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Screening and Functional Verification of Poplar Salt Tolerance Genes

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Abstract Through transcriptome analysis and functional screening, several key genes were identified and verified for their roles in salt tolerance. Notably, the *PeERF1* gene from *Populus euphratica* was found to significantly enhance salt tolerance when overexpressed in *Populus alba* × *Populus glandulosa*. Similarly, the *NAC13* gene was shown to improve salt tolerance in transgenic poplar lines. Overexpression of the *PtVP1.1* gene in *Populus trichocarpa* also conferred increased salt tolerance by enhancing ion homeostasis and reactive oxygen species (ROS) scavenging. Additionally, the *PsnHDZ63* and *PsnMYB108* genes were identified as important regulators of salt stress responses, with their overexpression leading to improved salt tolerance in transgenic poplar and tobacco, respectively. The *PtSOS2* gene was another significant finding, with its overexpression resulting in enhanced salt tolerance through improved Na^+ efflux and ROS scavenging. The identification and functional verification of these genes provide valuable insights into the genetic basis of salt tolerance in poplar. These findings have significant implications for the development of salt-tolerant poplar varieties through genetic engineering, which could be beneficial for forestry and environmental management in saline-affected areas.

Keywords Poplar (*Populus* L.); Salt tolerance; Gene screening; *PeERF1*; *NAC13*; *PtVP1.1*; *PsnHDZ63*; Genetic engineering

1 Introduction

Poplar trees (genus *Populus*) are widely recognized for their rapid growth and adaptability, making them valuable for agroforestry and environmental management. Their ability to thrive in diverse environments, including saline soils, is crucial for maintaining productivity and ecological balance in areas affected by salinity. Enhancing salt tolerance in poplar not only supports sustainable forestry practices but also contributes to soil stabilization, carbon sequestration, and the reclamation of degraded lands (Ge et al., 2022; Gao et al., 2022).

Despite the ecological and economic importance of poplar, improving salt tolerance in woody plants remains a significant challenge. The complexity of salt stress responses, which involve multiple physiological and molecular pathways, complicates the identification and functional validation of key tolerance genes. Traditional breeding methods are often time-consuming and less effective due to the long generation times of trees. Moreover, the genetic basis of salt tolerance in poplar is not fully understood, necessitating advanced genomic and biotechnological approaches to uncover and manipulate the underlying mechanisms (Ezawa and Tada, 2009; Ge et al., 2022; Gao et al., 2022).

The primary objective of this study is to identify and functionally verify key genes that contribute to salt tolerance in poplar. By leveraging transcriptome analysis and functional screening techniques, we aim to uncover candidate genes that enhance salt tolerance. This research will focus on the isolation and characterization of these genes, followed by the generation of transgenic poplar lines to validate their roles in salt stress response. Ultimately, this study hopes to provide valuable genetic resources for the development of salt-tolerant poplar varieties, thereby supporting sustainable agroforestry and environmental management practices.

2 Background on Salt Stress in Poplar

2.1 Physiological impacts of salt stress on poplar trees

Salt stress is a significant abiotic factor that adversely affects the growth and productivity of poplar trees. High salinity conditions lead to osmotic stress, ion toxicity, and oxidative stress, which collectively impair various

physiological processes. For instance, salt stress can disrupt ion homeostasis by causing an excessive accumulation of sodium (Na^+) and chloride (Cl^-) ions in plant tissues, which in turn inhibits the uptake of essential nutrients like potassium (K^+) and calcium (Ca^{2+}) (Yoon et al., 2018; Zhang et al., 2019b). This ionic imbalance can lead to reduced photosynthetic efficiency, stunted growth, and even cell death. Additionally, salt stress induces the production of reactive oxygen species (ROS), which can damage cellular components such as lipids, proteins, and nucleic acids (Guo et al., 2019; Ge et al., 2022). To mitigate these effects, poplar trees activate antioxidant enzymes like superoxide dismutase (SOD) and peroxidase (POD) to scavenge ROS and protect cellular integrity (Guo et al., 2019; Gao et al., 2022).

2.2 Overview of salt tolerance mechanisms in plants

Plants have evolved a variety of mechanisms to cope with salt stress, which can be broadly categorized into ion homeostasis, osmotic adjustment, and ROS scavenging. Ion homeostasis involves the regulation of ion transporters and channels to maintain a balance between Na+, K+, and Ca2+ ions. Key players in this process include the salt overly sensitive (SOS) pathway, which helps in the extrusion of Na+ from the cytoplasm and its sequestration into vacuoles (Yoon et al., 2018; Zhang et al., 2019b). Osmotic adjustment is achieved through the accumulation of compatible solutes such as proline, glycine betaine, and sugars, which help to maintain cell turgor and protect cellular structures (Guo et al., 2019; Ge et al., 2022). ROS scavenging mechanisms involve the activation of antioxidant enzymes and the synthesis of non-enzymatic antioxidants to neutralize ROS and prevent oxidative damage (Guo et al., 2019; Gao et al., 2022). Additionally, transcription factors like DREB and NAC play crucial roles in regulating the expression of stress-responsive genes, thereby enhancing the plant's ability to withstand salt stress (Guo et al., 2019; Zhang et al., 2019a).

2.3 Historical approaches to studying salt tolerance in poplar

Research on salt tolerance in poplar has a rich history, with early studies focusing on the physiological responses of different poplar species to salinity. Over time, advancements in molecular biology and genomics have enabled more detailed investigations into the genetic and molecular bases of salt tolerance. Transcriptome analyses have been instrumental in identifying key regulatory genes and pathways involved in salt stress responses (Zhang et al., 2019b; Ge et al., 2022). For example, the *PeERF1* gene from *Populus euphratica* has been shown to enhance salt tolerance when overexpressed in transgenic poplar lines (Ge et al., 2022). Similarly, downregulation of the PagSAP1 gene in *Populus alba* × *P. glandulosa* has been found to increase salt tolerance by improving ionic homeostasis and stress-responsive gene expression (Yoon et al., 2018). Recent studies have also explored the role of specific genes like *NAC13* and PtGSTF1 in conferring salt tolerance through mechanisms such as ROS scavenging and cell wall modification (Zhang et al., 2019a; Gao et al., 2022). These historical approaches have laid the foundation for current and future research aimed at developing salt-tolerant poplar varieties through genetic engineering and breeding programs.

3 Gene Screening Techniques

3.1 Advanced genomic tools used in salt tolerance gene screening

Advanced genomic tools have significantly enhanced the efficiency and accuracy of identifying salt tolerance genes in poplar. One such tool is the Weighted Gene Co-expression Network Analysis (WGCNA), which was used in combination with Genome-Wide Association Studies (GWAS) to identify key regulatory factors associated with salt resistance in *Populus euphratica*. This approach led to the discovery of the *PeERF1* gene, which showed significant differences in expression levels under salt stress conditions (Ge et al., 2022). Another powerful tool is RNA sequencing (RNA-seq), which has been employed to profile the expression patterns of various genes under salt stress. For instance, RNA-seq analysis identified 63 HD-Zip transcription factors in poplar, with *PsnHDZ63* being significantly up-regulated under salt stress (Guo et al., 2021). Additionally, transcriptomic and metabolomic analyses have been used to understand the molecular mechanisms underlying salt tolerance. For example, the transcriptomic analysis of transgenic Arabidopsis expressing glycine-rich RNA-binding proteins from Sporobolus virginicus revealed upregulation of stress-related pathways, providing insights into the roles of these proteins in salt tolerance (Tada et al., 2019).

3.2 Criteria and methods for selecting candidate genes

The selection of candidate genes for salt tolerance involves several criteria and methods. One primary criterion is the differential expression of genes under salt stress conditions. For example, the *NAC13* gene was selected based on its significant up-regulation in response to salt stress in poplar (Zhang et al., 2019a). Another criterion is the functional validation of candidate genes through overexpression and suppression studies. The *PeERF1* gene was functionally verified by transforming it into *Populus alba* × *Populus glandulosa*, where overexpressed plants exhibited better growth and physiological characteristics under salt stress compared to wild-type plants (Ge et al., 2022). Additionally, the physiological and biochemical responses of transgenic plants are evaluated to confirm the role of candidate genes in salt tolerance. For instance, transgenic poplar overexpressing PtGSTF1 showed improved shoot growth, wood formation, and optimized ion homeostasis under salt stress, confirming its role in enhancing salt tolerance (Figure 1) (Gao et al., 2022).

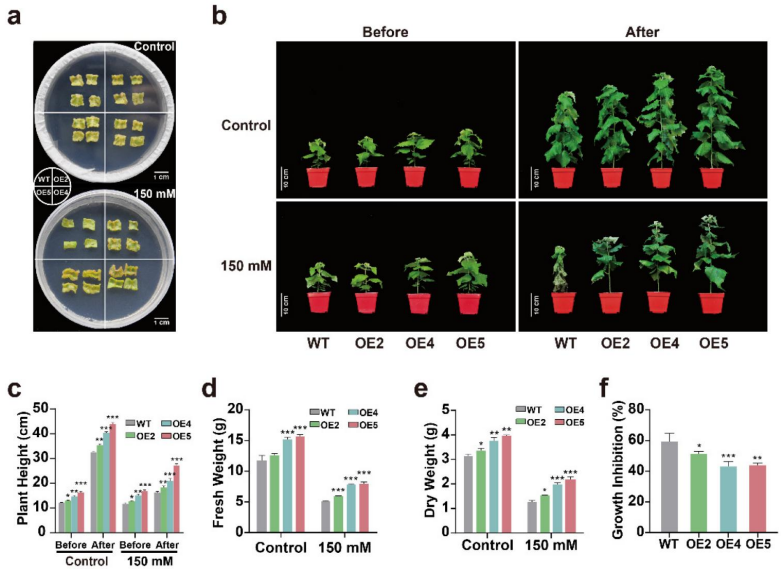

Figure 1 Overexpression of PtGSTF1 enhanced salt tolerance in transgenic plants (Adopted from Gao et al., 2022)

Imagine caption: (a) Callus induction analysis. Leaf explants from four-week-old wild type (WT) and PtGSTF1 transgenic plants were cultured on callus induction medium supplemented with 0 (control) or 150 mM NaCl for 1 week. (b) Growth phenotypes of WT and transgenic plants. Four-week-old plants grown in a greenhouse were treated with 0 (control) or 150 mM NaCl for another 4 weeks. (c–f) Plant heights, fresh and dry weights, and growth inhibitions before and after the salt treatment. WT, wild type; OE2, OE4 and OE5, different transgenic lines. Values are the mean ± SD from three independent experiments (n = 3). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (Adopted from Gao et al., 2022)

Gao et al. (2022) illustrates the impact of PtGSTF1 overexpression on salt tolerance in transgenic poplar plants compared to wild type (WT). Panel (a) shows callus formation in WT and transgenic leaf explants under normal and 150 mM NaCl conditions, highlighting better callus induction in transgenic lines under salt stress. Panel (b) displays the growth phenotypes of WT and transgenic plants before and after 150 mM NaCl treatment, with transgenic lines (OE2, OE4, OE5) exhibiting superior growth and less severe damage. Panels (c-f) quantify plant height, fresh weight, dry weight, and growth inhibition, respectively. The data reveal that transgenic lines significantly outperform WT in all measured traits under both normal and salt stress conditions, demonstrating that PtGSTF1 overexpression enhances salt tolerance in poplar plants.

3.3 Case studies: successful identification of salt tolerance genes in poplar

Several case studies highlight the successful identification and functional verification of salt tolerance genes in poplar. One notable example is the *PeERF1* gene from *Populus euphratica*, which was identified using a combination of WGCNA and GWAS. Transgenic *Populus alba* × *Populus glandulosa* plants overexpressing *PeERF1* exhibited enhanced salt tolerance, demonstrating the gene's potential for improving salt tolerance in poplar breeding (Ge et al., 2022). Another example is the *PsnHDZ63* gene, identified through RNA-seq analysis. Transgenic *Populus simonii* × *P. nigra* plants overexpressing *PsnHDZ63* displayed better morphological and

physiological indexes under salt stress, highlighting the gene's role in salt tolerance (Guo et al., 2021). Additionally, the *NAC13* gene was cloned and functionally characterized in poplar, where overexpression of *NAC13* significantly enhanced salt tolerance, while suppression of the gene increased salt sensitivity (Zhang et al., 2019a). These case studies underscore the importance of advanced genomic tools and rigorous selection criteria in identifying and validating salt tolerance genes in poplar.

4 Functional Verification of Identified Genes

4.1 Experimental strategies for gene function testing

To verify the function of identified salt tolerance genes in poplar, various experimental strategies were employed. One common approach involved the cloning and overexpression of target genes in transgenic poplar lines. For instance, the *NAC13* gene was cloned and overexpressed in *Populus alba* × *P. glandulosa*, resulting in enhanced salt tolerance compared to wild-type plants (Zhang et al., 2019a). Similarly, the *PeERF1* gene was overexpressed in *Populus alba* × *P. glandulosa*, leading to improved growth and physiological characteristics under salt stress (Ge et al., 2022). Another strategy included the use of RNA interference (RNAi) to suppress gene expression, as demonstrated with the *PagWRKY75* gene, where RNAi lines exhibited increased salt tolerance (Zhao et al., 2019).

4.2 Techniques for gene silencing and overexpression

Gene silencing and overexpression techniques are crucial for functional verification. Overexpression constructs typically involve the use of strong promoters such as CaMV35S to drive high levels of gene expression. For example, the *NAC13* gene was overexpressed using the CaMV35S promoter, resulting in significant salt tolerance in transgenic poplar (Zhang et al., 2019a). Conversely, gene silencing often employs RNAi or antisense suppression constructs. The *PagSAP1* gene was downregulated using RNAi, which increased salt tolerance in poplar by altering ionic homeostasis (Yoon et al., 2018). Additionally, virus-induced gene silencing (VIGS) was used to study the function of FcWRKY40 in salt tolerance, where silenced lines showed increased salt susceptibility (Dai et al., 2018).

4.3 Integration of physiological and molecular data to confirm gene function

The integration of physiological and molecular data is essential to confirm the function of identified genes. Physiological assays often include measurements of growth parameters, ion content, and stress-related enzyme activities. For instance, overexpression of PagERF072 in poplar led to increased activities of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT), along with reduced reactive oxygen species (ROS) levels under salt stress (Zhang et al., 2022). Molecular analyses, such as transcriptome profiling, provide insights into the regulatory networks and pathways affected by gene manipulation. Transcriptome analysis of *PeERF1* overexpressed lines revealed enrichment of stress response-related genes, supporting its role in salt tolerance (Ge et al., 2022). Similarly, the overexpression of PtGSTF1 in poplar was associated with upregulation of genes involved in ion homeostasis and ROS scavenging, corroborating its function in enhancing salt tolerance (Gao et al., 2022).

5 Case Studies: Functional Analysis of Salt Tolerance Genes

5.1 Detailed examination of specific salt tolerance genes in poplar

Several genes have been identified and characterized for their roles in enhancing salt tolerance in poplar species. For instance, the HD-Zip transcription factor family, particularly *PsnHDZ63*, has been shown to significantly improve salt tolerance when overexpressed in *Populus simonii* × *P. nigra*. This gene enhances reactive oxygen species (ROS) scavenging ability, which is crucial for mitigating salt-induced oxidative stress (Guo et al., 2021). Another key gene, *PeERF1*, identified in *Populus euphratica*, has been demonstrated to enhance salt tolerance in transgenic *Populus alba* × *Populus glandulosa* by regulating stress-related genes and improving antioxidant enzyme activity (Ge et al., 2022).

The *NAC13* gene, a transcription factor, has also been found to play a vital role in salt stress response. Overexpression of *NAC13* in *Populus alba* × *P. glandulosa* significantly enhances salt tolerance, while its suppression increases sensitivity to salt stress (Zhang et al., 2019a). Additionally, the *PalERF109* gene in *Populus alba* var. pyramidalis has been shown to enhance salt tolerance by upregulating the high-affinity K+ transporter

gene *PalHKT1;2*, which is involved in ion transport and homeostasis (Chen et al., 2020). Lastly, the *PtGSTF1* gene, encoding a Glutathione S-transferase, improves both biomass production and salt tolerance in *Populus trichocarpa* by regulating xylem cell proliferation, ion homeostasis, and ROS scavenging (Gao et al., 2022).

5.2 Outcomes of functional verification experiments

Functional verification experiments have provided substantial evidence supporting the roles of these genes in salt tolerance. For example, transgenic *Populus simonii* × *P. nigra* plants overexpressing *PsnHDZ63* displayed better morphological and physiological indexes under salt stress compared to wild-type plants, indicating enhanced salt tolerance (Guo et al., 2021). Similarly, *PeERF1* overexpression in *Populus alba* × *Populus glandulosa* resulted in improved growth and physiological characteristics under salt stress, further validating its role in salt tolerance (Figure 2) (Ge et al., 2022).

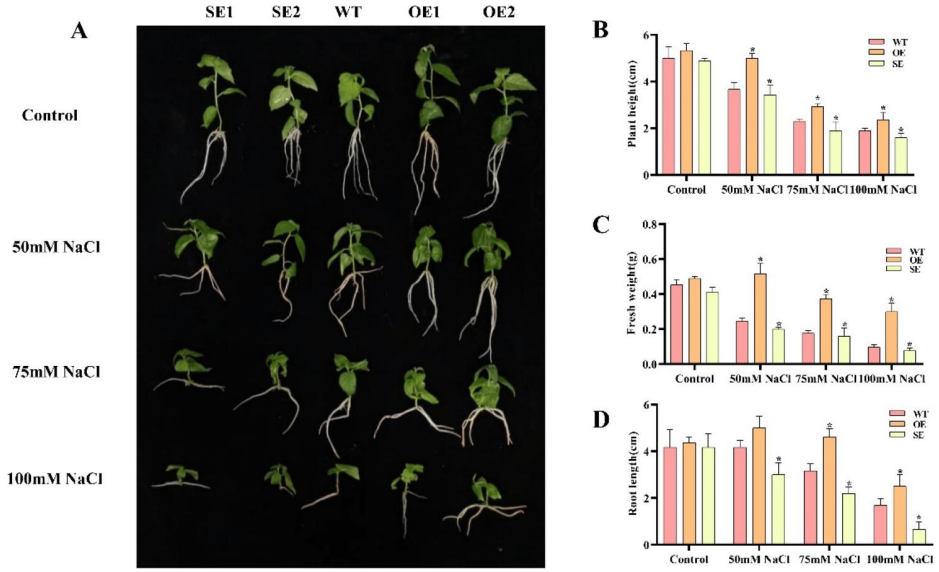

Figure 2 Transgenic *P. alba* × *P. glandulosa* morphological traits under salt stress (Adopted from Ge et al., 2022)

Imagine caption: SE1, SE2, OE1, OE2: various transgenic poplar lines; WT, wild type poplar. (A-D) 1-month-old *P. alba* × *P. glandulosa* phenotypes on 0, 50, 75, and 100 mM NaCl rooting media. Under salt stress, the height, root length, and fresh weight were measured in transgenic and WT. The standard deviation is shown by the error bar. The presence of an asterisk implies that there is a substantial difference between transgenic and WT (t-test, * $p < 0.05$) (Adopted from Ge et al., 2022)

Ge et al. (2022) demonstrates the impact of salt stress on transgenic *P. alba* × *P. glandulosa* lines compared to wild type (WT). Panel (A) shows the phenotypes of transgenic and WT poplars under different NaCl concentrations (0, 50, 75, 100 mM). Panels (B), (C), and (D) illustrate the measurements of plant height, fresh weight, and root length, respectively. The data reveal that the 35S: *PeERF1* transgenic lines exhibit superior growth under salt stress compared to WT, showing significant increases in height, weight, and root length. Conversely, the 35S: SRDX-*PeERF1* transgenic lines perform worse than WT under the same conditions. This indicates that overexpression of *PeERF1* enhances salt tolerance, while SRDX-*PeERF1* reduces it. The error bars and asterisks indicate statistical significance ($p < 0.05$), highlighting the reliability of the results. This research underscores the potential of genetic modifications in improving salt stress tolerance in poplars.

In the case of *NAC13*, overexpression in *Populus alba* × *P. glandulosa* led to significant enhancement in salt tolerance, while antisense suppression resulted in increased sensitivity to salt stress, highlighting the gene's critical function in stress response (Zhang et al., 2019a). Overexpression of PalERF109 in *Populus alba* var. pyramidalis also confirmed its role in enhancing salt tolerance through the upregulation of PalHKT1;2 (Chen et al., 2020). Furthermore, transgenic *Populus trichocarpa* plants overexpressing *PtGSTF1* showed improved shoot growth, wood formation, and salt tolerance, demonstrating the gene's multifaceted role in stress adaptation (Gao et al., 2022).

5.3 Comparative analysis with other tree species exhibiting salt tolerance

Comparative analysis with other tree species reveals that similar mechanisms are employed across different species to confer salt tolerance. For instance, the glycine-rich RNA-binding proteins (GRPs) identified in the halophyte Sporobolus virginicus have been shown to enhance salt tolerance in transgenic Arabidopsis, suggesting a conserved role of GRPs in salt stress response across different plant species (Tada et al., 2019). Additionally, the downregulation of stress-associated protein 1 (*PagSAP1*) in *Populus alba* × *P. glandulosa* increases salt tolerance by maintaining cellular ionic homeostasis, a mechanism also observed in other salt-tolerant species (Yoon et al., 2018).

The WRKY transcription factor family, including PagWRKY75 and PalWRKY77, has been shown to play significant roles in salt and osmotic stress responses in poplar, with similar regulatory functions observed in other plant species (Zhao et al., 2019; Jiang et al., 2020). These findings indicate that while specific genes and their regulatory networks may vary, the underlying mechanisms of salt tolerance, such as ion homeostasis, ROS scavenging, and stress-responsive gene regulation, are conserved across different tree species.

6 Technological Advances in Genetic Engineering

6.1 Role of CRISPR-Cas9 and other genome editing tools in enhancing salt tolerance

CRISPR-Cas9 has revolutionized the field of genetic engineering by providing a precise and efficient method for genome editing. This technology allows for the targeted modification of specific genes, which is crucial for enhancing salt tolerance in plants. For instance, CRISPR-Cas9 has been successfully used to edit genes in rice, leading to improved salt stress tolerance by targeting key molecular pathways involved in salt stress response (Farhat et al., 2019; Tran et al., 2020; Nazir et al., 2022). In poplar, CRISPR-Cas9 has been applied to create targeted mutations, demonstrating its potential in woody plants as well (Wang et al., 2021). The ability to perform multiplexed editing, as shown in tomato, further highlights the versatility of CRISPR-Cas9 in addressing complex traits like salt tolerance (Nazir et al., 2022).

6.2 Advances in transgenic approaches and their application in poplar

Transgenic approaches have also made significant strides in enhancing salt tolerance in poplar. Overexpression of specific genes, such as the H+-pyrophosphatase gene *PtVP1.1*, has been shown to confer salt tolerance by improving ion homeostasis and reactive oxygen species scavenging (Kerek et al., 2021). Similarly, the overexpression of the HD-Zip transcription factor *PsnHDZ63* in *Populus simonii* × *P. nigra* has resulted in better morphological and physiological responses under salt stress (Chan et al., 2022). These advances demonstrate the potential of transgenic approaches in developing salt-tolerant poplar varieties by manipulating key genes involved in stress response pathways.

6.3 Challenges and future prospects for genetic engineering in poplar

Despite the promising advances, several challenges remain in the genetic engineering of poplar for salt tolerance. One major challenge is the complexity of the poplar genome, which can complicate the identification and manipulation of target genes. Additionally, the long generation time of woody plants poses a significant hurdle for rapid genetic improvements. However, the development of high-throughput functional genomics tools, such as CRISPR-Cas9, offers new opportunities to overcome these challenges (Zafar et al., 2020). Future prospects include the integration of genome-wide association studies (GWAS) with genome editing to identify and target novel genes associated with salt tolerance. Moreover, the combination of CRISPR-Cas9 with other emerging technologies, such as RNA interference (RNAi) and transcriptional modulation, could further enhance the precision and efficiency of genetic engineering in poplar (Tran et al., 2020; Han et al., 2022).

7 Implications for Breeding and Conservation

7.1 Using genetic insights to develop salt-tolerant poplar varieties

The identification and functional verification of salt tolerance genes in poplar species provide a robust foundation for breeding programs aimed at developing salt-tolerant varieties. For instance, the overexpression of the *PeERF1* gene in *Populus alba* × *Populus glandulosa* has demonstrated significant improvements in salt tolerance, highlighting its potential as a candidate gene for breeding (Ge et al., 2022). Similarly, the *PtGSTF1* gene has been

shown to enhance both biomass production and salt tolerance through mechanisms such as ion homeostasis and reactive oxygen species (ROS) scavenging, making it another valuable target for genetic improvement (Gao et al., 2022). The *NAC13* gene also plays a crucial role in salt stress response, with overexpression leading to enhanced salt tolerance in transgenic poplar (Zhang et al., 2019a). These genetic insights can be leveraged to develop new poplar varieties that are better suited to saline environments, thereby expanding the range of habitats where poplar can be cultivated.

7.2 Impact of improved salt tolerance on poplar's ecological and economic value

Enhancing the salt tolerance of poplar species has significant ecological and economic implications. Ecologically, salt-tolerant poplars can be planted in saline soils, which are often unsuitable for other crops, thereby contributing to land reclamation and ecosystem restoration efforts. For example, the overexpression of the AhDREB transcription factor in *Populus tomentosa* has been shown to confer salt tolerance without growth reduction, making it a promising candidate for such applications (Guo et al., 2019). Economically, salt-tolerant poplars can improve biomass production in saline environments, as demonstrated by the *PtGSTF1* gene, which enhances both growth and salt tolerance (Gao et al., 2022). This dual benefit can lead to increased wood and biomass yields, providing a sustainable source of raw materials for various industries, including bioenergy, paper, and timber.

7.3 Strategies for the conservation of salt-tolerant poplar genotypes

Conserving salt-tolerant poplar genotypes is essential for maintaining genetic diversity and ensuring the long-term success of breeding programs. One strategy involves the establishment of germplasm banks that store seeds or tissue samples of salt-tolerant varieties, such as those identified through transcriptome and genome-wide association studies (Sun et al., 2018; Ge et al., 2022). Additionally, in situ conservation efforts, such as planting salt-tolerant poplars in their natural habitats, can help preserve these valuable genotypes. The use of molecular markers to identify and select for salt tolerance traits, as demonstrated in studies on cotton and rice, can also be applied to poplar breeding programs to ensure the propagation of salt-tolerant genotypes (Sun et al., 2018; Geng et al., 2023). By combining these strategies, we can safeguard the genetic resources necessary for future breeding efforts and the continued adaptation of poplar species to changing environmental conditions.

8 Integrating Genomic Insights into Forestry Practices

8.1 Practical applications of genomic research in forestry

Genomic research has significantly advanced our understanding of the genetic basis of traits such as salt tolerance in poplars, which are crucial for forestry practices. For instance, the downregulation of the *PagSAP1* gene in hybrid poplar (*Populus alba* × *P. glandulosa*) has been shown to enhance salt tolerance by maintaining cellular ionic homeostasis, making these genetically modified trees more suitable for planting in marginal lands (Yoon et al., 2018). Similarly, the overexpression of the *PeERF1* gene in *Populus alba* × *Populus glandulosa* has demonstrated improved growth and physiological characteristics under salt stress, highlighting the potential of transcriptome analysis in identifying key regulatory factors for stress resistance (Ge et al., 2022). Additionally, the *PtGSTF1* gene from *P. trichocarpa* has been found to improve both biomass production and salt tolerance through the regulation of xylem cell proliferation, ion homeostasis, and reactive oxygen species scavenging (Gao et al., 2022). These findings underscore the practical applications of genomic research in developing poplar varieties that can thrive in challenging environmental conditions.

8.2 Role of salt tolerance research in sustainable forest and land management

Salt tolerance research plays a pivotal role in sustainable forest and land management by enabling the cultivation of trees in saline soils, which are often unsuitable for traditional forestry. For example, the overexpression of the *NAC13* gene in poplar has been shown to significantly enhance salt tolerance, making these transgenic plants more resilient to abiotic stress (Zhang et al., 2019a). The introduction of the *NsNHX1* gene from the halophytic shrub *Nitraria sibirica* into poplar has also resulted in improved salt tolerance and root development, further supporting the use of genetically modified trees in saline environments (Geng et al., 2020). Moreover, the *PeWRKY31* gene from *Populus* × *euramericana* has been found to enhance both salt and insect resistance in transgenic tobacco, indicating its potential for producing stress-resistant poplars (Yu et al., 2021). These

advancements in salt tolerance research contribute to the sustainable management of forests by expanding the range of environments where trees can be successfully cultivated.

8.3 Policy implications and recommendations for using genetically modified poplars

The use of genetically modified poplars in forestry practices has significant policy implications, particularly concerning environmental safety, biodiversity, and public acceptance. Policymakers should consider the following recommendations. Conduct thorough environmental impact assessments to evaluate the potential effects of genetically modified poplars on local ecosystems and biodiversity. For instance, the overexpression of the *PsnHDZ63* gene in *Populus simonii* × *P. nigra* has been shown to enhance salt tolerance, but its long-term ecological impact needs careful evaluation (Guo et al., 2021). Develop and implement robust regulatory frameworks that ensure the safe deployment of genetically modified trees. This includes monitoring and managing gene flow to wild relatives and other non-target species.

Engage with the public and stakeholders to address concerns and provide transparent information about the benefits and risks associated with genetically modified poplars. Highlighting successful case studies, such as the improved salt tolerance in transgenic poplars through the overexpression of *PtGSTF1* (Gao et al., 2022), can help build public trust and acceptance. Promote the integration of genetically modified poplars into sustainable forestry practices that prioritize environmental conservation and resource efficiency. For example, the use of salt-tolerant poplars in afforestation programs can help reclaim saline soils and enhance land productivity (Zhang et al., 2019b).

9 Future Research Directions

9.1 Emerging areas in poplar salt tolerance research

Recent studies have significantly advanced our understanding of the molecular and physiological mechanisms underlying salt tolerance in poplars. However, several emerging areas warrant further exploration. One promising direction is the detailed functional analysis of newly identified salt tolerance genes, such as *PeERF1*, which has shown potential in enhancing salt tolerance in transgenic poplars (Ge et al., 2022). Additionally, the role of stress-associated proteins like PagSAP1 in maintaining cellular ionic homeostasis under salt stress conditions presents another intriguing area for future research (Yoon et al., 2018). The identification and functional characterization of transcription factors, such as *NAC13* and *PalERF109*, which regulate key stress response pathways, also offer valuable insights into the complex regulatory networks involved in salt tolerance (Zhang et al., 2019a; Chen et al., 2020).

9.2 Opportunities for multi-disciplinary collaborations

The complexity of salt tolerance mechanisms in poplars necessitates a multi-disciplinary approach to fully elucidate the underlying processes. Collaborations between molecular biologists, geneticists, and plant physiologists can facilitate the integration of transcriptomic, proteomic, and metabolomic data to provide a comprehensive understanding of salt stress responses (Zhang et al., 2019b). Furthermore, partnerships with bioinformaticians can enhance the analysis of large-scale data sets, such as those generated from high-throughput sequencing and genome-wide association studies (GWAS) (Wang et al., 2021; Ge et al., 2022). Collaborative efforts with agronomists and ecologists can also help translate laboratory findings into practical applications for improving poplar cultivation in saline environments (Zhou et al., 2020).

9.3 Technological innovations and their potential impact on future studies

Advancements in genetic engineering and genome editing technologies, such as CRISPR/Cas9, hold great promise for the development of salt-tolerant poplar varieties. These tools can be used to precisely modify key genes involved in salt stress responses, such as those encoding transcription factors and ion transporters (Guo et al., 2019; Zhao et al., 2020). Additionally, the use of omics technologies, including transcriptomics, proteomics, and metabolomics, can provide a holistic view of the molecular changes occurring in poplars under salt stress (Zhang et al., 2019b; Ge et al., 2022). The integration of these technologies with advanced phenotyping methods, such as high-throughput imaging and remote sensing, can significantly accelerate the identification and characterization of salt tolerance traits in poplars (Yoon et al., 2018; Zhang et al., 2019a).

10 Concluding Remarks

Recent research has significantly advanced our understanding of the genetic mechanisms underlying salt tolerance in poplar species. Various genes have been identified and functionally characterized for their roles in enhancing salt tolerance. For instance, the *PeERF1* gene from *Populus euphratica* has been shown to improve salt tolerance when overexpressed in transgenic *Populus alba* × *Populus glandulosa*. Similarly, the *NAC13* gene has been demonstrated to enhance salt tolerance in transgenic poplar through overexpression, while its suppression leads to increased salt sensitivity. The *PtGSTF1* gene has been found to improve both biomass production and salt tolerance by regulating xylem cell proliferation, ion homeostasis, and reactive oxygen species (ROS) scavenging. Additionally, the PeSTZ1 transcription factor has been shown to confer salt stress tolerance by regulating the expression of PeZAT12 and PeAPX2, which are involved in ROS scavenging. Other notable genes include ERF38, which enhances salt and osmotic tolerance, and PalERF109, which regulates salt tolerance via the PalHKT1;2 transporter. These findings collectively highlight the diverse genetic strategies employed by poplar species to cope with salt stress.

The research on salt tolerance genes in poplar has made substantial contributions to forestry science and genetic technology. By identifying and characterizing key genes involved in salt stress responses, these studies provide valuable genetic resources for breeding salt-tolerant poplar varieties. For example, the overexpression of PtGSTF1 not only enhances salt tolerance but also improves biomass production, making it a promising candidate for genetic improvement programs aimed at increasing both stress resilience and growth performance. The functional verification of genes such as *NAC13* and *PeERF1* in transgenic poplar lines demonstrates the practical applicability of these findings in developing stress-tolerant forestry species. Moreover, the elucidation of regulatory networks, such as the PeSTZ1-PeZAT12-PeAPX2 pathway, offers insights into the complex molecular mechanisms underlying stress responses, paving the way for more targeted genetic interventions. These advancements contribute to the sustainable management of forest ecosystems and the development of resilient tree species capable of thriving in saline environments.

Future research should focus on several key areas to further enhance the understanding and application of salt tolerance genes in poplar. First, comprehensive genome-wide association studies (GWAS) and transcriptome analyses should be conducted to identify additional candidate genes and regulatory elements involved in salt stress responses. Second, the functional roles of these genes should be validated in diverse poplar species and under various environmental conditions to ensure their broad applicability. Third, the development of advanced genetic engineering techniques, such as CRISPR/Cas9, could facilitate precise editing of salt tolerance genes, enabling the creation of highly resilient poplar varieties. Additionally, field trials should be conducted to evaluate the performance of transgenic poplar lines in real-world agroforestry settings, assessing their growth, survival, and ecological impact under saline conditions. Finally, interdisciplinary collaborations between geneticists, ecologists, and forestry practitioners are essential to translate these genetic advancements into practical solutions for sustainable forestry and agroforestry management.

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Conflict of Interest Disclosure

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

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