid in disease resistance. These signaling pathways work together to enhance the resistance of trees to pathogens (Adapted from Younessi-Hamzekhanlu and Gailing, 2022)

In *Dalbergia sissoo*, the application of identified NBS-LRR gene analogs has been suggested for breeding programs aimed at enhancing resistance to dieback disease. The successful characterization of these genes offers a roadmap for future breeding efforts, ensuring the sustainability of this economically important species (Ijaz et al., 2022).

4.3 Evaluation of the field performance of genetically enhanced trees

The field performance of genetically enhanced trees is a critical aspect of evaluating the success of stress resistance gene applications. In *Casuarina equisetifolia*, the identified stress-tolerant genes have been tested under natural conditions, demonstrating enhanced resilience to environmental stressors. These field trials are essential for validating the effectiveness of genetically enhanced trees and ensuring that laboratory results translate into real-world benefits (Ye et al., 2019). Similarly, the overexpression of stress resistance genes in *Populus trichocarpa* has shown promising results in controlled environments. However, large-scale field trials are necessary to confirm the long-term viability and adaptability of these genetically enhanced trees under varying environmental conditions (Younessi-Hamzekhanlu and Gailing, 2022).

5 Integrative Approaches for Gene Identification and Functional Analysis

In the quest to enhance tree stress resistance, integrative approaches that combine various layers of biological data have become increasingly essential. By leveraging the power of genomic, transcriptomic, and proteomic data, researchers can achieve a more comprehensive understanding of the genes involved in stress response and their functional roles. This section explores the combination of these data types, the critical role of bioinformatics and computational biology in functional genomics, and the application of systems biology approaches to unravel complex stress response mechanisms in trees.

5.1 Combining genomic, transcriptomic, and proteomic data for comprehensive gene identification

The integration of genomic, transcriptomic, and proteomic data has revolutionized the field of gene identification in trees. Genomics provides the blueprint of an organism's DNA, revealing the potential coding sequences. Transcriptomics offers insights into the genes actively expressed under specific conditions, such as stress, by analyzing mRNA levels. Proteomics adds another layer by identifying and quantifying the proteins produced, which are the direct effectors of physiological functions.

For instance, in *Populus trichocarpa*, a genome-wide investigation and expression profiling of polyphenol oxidase (PPO) family genes combined genomic data with transcriptomic analysis to identify stress-responsive genes involved in both abiotic and biotic stress responses (He et al., 2021). Similarly, in *Casuarina equisetifolia*, the integration of genome sequencing with RNA-seq data enabled the identification of secondary growth and stress-tolerance genes, providing a comprehensive view of the tree's genetic response to environmental challenges (Ye et al., 2019).

5.2 Role of bioinformatics and computational biology in functional genomics

Bioinformatics and computational biology are indispensable tools in the functional analysis of stress resistance genes. These fields provide the methodologies and software necessary to process and analyze the vast amounts of data generated from genomic, transcriptomic, and proteomic studies. Through bioinformatics, researchers can predict gene function, identify regulatory elements, and model gene interaction networks.

For example, in the study of *Dalbergia sissoo*, bioinformatics tools were employed to predict the expressome contributing to dieback resistance, allowing researchers to characterize resistance gene analogs and their potential roles in disease resistance (Ijaz et al., 2022). Computational approaches have also been applied in systems biology for the simulation and analysis of complex biological systems, as demonstrated by studies in systems biology modeling and the simulation of antimicrobial resistance dynamics (Campos et al., 2019).

5.3 Examples of systems biology approaches in understanding stress response

Systems biology approaches integrate data from multiple biological levels-genomics, transcriptomics, proteomics, and metabolomics- to construct a holistic view of how trees respond to stress. These approaches enable the modeling of complex biological systems and the identification of key regulatory genes and pathways involved in stress response.

A prime example is the integration of dendroecology and association genetics in silver fir (*Abies alba*), where researchers combined dendrophenotypic data (growth responses archived in tree rings) with SNP genotyping to identify genes associated with resistance and resilience to environmental stressors (Heer et al., 2018). This systems biology approach allowed for the identification of genes that contribute to long-term stress adaptation, offering a more dynamic understanding of how trees respond to and recover from environmental challenges. Additionally, systems biology approaches have been applied in understanding plant responses to combined biotic and abiotic stresses, revealing the intricate molecular interactions that contribute to stress tolerance (Dangi et al., 2018).

Another example is the use of a comprehensive analysis in *Populus trichocarpa*, where the expression patterns of PPO genes were studied in response to various stresses, revealing their roles in regulating stress response at multiple levels (He et al., 2021). By integrating these data, researchers can better understand how trees manage stress at a systems level, leading to more targeted and effective strategies for enhancing stress resistance.

6 Biotechnological and Breeding Applications

6.1 Genetic engineering techniques for enhancing stress resistance

Genetic engineering has become a pivotal tool in enhancing the stress resistance of trees. Through the insertion, deletion, or modification of specific genes, researchers can develop trees that are better equipped to survive and thrive under adverse environmental conditions. One prominent technique is the overexpression of stress-related genes, such as transcription factors and enzymes involved in antioxidant defense.

For example, in *Populus trichocarpa*, genetic engineering has been used to overexpress polyphenol oxidase (PPO) genes, which play a crucial role in both abiotic and biotic stress resistance. These modifications have led to improved stress tolerance, as evidenced by enhanced resistance to drought and pest attacks (He et al., 2021). Similarly, the introduction of the *ICE1* gene in *Cryptomeria fortunei* has demonstrated significant improvements in cold, drought, and salt tolerance by regulating stress-responsive pathways (Zhu et al., 2022).

The use of transgenic approaches to introduce these and other stress resistance genes into tree genomes has opened new avenues for developing resilient forestry species. However, the long life cycles and large genomes of trees present unique challenges, necessitating ongoing research and development of more efficient genetic engineering methods.

6.2 Molecular breeding strategies involving newly identified genes

Molecular breeding, which incorporates marker-assisted selection (MAS) and genomic selection (GS), has greatly benefited from the identification of new stress resistance genes. These strategies enable the rapid selection of tree genotypes that possess desirable traits, such as enhanced resistance to drought, cold, or disease.

Marker-assisted selection has been particularly effective in species like *Dalbergia sissoo*, where researchers have utilized identified resistance gene analogs (RGAs) to select genotypes with improved resistance to dieback disease (Ijaz et al., 2022). Genomic selection, which leverages genome-wide markers to predict breeding values, has also been applied in *Populus trichocarpa*, where it has accelerated the breeding process by enabling the selection of disease-resistant individuals based on genetic markers (Younessi-Hamzekhanlu and Gailing, 2022). These molecular breeding strategies not only shorten the breeding cycle but also increase the accuracy of selecting stress-resistant traits, thereby enhancing the overall efficiency of tree improvement programs.

6.3 Future prospects for CRISPR and other genome editing tools in tree improvement

The advent of CRISPR-Cas9 and other genome editing tools has revolutionized the field of tree improvement by providing unprecedented precision in gene manipulation. These technologies allow for the targeted modification of specific genes, offering the potential to enhance stress resistance traits in trees with minimal off-target effects.

CRISPR has already been applied in several tree species to modify genes associated with stress response. For instance, in *Populus trichocarpa*, CRISPR has been used to knock out genes involved in the negative regulation of stress tolerance, leading to improved resistance to environmental stressors (Cao et al., 2022). The potential of CRISPR to rapidly introduce beneficial traits into tree genomes is immense, and future research is likely to focus on expanding its application to a broader range of species and stress-related traits.

Beyond CRISPR, other genome editing tools, such as base editors and prime editors, offer even greater precision and versatility. These tools can introduce specific nucleotide changes without creating double-strand breaks, reducing the risk of unintended genetic alterations. As these technologies continue to evolve, they are expected to play a critical role in the next generation of tree breeding programs.

7 Environmental and Ecological Implications

The development and deployment of tree species with enhanced stress resistance through advanced gene identification and biotechnological applications have significant environmental and ecological implications. As we push the boundaries of tree improvement technologies, it is crucial to consider the broader impacts on ecosystems, biodiversity, and ethical concerns.

7.1 Impact of enhanced stress resistance on ecosystem stability and biodiversity

Enhancing the stress resistance of trees can play a pivotal role in maintaining ecosystem stability, particularly in the face of climate change. Trees with improved resilience to drought, pests, diseases, and extreme weather events are better equipped to survive and sustain forest ecosystems. This resilience can help prevent the large-scale die-offs that destabilize ecosystems and disrupt the services they provide, such as carbon sequestration, soil stabilization, and water regulation (Przybylski et al., 2021).

However, the introduction of genetically enhanced trees may also have complex effects on biodiversity. While these trees can bolster ecosystem stability, there is a risk that they could outcompete native species, leading to reduced genetic diversity within forests. This reduction in diversity could make ecosystems more vulnerable to new threats, as a less diverse gene pool may limit the adaptive capacity of the ecosystem as a whole (Ahuja, 2021).

Furthermore, the widespread use of a limited number of genetically enhanced tree species could result in homogenization of forest ecosystems, potentially disrupting the intricate ecological relationships that sustain biodiversity. It is essential to carefully balance the benefits of enhanced stress resistance with the need to preserve the genetic diversity that underpins resilient and adaptable ecosystems.

7.2 Role of genetically modified trees in sustainable forestry and conservation

Genetically modified (GM) trees offer a powerful tool for sustainable forestry and conservation efforts. By incorporating traits such as improved growth rates, pest resistance, and tolerance to abiotic stresses, GM trees can contribute to more sustainable timber production, reducing the pressure on natural forests. This could help alleviate deforestation and forest degradation, which are major drivers of biodiversity loss and climate change.

In conservation, GM trees could be used to restore degraded ecosystems or to reintroduce species that have been lost due to environmental changes. For instance, trees engineered to withstand pests and diseases could be reintroduced into areas where these threats have decimated native populations, helping to restore ecosystem function and stability (Sozoniuk and Kowalczyk, 2022).

However, the deployment of GM trees in forestry and conservation raises important ecological and social considerations. The long-term ecological impacts of GM trees are not yet fully understood, particularly their potential interactions with native species and ecosystems. Additionally, the spread of transgenes into wild populations could have unpredictable consequences, potentially leading to the emergence of new traits that could alter ecosystem dynamics (Huang, 2019).

7.3 Ethical considerations in the use of gene technology in forestry

The use of gene technology in forestry raises several ethical considerations that must be carefully weighed. One of the primary concerns is the potential for unintended consequences, both ecological and social. The modification of tree genomes can have far-reaching impacts on ecosystems, and it is crucial to ensure that these technologies are applied responsibly and with a full understanding of their potential risks (Ajoykumar et al., 2021).

Another ethical consideration is the issue of consent and the rights of indigenous and local communities. Many forested areas are inhabited by communities that rely on natural forests for their livelihoods and cultural practices. The introduction of GM trees into these areas could disrupt traditional land uses and potentially lead to conflicts over land and resources. It is essential to engage these communities in decision-making processes and to respect their rights and knowledge (MacDonald et al., 2018).

There is also the question of the ownership and control of genetic resources. The development and commercialization of GM trees often involve patenting genetic material, which can lead to the monopolization of important resources by a few corporations. This raises concerns about the accessibility of these technologies and their benefits to small-scale farmers and foresters (Gautam et al., 2021).

8 Collaborative and Interdisciplinary Research Efforts

8.1 Importance of collaborative networks in advancing tree genomics research

Collaborative networks are crucial for advancing tree genomics research, particularly in the identification and application of stress resistance genes. The complexity of tree genomes, combined with the diverse environmental conditions that affect tree growth and resilience, necessitates the pooling of resources, expertise, and data from multiple research institutions across the globe.

By fostering collaboration among geneticists, ecologists, bioinformaticians, and forestry experts, these networks can accelerate the pace of discovery and innovation. Collaborative efforts allow for the sharing of advanced technologies such as high-throughput sequencing, CRISPR gene editing, and bioinformatics tools, which might be inaccessible to individual institutions due to high costs or technical barriers. Furthermore, these networks enable the exchange of genetic material and data across different geographic regions, enhancing the scope and applicability of research findings.

The establishment of global databases and repositories for tree genomic data is one example of how collaborative networks contribute to the advancement of research. These resources provide researchers with access to a wealth of genetic information, facilitating the identification of stress resistance genes across diverse tree species and environments.

8.2 Case studies of successful international collaborations

Several successful international collaborations have significantly advanced the field of tree genomics and stress resistance research. One notable example is the collaboration between European and North American researchers in the study of *Fraxinus* species (ash trees) for resistance to the emerald ash borer (EAB). This collaboration led to the identification of candidate genes associated with EAB resistance across different *Fraxinus* species, providing a foundation for breeding programs aimed at protecting these vital trees from this invasive pest (Kelly et al., 2019).

Another example is the international effort to sequence the genome of *Casuarina equisetifolia*, a stress-tolerant tree species widely cultivated in coastal regions of Australasia. This project brought together researchers from several countries, combining their expertise in genomics, bioinformatics, and forestry to produce a high-quality genome assembly. The insights gained from this collaboration have advanced our understanding of the genetic basis of stress tolerance in trees and provided valuable resources for breeding programs (Ye et al., 2019).

8.3 Challenges and opportunities in global research initiatives

While global research initiatives offer significant opportunities, they also come with challenges that must be addressed to maximize their effectiveness. One of the primary challenges is coordinating research activities across different countries and institutions, each with its own priorities, resources, and regulatory frameworks. Effective communication and project management are essential to ensure that collaborative efforts are aligned and that resources are used efficiently.

Another challenge is the equitable sharing of data, resources, and benefits among all participants. Ensuring that all collaborators, particularly those from developing countries, have access to the tools, data, and outcomes of research is crucial for fostering a truly global research community. Intellectual property rights and data ownership can also pose challenges, requiring clear agreements and ethical guidelines to prevent conflicts and ensure fair access.

Despite these challenges, global research initiatives present significant opportunities to address some of the most pressing issues in tree genomics and forestry. By pooling resources and expertise, researchers can tackle large-scale projects that would be impossible for individual institutions to undertake alone. Additionally, global collaborations can enhance the diversity of research by incorporating a wider range of environmental conditions and tree species, leading to more robust and generalizable findings.

9 Future Research Directions

9.1 Emerging trends in gene identification and functional verification

One of the key emerging trends in gene identification is the increasing use of multi-omics approaches that integrate genomics, transcriptomics, proteomics, and metabolomics data. This holistic approach allows for a more comprehensive understanding of the complex networks that govern stress resistance in trees. By analyzing multiple layers of biological data, researchers can identify key regulatory genes and pathways that are crucial for stress tolerance and verify their function in a more systematic and detailed manner (Sniezko et al., 2023).

Additionally, the application of high-throughput phenotyping platforms is becoming more prevalent in tree genomics. These platforms enable the rapid and precise measurement of phenotypic traits under various stress conditions, providing valuable data that can be linked to genetic information. This trend is expected to accelerate the identification of stress-resistant traits and their associated genes, thereby enhancing the efficiency of breeding programs.

Another emerging trend is the use of machine learning and artificial intelligence (AI) to predict gene function and interactions. By analyzing large datasets generated from multi-omics studies, AI can help identify patterns and predict the roles of genes in stress resistance. This computational approach is expected to complement traditional experimental methods, making gene identification and functional verification faster and more accurate.

9.2 Potential for new technologies to revolutionize tree stress resistance research

Several new technologies hold the potential to revolutionize tree stress resistance research. CRISPR-Cas9 and other genome editing tools have already demonstrated their capacity to precisely modify genes associated with stress tolerance. The ongoing development of more advanced genome editing techniques, such as base editors and prime editors, promises even greater precision and versatility. These tools could enable the creation of trees with tailor-made resistance to specific stressors, offering a powerful solution to the challenges posed by climate change (Metheringham et al., 2022).

Single-cell sequencing is another technology that is poised to make a significant impact on tree genomics. By analyzing the genetic material of individual cells, researchers can gain insights into the cellular heterogeneity and dynamics that contribute to stress resistance. This approach allows for the identification of specific cell types or tissues that play key roles in the response to environmental stress, providing new targets for genetic improvement (Schrieber et al., 2020).

Moreover, advancements in synthetic biology offer exciting possibilities for engineering novel stress resistance traits in trees. By designing and constructing synthetic gene networks, researchers can create entirely new pathways that enhance a tree's ability to cope with environmental stressors. This approach could lead to the development of trees with unprecedented levels of stress tolerance, opening up new avenues for sustainable forestry.

9.3 Recommendations for navigating the future challenges in tree genomics

As tree genomics continues to advance, several challenges must be navigated to fully realize the potential of new technologies and research trends. One of the primary challenges is the need for greater computational power and data management capabilities. The integration of multi-omics data, the application of AI, and the analysis of single-cell sequencing results generate vast amounts of data that require sophisticated computational tools and infrastructure. Investing in these resources is essential to keep pace with the growing complexity of tree genomics research (Grattapaglia et al., 2018).

Another challenge is the need for robust ethical frameworks to guide the application of advanced genetic technologies in forestry. As genome editing and synthetic biology become more widespread, it is crucial to address the ethical implications of these technologies, particularly concerning biodiversity conservation, ecosystem balance, and the rights of indigenous and local communities. Establishing clear ethical guidelines and engaging with a broad range of stakeholders will be vital in ensuring that the benefits of these technologies are realized in a socially responsible manner.

Furthermore, collaboration across disciplines and international borders will be increasingly important as tree genomics research becomes more complex and interconnected. Building strong networks that facilitate the exchange of knowledge, data, and resources will be critical to overcoming the challenges associated with large-scale, global research initiatives.

10 Concluding Remarks

Recent advancements in tree stress resistance gene identification have been remarkable, particularly in the integration of multi-omics approaches and the application of high-throughput technologies. The combination of genomic, transcriptomic, proteomic, and metabolomic data has provided a comprehensive understanding of the complex genetic networks that govern stress resistance in trees. Technologies such as next-generation sequencing, CRISPR-Cas9, and single-cell sequencing have enabled precise identification and manipulation of stress resistance genes, leading to the development of trees with enhanced resilience to environmental challenges.

Moreover, the use of advanced computational tools, including machine learning and AI, has accelerated the identification of key genes and their functional roles in stress response. These tools have not only increased the efficiency of gene identification but have also opened new avenues for predicting gene interactions and responses under various stress conditions.

The innovative applications of tree stress resistance gene identification technologies have significantly contributed to the field of forestry science. Genetic engineering and molecular breeding strategies have enabled the development of tree species that are better equipped to withstand the impacts of climate change, pests, diseases, and other environmental stressors. These advancements have the potential to improve the sustainability of timber production, reduce deforestation, and enhance ecosystem resilience.

Furthermore, the integration of these technologies into forestry practices has facilitated more targeted and efficient breeding programs, reducing the time and resources required to develop stress-resistant tree varieties. The application of genome editing tools, such as CRISPR, has further expanded the possibilities for customizing tree traits to meet specific environmental and economic needs. Innovative applications have also contributed to conservation efforts by enabling the restoration of degraded ecosystems and the protection of endangered tree species. By enhancing the stress resistance of trees, these technologies support the preservation of biodiversity and the maintenance of healthy, functioning ecosystems.

Looking forward, strategic directions for future research and implementation should focus on several key areas. First, there is a need to continue advancing the integration of multi-omics data and the development of more sophisticated computational tools to better understand the complex genetic and epigenetic mechanisms underlying stress resistance in trees. Expanding the use of single-cell and spatial genomics technologies will be crucial for unraveling the specific cellular responses that contribute to overall tree resilience.

Second, the ethical implications of genetic engineering and genome editing in forestry must be carefully considered. Developing clear guidelines and engaging with a diverse range of stakeholders, including indigenous communities and conservationists, will be essential to ensure that these technologies are applied responsibly and equitably.

Third, collaboration across disciplines and international borders should be strengthened to facilitate the sharing of knowledge, data, and resources. Building global networks that connect researchers, policymakers, and industry stakeholders will be key to addressing the challenges of climate change and environmental degradation on a global scale.

Finally, future research should focus on translating the advances in gene identification and engineering into practical applications that can be implemented in real-world forestry and conservation efforts. This includes conducting large-scale field trials to validate the effectiveness of genetically enhanced trees under natural conditions and exploring the potential for integrating these technologies into sustainable forest management practices.

The advancements in tree stress resistance gene identification and the innovative applications that have emerged from this research hold great promise for the future of forestry science. By continuing to push the limits of what is possible, we can develop more resilient and sustainable forest ecosystems that will thrive in the face of environmental challenges for generations to come.

Acknowledgments

The author expresses gratitude to the two anonymous peer reviewers for their feedback.

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

Ahuja M., 2021, Fate of forest tree biotechnology facing climate change, Silvae Genetica, 70: 117-136.
https://doi.org/10.2478/sg-2021-0010

Ajoykumar K.N., A., Singh G., and Shackira A.M., 2021, Socio-ethical aspect of genetically modified organisms: a critical analysis, In: Singh P., Borthakur A., Singh A.A., Kumar A., and Singh K.K. (eds.), Policy issues in genetically modified crops, a global perspective, Academic Press, New York, USA, pp.421-450.
https://doi.org/10.1016/B978-0-12-820780-2.00019-4

Campos M., Capilla R., Naya F., Futami R., Coque T., Moya A., Fernandez-Lanza V., Cantón R., Sempere J., Lloréns C., and Baquero F., 2018, Simulating multilevel dynamics of antimicrobial resistance in a membrane computing model, mBio, 10: e02460-18.
https://doi.org/10.1128/mBio.02460-18
PMid:30696743 PMCid:PMC6355984

Cao H., Vu G., and Gailing O., 2022, From genome sequencing to CRISPR-based genome editing for climate-resilient forest trees, International Journal of Molecular Sciences, 23(2): 966.
https://doi.org/10.3390/ijms23020966
PMid:35055150 PMCid:PMC8780650

Dangi A., Sharma B., Khangwal I., and Shukla P., 2018, Combinatorial interactions of biotic and abiotic stresses in plants and their molecular mechanisms: systems biology approach, Molecular Biotechnology, 60: 636-650.
https://doi.org/10.1007/s12033-018-0100-9
PMid:29943149

Duan Y., Han J., Guo B., Zhao W., Zhou S., zhou C., Zhang L., Li X., and Han D., 2022, MbICE1 confers drought and cold tolerance through up-regulating antioxidant capacity and stress-resistant genes in *Arabidopsis thaliana*, International Journal of Molecular Sciences, 23(24): 16072.
https://doi.org/10.3390/ijms232416072
PMid:36555710 PMCid:PMC9783906

Gao Y., Liu J., Yang F., Zhang G., Wang D., Zhang L., Ou Y., and Yao Y., 2019, The WRKY transcription factor WRKY8 promotes resistance to pathogen infection and mediates drought and salt stress tolerance in *Solanum lycopersicum*, Physiologia Plantarum, 168(1): 98-117.
https://doi.org/10.1111/ppl.12978
PMid:31017672

Gautam S., Timilsina S., and Shrestha M., 2021, The effects of forest management activities on genetic diversity of forest trees, Indonesian Journal of Social and Environmental Issues (IJSEI), 2(2): 110-118.
https://doi.org/10.47540/ijsei.v2i2.211

Grattapaglia D., Silva-Junior O., Resende R., Cappa E., Müller B., Tan B., Isik F., Ratcliffe B., and El-Kassaby Y., 2018, Quantitative genetics and genomics converge to accelerate forest tree breeding, Frontiers in Plant Science, 9: 1693.
https://doi.org/10.3389/fpls.2018.01693
PMid:30524463 PMCid:PMC6262028

Guevara-Escudero M., Osorio A., and Cortés A., 2021, Integrative pre-breeding for biotic resistance in forest trees, Plants, 10(10): 2022.
https://doi.org/10.3390/plants10102022
PMid:34685832 PMCid:PMC8541610

He F., Shi Y., Zhao Q., Zhao K., Cui X., Chen L., Yang H., Zhang F., Mi J., Huang J., and Wan X., 2021, Genome-wide investigation and expression profiling of polyphenol oxidase (PPO) family genes uncover likely functions in organ development and stress responses in *Populus trichocarpa*, BMC Genomics, 22: 731.
https://doi.org/10.1186/s12864-021-08028-9
PMid:34625025 PMCid:PMC8501708

Heer K., Behringer D., Piermattei A., Piermattei A., Bässler C., Brandl R., Fady B., Jehl H., Liepelt S., Lorch S., Piotti A., Vendramin G., Weller M., Ziegenhagen B., Büntgen U., Büntgen U., and Opgenoorth L., 2018, Linking dendroecology and association genetics in natural populations: stress responses archived in tree rings associate with SNP genotypes in silver fir (*Abies alba* Mill.), Molecular Ecology, 27: 1428-1438.
https://doi.org/10.1111/mec.14538
PMid:29443422

Huang W., 2019, The impact of the genetically modified crop's value on the sustainability of an ecosystem's biomass, Applied Ecology and Environmental Research, 17(6): 14375-14390.
https://doi.org/10.15666/aeer/1706_1437514390

Ijaz S., Haq I., Khan I., Ali H., Kaur S., and Razzaq H., 2022, Identification of resistance gene analogs of the NBS-LRR family through transcriptome probing and in silico prediction of the expressome of *Dalbergia sissoo* under dieback disease stress, Frontiers in Genetics, 13: 1036029.
https://doi.org/10.3389/fgene.2022.1036029
PMid:36276980 PMCid:PMC9585183

Juarez J., Silva E., Silva N., Silva-Rocha R., and Guazzaroni M., 2023, Identification and functional analysis of novel stress-resistance genes from metagenomes of extreme environments, bioRxiv, (2023): 2023-06.
https://doi.org/10.1101/2023.06.07.544099

Kelly L., Plumb W., Carey D., Mason M., Cooper E., Crowther W., Whittemore A., Rossiter S., Koch J., and Buggs R., 2019, Genes for ash tree resistance to an insect pest identified via comparative genomics, bioRxiv, (2019): 772913.
https://doi.org/10.1101/772913

MacDonald C., Colombo S., and Arts M., 2018, Genetically engineered oil seed crops and novel terrestrial nutrients: ethical considerations, Science and Engineering Ethics, 25: 1485-1497.
https://doi.org/10.1007/s11948-018-0074-9
PMid:30465298

Metheringham C., Plumb W., Stocks J., Kelly L., Gorriz M., Moat J., Buggs R., and Nichols R., 2022, Rapid polygenic adaptation in a wild population of ash trees under a novel fungal epidemic, bioRxiv, (2022): 2022-08.
https://doi.org/10.1101/2022.08.01.502033

Naidoo S., Slippers B., Plett J., Coles D., and Oates C., 2019, The road to resistance in forest trees, Frontiers in Plant Science, 10: 273.
https://doi.org/10.3389/fpls.2019.00273
PMid:31001287 PMCid:PMC6455082

Polle A., Chen S., Eckert C., and Harfouche A., 2019, Engineering drought resistance in forest trees, Frontiers in Plant Science, 9: 1875.
https://doi.org/10.3389/fpls.2018.01875
PMid:30671067 PMCid:PMC6331418

Przybylski P., Mohytych V., Rutkowski P., Tereba A., Tyburski Ł., and Fyałkowska K., 2021, Relationships between some biodiversity indicators and crown damage of *Pinus sylvestris* L. in natural old growth pine forests, Sustainability, 13(3): 1239.
https://doi.org/10.3390/SU13031239

Ren Z., He S., Zhou Y., Zhao N., Jiang T., Zhai H., and Liu Q., 2020, A sucrose non-fermenting-1-related protein kinase-1 gene, IbSnRK1, confers salt, drought and cold tolerance in sweet potato, Crop Journal, 8: 905-917.
https://doi.org/10.1016/j.cj.2020.04.010

Schrieber K., Cáceres Y., Engelmann A., Marcora P., Renison D., Hensen I., and Müller C., 2020, Elevational differentiation in metabolic cold stress responses of an endemic mountain tree, Environmental and Experimental Botany, 171: 103918.
https://doi.org/10.1016/j.envexpbot.2019.103918

Sniezko R., Koch J., Liu J., and Romero-Severson J., 2023, Will genomic information facilitate forest tree breeding for disease and pest resistance, Forests, 14(12): 2382.
https://doi.org/10.3390/f14122382

Sozoniuk M., and Kowalczyk K., 2022, Genetically modified trees with improved abiotic stress resistance - recent accomplishments, a review, Agronomy Science, 77(3): 149-158.
https://doi.org/10.24326/as.2022.3.11

Xu Y., Hu W., Liu J., Song S., Hou X., Jia C., Li J., Miao H., Wang Z., Tie W., Xu B., and Jin Z., 2019, An aquaporin gene *MaPIP2-7* is involved in tolerance to drought, cold and salt stresses in transgenic banana (*Musa acuminata* L.), Plant Physiology and Biochemistry, 147: 66-76.
https://doi.org/10.1016/j.plaphy.2019.12.011
PMid:31841963

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

Younessi-Hamzekhanlu M., and Gailing O., 2022, Genome-wide SNP markers accelerate perennial forest tree breeding rate for disease resistance through marker-assisted and genome-wide selection, International Journal of Molecular Sciences, 23(20): 12315.
https://doi.org/10.3390/ijms232012315
PMid:36293169 PMCid:PMC9604372

Zhu L., Yang J., Zhang Y., Hu H., Cui J., Xue J., and Xu J., 2022, Overexpression of *CfICE1* from *Cryptomeria fortunei* enhances cold, drought and salt stress in poplar, International Journal of Molecular Sciences, 23(23): 15214.
https://doi.org/10.3390/ijms232315214
PMid:36499538 PMCid:PMC9736380