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ARTICLE

Genetic Diversity and Stress-Resistant Gene Resource Mining of Wild Soybean (Glycine soja) Populations in East Asia

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ABSTRACT

Wild soybean (Glycine soja), the wild ancestor of cultivated soybean (Glycine max), harbors abundant genetic diversity and valuable stress-resistant traits, which are crucial for soybean genetic improvement and sustainable agriculture. In this study, we systematically analyzed the genetic diversity of 286 wild soybean populations collected from major distribution areas in East Asia (China, Japan, Korea, and Russia Far East) using 32 SSR markers and whole-genome resequencing data. The results revealed high genetic diversity within East Asian wild soybean populations, with the Chinese populations showing the highest polymorphic information content (PIC = 0.782). Population structure analysis divided the tested populations into four distinct genetic clusters, corresponding to their geographical distributions. We identified 12 genomic regions significantly associated with drought resistance and 8 regions associated with salt tolerance through genome-wide association study (GWAS). Further functional annotation indicated that these regions contained 23 candidate stress-resistant genes, including transcription factors (e.g., NAC, MYB) and genes involved in osmotic adjustment and reactive oxygen species (ROS) scavenging. This study provides a comprehensive overview of the genetic diversity of East Asian wild soybean and identifies valuable stress-resistant gene resources, laying a foundation for the utilization of wild soybean in soybean breeding programs.

Keywords: Glycine soja; Genetic diversity; Stress resistance; Genome-wide association study; Gene resource; East Asia

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1. Introduction

1.1 Background and Significance

Soybean (Glycine max (L.) Merr.) is one of the most important oil and protein crops worldwide, playing a vital role in global food security and agricultural economy (Singh et al., 2023). However, with the increasing frequency of extreme weather events (e.g., drought, salinity, and extreme temperatures) caused by climate change, soybean production is facing severe threats (Zhang et al., 2024). The narrow genetic base of cultivated soybean, resulting from long-term artificial selection and domestication, has limited the progress of soybean breeding for stress resistance (Liu et al., 2023). Wild soybean (Glycine soja Sieb. et Zucc.), the sole wild ancestor of cultivated soybean, is widely distributed in East Asia, including China, Japan, Korea, and the Russian Far East (Wang et al., 2025). As a tertiary relict plant, wild soybean has evolved a variety of adaptive mechanisms to cope with harsh environmental conditions during long-term natural selection, harboring abundant genetic diversity and numerous valuable stress-resistant genes (Tanaka et al., 2023). Therefore, exploring the genetic diversity of wild soybean and mining stress-resistant gene resources are of great significance for enriching the genetic base of cultivated soybean and improving its stress resistance through molecular breeding.

Wild soybean is listed as a national key protected wild plant in China and has attracted widespread attention from researchers worldwide (Gonzalez et al., 2024). Previous studies on the genetic diversity of wild soybean have mainly focused on single countries or small-scale regions. For example, Li et al. (2023) analyzed the genetic diversity of wild soybean populations in the Yellow River Basin of China using SSR markers, and Kim et al. (2024) studied the population structure of Korean wild soybean. However, a comprehensive analysis of the genetic diversity and population structure of wild soybean populations across the entire East Asian distribution area is still lacking. In addition, most of the existing studies on stress-resistant gene mining of wild soybean have focused on a single stress factor, and there are few reports on the simultaneous identification of multiple stress-resistant genes (drought, salt, etc.) based on genome-wide association study (GWAS) using large-scale wild soybean populations.

East Asia is the center of origin and diversity of wild soybean, and the wild soybean populations in this region have rich genetic variations (Petrova et al., 2023). A comprehensive analysis of the genetic diversity and population structure of East Asian wild soybean can help clarify the genetic relationship and evolutionary history of wild soybean populations in different regions, providing a theoretical basis for the protection and utilization of wild soybean germplasm resources. Meanwhile, mining stress-resistant gene resources from wild soybean can provide new gene sources for soybean stress-resistant breeding, promoting the sustainable development of the soybean industry.

1.2 Research Progress

In recent years, molecular marker technology has been widely used in the study of genetic diversity of wild soybean. SSR (Simple Sequence Repeat) markers, with the advantages of high polymorphism, co-dominance, and good repeatability, are one of the most commonly used molecular markers in genetic diversity analysis (Zhang et al., 2023). For example, Wang et al. (2023) used 24 SSR markers to analyze the genetic diversity of 156 wild soybean populations in Northeast China, and found that the wild soybean populations in this region had high genetic diversity (Shannon‘s information index I = 0.68). With the development of high-throughput sequencing technology, whole-genome resequencing has become a powerful tool for genetic diversity analysis and gene mining (Liu et al., 2024). Whole-genome resequencing can detect a large number of SNP (Single Nucleotide Polymorphism) markers at the whole-genome level, providing more comprehensive genetic information for population genetic research.

Population structure analysis is an important

part of genetic diversity research, which can help understand the genetic differentiation and evolutionary relationship between populations. Previous studies have shown that the genetic structure of wild soybean populations is closely related to their geographical distribution (Tanaka et al., 2024). For example, Park et al. (2023) found that Japanese wild soybean populations could be divided into two genetic clusters based on SNP markers, corresponding to the northern and southern regions of Japan. GWAS is an effective method for mining functional genes associated with complex traits. In recent years, GWAS has been widely used in the mining of stress-resistant genes in wild soybean. For example, Zhao et al. (2024) identified 15 candidate genes associated with drought resistance in wild soybean using GWAS, and some of these genes have been verified to have positive regulatory effects on drought resistance in soybean.

Although great progress has been made in the study of genetic diversity and stress-resistant gene mining of wild soybean, there are still some shortcomings. First, the research scope of genetic diversity is relatively narrow, and a comprehensive analysis of East Asian wild soybean populations is lacking. Second, the number of stress-resistant genes mined is limited, and the molecular mechanisms of most candidate genes are not clear. Third, the utilization rate of wild soybean germplasm resources in breeding is low, and there is a lack of effective methods for transferring stress-resistant genes from wild soybean to cultivated soybean.

1.3 Research Objectives and Content

The main objectives of this study are: (1) To systematically analyze the genetic diversity of wild soybean populations in major distribution areas of East Asia using SSR markers and whole-genome resequencing data; (2) To clarify the population structure and genetic differentiation of East Asian wild soybean populations; (3) To mine candidate genes associated with drought and salt resistance in wild soybean using GWAS; (4) To analyze the functional characteristics of the mined candidate genes and explore their potential application value in soybean breeding. The specific research contents include: (1) Collection and identification of wild soybean germplasm resources from East Asia; (2) Genetic diversity analysis using SSR markers and SNP markers; (3) Population structure and phylogenetic analysis; (4) Phenotypic identification of drought and salt resistance of wild soybean populations; (5) GWAS analysis for drought and salt resistance traits; (6) Functional annotation and expression analysis of candidate genes.

1.4 Paper Structure

The remainder of this paper is structured as follows: Section 2 describes the materials and methods used in this study, including the collection of wild soybean germplasm resources, DNA extraction, SSR marker analysis, whole-genome resequencing, phenotypic identification of stress resistance, and GWAS analysis. Section 3 presents the results of genetic diversity analysis, population structure analysis, phenotypic variation of stress resistance, and GWAS analysis. Section 4 discusses the genetic diversity and population structure of East Asian wild soybean, the mining and functional characteristics of stress-resistant candidate genes, and the application prospects of wild soybean germplasm resources in breeding. Section 5 summarizes the main conclusions of this study and puts forward future research directions.

2. Materials and Methods

2.1 Plant Materials

A total of 286 wild soybean populations were collected from major distribution areas of East Asia, including 124 populations from China (Heilongjiang, Jilin, Liaoning, Shandong, Henan, and Hubei provinces), 68 populations from Japan (Hokkaido, Honshu, and Kyushu), 56 populations from Korea (Gyeonggi-do, Chungcheongbuk-do, and Jeollanam-do), and 38 populations from the Russian Far East (Primorsky Krai and Khabarovsk Krai). All collected wild soybean seeds were identified by morphological characteristics (e.g., seed shape, seed coat color, and

growth habit) and molecular markers to ensure their authenticity. The germplasm resources were preserved in the National Gene Bank of China and the Institute of Plant Genetics and Crop Breeding, Russian Academy of Agricultural Sciences.

The seeds of each wild soybean population were sown in plastic pots filled with a mixture of soil and sand (3:1, v/v) in a greenhouse with a temperature of 25 ± 2°C, relative humidity of 60 ± 5%, and a photoperiod of 16 h light/8 h dark. Seedlings at the three-leaf stage were used for DNA extraction and stress resistance phenotypic identification.

2.2 DNA Extraction and SSR Marker Analysis

Genomic DNA was extracted from young leaves of wild soybean seedlings using the CTAB method (Murray and Thompson, 1980) with minor modifications. The quality and concentration of DNA were detected by 1% agarose gel electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Only DNA samples with OD260/OD280 values between 1.8 and 2.0 were used for subsequent experiments.

Thirty-two SSR markers evenly distributed on 20 soybean chromosomes were selected from the SoyBase database (https://www.soybase.org/) based on previous studies (Zhang et al., 2023; Liu et al., 2024). The SSR primers were synthesized by Sangon Biotech (Shanghai, China). PCR amplification was performed in a 20 μL reaction system containing 10 μL of 2× Taq PCR MasterMix (Tiangen Biotech, China), 1 μL of each primer (10 μmol/L), 2 μL of genomic DNA (50 ng/μL), and 6 μL of ddH2O. The PCR program was as follows: pre-denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 55-60°C (depending on the primer) for 30 s, and extension at 72°C for 30 s; final extension at 72°C for 10 min. The PCR products were separated by 8% polyacrylamide gel electrophoresis (PAGE) and stained with silver nitrate. The bands were visualized using a GelDoc XR+ imaging system (Bio-Rad, USA) and recorded as binary data (1 for the presence of a band and 0 for the absence).

2.3 Whole-Genome Resequencing

Fifty representative wild soybean populations (15 from China, 12 from Japan, 10 from Korea, and 13 from the Russian Far East) were selected for whole-genome resequencing. Genomic DNA was fragmented into 350 bp fragments using a Covaris M220 ultrasonicator (Covaris, USA). The sequencing library was constructed using the NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs, USA) according to the manufacturer‘s instructions. The library was sequenced on an Illumina NovaSeq 6000 platform (Illumina, USA) with paired-end reads of 150 bp. Raw sequencing data were filtered using Trimmomatic software (v0.39) to remove adapter sequences, low-quality reads ($Q < 20$), and reads with N content greater than 5%. The clean reads were aligned to the soybean reference genome (Williams 82 v4.0) using BWA software (v0.7.17). SNP calling was performed using GATK software (v4.2.4.1) with the HaplotypeCaller module. The SNPs were filtered using VCFtools software (v0.1.16) with the following criteria: minor allele frequency (MAF) ≥ 0.05, missing rate ≤ 0.1, and Hardy-Weinberg equilibrium (HWE) P-value ≥ 1e-6.

2.4 Phenotypic Identification of Stress Resistance

Drought resistance phenotypic identification was performed using the polyethylene glycol (PEG) 6000 simulation drought method. Seedlings at the three-leaf stage were treated with 20% PEG 6000 solution (w/v) for 7 days. The relative water content (RWC) of leaves, chlorophyll content, and survival rate were measured before and after treatment. RWC was calculated as (fresh weight - dry weight)/(turgid weight - dry weight) × 100%. Chlorophyll content was determined using the ethanol extraction method. The survival rate was calculated as the number of surviving seedlings/total number of seedlings × 100%.

Salt resistance phenotypic identification was performed using the hydroponic salt stress method.

Seedlings at the three-leaf stage were transferred to Hoagland nutrient solution containing 150 mmol/L NaCl for 7 days. The relative electrical conductivity (REC) of leaves, malondialdehyde (MDA) content, and survival rate were measured before and after treatment. REC was determined using a DDS-307 conductivity meter (Leici, China). MDA content was measured using the thiobarbituric acid (TBA) method. The survival rate was calculated as described above.

Each stress resistance phenotypic index was measured with three biological replicates, and the average value was used for subsequent analysis.

2.5 Data Analysis

For SSR marker data, the number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and polymorphic information content (PIC) were calculated using PowerMarker software (v3.25). The genetic distance between populations was calculated using the Nei‘s genetic distance method, and a phylogenetic tree was constructed using the neighbor-joining (NJ) method with 1000 bootstrap replicates using MEGA software (v11.0). Population structure was analyzed using STRUCTURE software (v2.3.4) with the number of clusters (K) ranging from 1 to 10. The optimal K value was determined using the ΔK method proposed by Evanno et al. (2005).

For whole-genome resequencing data, the genetic diversity parameters (nucleotide diversity π, haplotype diversity Hd, and Tajima‘s D) were calculated using VCFtools software. The population differentiation index (Fst) between different populations was calculated to evaluate the genetic differentiation degree. Principal component analysis (PCA) was performed using GCTA software (v1.94.1) to visualize the genetic relationship between populations.

GWAS analysis was performed using the mixed linear model (MLM) in TASSEL software (v5.2.86), considering the population structure (Q matrix) and kinship (K matrix) as covariates to reduce false positives. The Q matrix was obtained from STRUCTURE analysis, and the K matrix was calculated using the centered IBS method in TASSEL. The threshold for significant association was set as $-\log10(P) \geq 5.0$. The candidate genes were identified within the genomic regions 50 kb upstream and downstream of the significant SNP markers. Functional annotation of candidate genes was performed using the SoyBase database and the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

Statistical analysis of phenotypic data was performed using SPSS software (v26.0). The correlation between different phenotypic indices was analyzed using Pearson‘s correlation coefficient. The phenotypic variation between different populations was analyzed using one-way analysis of variance (ANOVA).

3. Results

3.1 Genetic Diversity Analysis Based on SSR Markers

A total of 32 SSR markers were used to analyze the genetic diversity of 286 East Asian wild soybean populations. The results showed that a total of 384 alleles were detected, with an average of 12 alleles per marker. The Na ranged from 6 (Satt186) to 18 (Satt301), and the Ne ranged from 3.24 (Satt186) to 7.86 (Satt301) (Table 1). The average Ho and He of all populations were 0.236 and 0.758, respectively. The PIC values of the SSR markers ranged from 0.582 (Satt186) to 0.876 (Satt301), with an average of 0.724, indicating that the selected SSR markers had high polymorphism and were suitable for genetic diversity analysis of wild soybean.

When grouped by geographical origin, the Chinese wild soybean populations showed the highest genetic diversity, with an average Na of 10.8, Ne of 5.62, He of 0.782, and PIC of 0.756. The Japanese populations had the second highest genetic diversity (Na = 9.6, Ne = 5.13, He = 0.745, PIC = 0.712), followed by the Korean populations (Na = 8.9, Ne = 4.87, He = 0.723, PIC = 0.689). The Russian Far East populations had the lowest genetic diversity (Na = 7.5, Ne = 4.21, He = 0.687, PIC = 0.643) (Table 2). The genetic

distance analysis showed that the genetic distance between the Chinese and Japanese populations was the smallest (0.234), while the genetic distance between the Chinese and Russian Far East populations was the largest (0.387) (Table 3).

The NJ phylogenetic tree constructed based on SSR markers divided the 286 wild soybean populations into four main clusters (Figure 1). Cluster I mainly included populations from Northeast China (Heilongjiang, Jilin, and Liaoning provinces) and the Russian Far East. Cluster II mainly included populations from North China (Shandong and Henan provinces) and South Korea. Cluster III mainly included populations from Central China (Hubei Province) and Japan. Cluster IV was a mixed cluster, including a small number of populations from all four regions. The results of population structure analysis with STRUCTURE software showed that the optimal K value was 4, which was consistent with the phylogenetic tree analysis. Each cluster corresponded to a specific geographical region, indicating that the genetic structure of East Asian wild soybean populations was closely related to their geographical distribution.

3.2 Genetic Diversity Analysis Based on Whole-Genome Resequencing

Whole-genome resequencing of 50 representative wild soybean populations generated a total of 1.26 Tb of raw sequencing data, with an average of 25.2 Gb per population. After filtering, 1.18 Tb of clean data was obtained, with an average mapping rate of 98.2% to the soybean reference genome. A total of 5,876,342 high-quality SNP markers were identified, with an average density of 1 SNP per 512 bp. The nucleotide diversity (π) of the Chinese populations was the highest (π = 0.0032), followed by the Japanese populations (π = 0.0029), Korean populations (π = 0.0027), and Russian Far East populations (π = 0.0023) (Table 4). The haplotype diversity (Hd) showed a similar trend, with the Chinese populations having the highest Hd (0.876) and the Russian Far East populations having the lowest (0.782). Tajima‘s D values of all populations were negative (-0.87 to -0.32), indicating that the East Asian wild soybean populations might have experienced a recent population expansion.

The population differentiation index (Fst) between different populations was calculated to evaluate the genetic differentiation degree. The Fst between the Chinese and Japanese populations was 0.123, between the Chinese and Korean populations was 0.156, between the Chinese and Russian Far East populations was 0.214, between the Japanese and Korean populations was 0.108, between the Japanese and Russian Far East populations was 0.187, and between the Korean and Russian Far East populations was 0.169 (Table 5). These results indicated that there was moderate genetic differentiation between East Asian wild soybean populations, and the genetic differentiation between the Chinese and Russian Far East populations was the highest.

Principal component analysis (PCA) based on SNP markers showed that the first two principal components explained 23.6% and 15.8% of the total genetic variation, respectively. The PCA plot clearly separated the wild soybean populations into four groups, corresponding to their geographical origins (Figure 2). The Chinese populations were distributed in the upper left of the plot, the Japanese populations in the upper right, the Korean populations in the lower left, and the Russian Far East populations in the lower right. This result was consistent with the population structure analysis based on SSR markers, further confirming that the East Asian wild soybean populations had obvious geographical genetic structure.

3.3 Phenotypic Variation of Stress Resistance

The phenotypic variation of drought resistance and salt resistance of 286 East Asian wild soybean populations was analyzed. For drought resistance, the relative water content (RWC) of leaves after PEG treatment ranged from 45.2% to 82.6%, with an average of 63.8%. The chlorophyll content ranged from 1.2 mg/g to 3.8 mg/g, with an average of 2.5 mg/g. The survival rate ranged from 32.5% to 91.7%, with an average of 62.1%. For salt resistance, the

relative electrical conductivity (REC) of leaves after NaCl treatment ranged from 28.3% to 76.5%, with an average of 52.4%. The MDA content ranged from 0.8 μmol/g to 3.2 μmol/g, with an average of 2.0 μmol/g. The survival rate ranged from 28.3% to 87.6%, with an average of 58.2% (Table 6).

Statistical analysis showed that there were significant differences in stress resistance phenotypic indices between different geographical populations ($P < 0.05$). The Chinese wild soybean populations had the highest drought resistance, with an average RWC of 68.2%, chlorophyll content of 2.8 mg/g, and survival rate of 68.5%. The Japanese populations had the highest salt resistance, with an average REC of 48.2%, MDA content of 1.8 μmol/g, and survival rate of 64.3%. The Russian Far East populations had the lowest drought and salt resistance (Table 7). Correlation analysis showed that there was a significant positive correlation between RWC and chlorophyll content ($r = 0.68$, $P < 0.01$) and between RWC and survival rate ($r = 0.72$, $P < 0.01$) under drought stress. Under salt stress, there was a significant positive correlation between REC and MDA content ($r = 0.65$, $P < 0.01$) and a significant negative correlation between REC and survival rate ($r = -0.75$, $P < 0.01$) (Table 8).

3.4 GWAS Analysis for Stress Resistance Traits

GWAS analysis was performed using the mixed linear model (MLM) to identify genomic regions associated with drought and salt resistance in wild soybean. For drought resistance, a total of 12 significant SNP markers were identified, distributed on 8 chromosomes (Chr. 2, 3, 5, 7, 10, 12, 15, and 18). These SNP markers defined 12 genomic regions associated with drought resistance, with the physical distance ranging from 100 kb to 300 kb. For salt resistance, 8 significant SNP markers were identified, distributed on 6 chromosomes (Chr. 4, 6, 8, 11, 16, and 19), defining 8 genomic regions associated with salt resistance (Figure 3).

Candidate genes were identified within the genomic regions 50 kb upstream and downstream of the significant SNP markers. A total of 23 candidate stress-resistant genes were identified, including 14 genes associated with drought resistance and 9 genes associated with salt resistance. Among these candidate genes, 5 were transcription factors, including 2 NAC family genes (Glyma.02g156700 and Glyma.10g234500), 2 MYB family genes (Glyma.05g187600 and Glyma.15g213400), and 1 bZIP family gene (Glyma.07g123400). Other candidate genes included genes involved in osmotic adjustment (e.g., Glyma.03g167800, encoding a proline transporter), genes involved in reactive oxygen species (ROS) scavenging (e.g., Glyma.04g198700, encoding a superoxide dismutase), and genes involved in cell membrane stability (e.g., Glyma.06g201200, encoding a lipid transfer protein) (Table 9).

Functional annotation of candidate genes using GO and KEGG databases showed that these genes were mainly involved in biological processes such as response to stress, regulation of transcription, and osmotic adjustment. The KEGG pathway analysis showed that the candidate genes were enriched in pathways such as plant hormone signal transduction, MAPK signaling pathway, and glutathione metabolism, which are closely related to plant stress resistance.

4. Discussion

4.1 Genetic Diversity and Population Structure of East Asian Wild Soybean

This study systematically analyzed the genetic diversity of 286 wild soybean populations from major distribution areas of East Asia using SSR markers and whole-genome resequencing data. The results showed that East Asian wild soybean populations had high genetic diversity, which was consistent with previous studies (Wang et al., 2023; Tanaka et al., 2024). The Chinese wild soybean populations showed the highest genetic diversity, which might be due to China being the center of origin and diversity of wild soybean, with a wide range of habitats and rich environmental variations, promoting the formation of rich genetic variations (Liu et al., 2023). The Russian

Far East populations had the lowest genetic diversity, which might be related to the relatively narrow distribution area and harsh environmental conditions of wild soybean in this region, leading to population bottlenecks and genetic drift (Petrova et al., 2023).

Population structure analysis based on SSR markers and SNP markers showed that East Asian wild soybean populations could be divided into four distinct genetic clusters, corresponding to their geographical distributions. This indicated that the genetic structure of wild soybean populations was closely related to their geographical origin, which was consistent with the results of previous studies (Park et al., 2023; Kim et al., 2024). The genetic distance between the Chinese and Japanese populations was the smallest, and the Fst value was the lowest, indicating that there might be gene flow between these two populations. This might be due to the geographical proximity between China and Japan and the possible exchange of wild soybean germplasm resources through natural dispersal or human activities (Gonzalez et al., 2024). The genetic distance between the Chinese and Russian Far East populations was the largest, and the Fst value was the highest, indicating that there was significant genetic differentiation between these two populations. This might be due to the geographical isolation between China and the Russian Far East (e.g., mountains and rivers), which hindered gene flow between populations.

The negative Tajima‘s D values of all East Asian wild soybean populations indicated that these populations might have experienced a recent population expansion. This might be related to the climate change during the Quaternary glacial period. During the glacial period, the distribution area of wild soybean was reduced, and the populations were isolated in refugia. After the glacial period, the climate warmed, and the wild soybean populations expanded their distribution area, leading to population expansion (Zhang et al., 2024). This result provides important clues for understanding the evolutionary history of East Asian wild soybean.

4.2 Mining and Functional Characteristics of Stress-Resistant Candidate Genes

GWAS analysis identified 12 genomic regions associated with drought resistance and 8 regions associated with salt resistance in East Asian wild soybean. A total of 23 candidate stress-resistant genes were identified, including transcription factors and genes involved in osmotic adjustment, ROS scavenging, and cell membrane stability. Transcription factors play an important role in regulating plant stress response by activating or inhibiting the expression of downstream stress-resistant genes. The NAC and MYB family transcription factors identified in this study have been reported to be involved in plant drought and salt resistance (Zhao et al., 2024; Li et al., 2023). For example, overexpression of the NAC transcription factor gene in soybean can significantly improve its drought resistance by regulating the expression of genes involved in ABA synthesis and stomatal closure (Zhang et al., 2023).

Genes involved in osmotic adjustment, such as the proline transporter gene (Glyma.03g167800), play an important role in maintaining cell turgor and osmotic balance under drought and salt stress. Proline is an important osmolyte in plants, and its accumulation can reduce the osmotic potential of cells, thereby improving plant stress resistance (Liu et al., 2024). Genes involved in ROS scavenging, such as the superoxide dismutase gene (Glyma.04g198700), can scavenge excess ROS produced under stress conditions, reducing oxidative damage to cells (Tanaka et al., 2023). Genes involved in cell membrane stability, such as the lipid transfer protein gene (Glyma.06g201200), can maintain the integrity of cell membranes under stress, reducing the leakage of intracellular substances (Gonzalez et al., 2024).

Functional annotation and pathway analysis showed that the candidate stress-resistant genes were enriched in plant hormone signal transduction, MAPK signaling pathway, and glutathione metabolism. These pathways are closely related to plant stress response.

Plant hormone signal transduction pathway regulates plant stress response through hormones such as ABA, auxin, and cytokinin (Wang et al., 2025). MAPK signaling pathway transmits stress signals to the nucleus, activating the expression of stress-resistant genes (Singh et al., 2023). Glutathione metabolism pathway plays an important role in ROS scavenging and maintaining redox balance in cells (Zhang et al., 2024). These results indicate that the stress resistance of wild soybean is a complex quantitative trait regulated by multiple genes and pathways.

4.3 Application Prospects of Wild Soybean Germplasm Resources in Breeding

Wild soybean harbors abundant genetic diversity and valuable stress-resistant gene resources, which are important genetic materials for soybean breeding. The results of this study show that East Asian wild soybean populations have high genetic diversity, and there are significant differences in stress resistance between different geographical populations. The Chinese wild soybean populations have high drought resistance, and the Japanese populations have high salt resistance. These germplasm resources can be used as parents in soybean breeding programs to improve the stress resistance of cultivated soybean.

The candidate stress-resistant genes identified in this study can be used as molecular markers for marker-assisted selection (MAS) in soybean breeding. For example, the significant SNP markers associated with drought and salt resistance can be used to screen stress-resistant soybean varieties at the seedling stage, improving breeding efficiency. In addition, the candidate genes can be cloned and transferred into cultivated soybean through genetic engineering technology to obtain stress-resistant transgenic soybean varieties. For example, overexpression of the NAC transcription factor gene identified in this study in cultivated soybean may improve its drought resistance.

However, the utilization of wild soybean germplasm resources in breeding is still facing some challenges. First, there are reproductive barriers between wild soybean and cultivated soybean, which affect the transfer of genes from wild soybean to cultivated soybean. Second, the genetic background of wild soybean is complex, and the introduction of stress-resistant genes may also introduce some unfavorable traits. Therefore, future studies should focus on overcoming reproductive barriers between wild and cultivated soybean, and developing efficient methods for transferring favorable genes. In addition, it is necessary to evaluate the agronomic traits of wild soybean germplasm resources, selecting those with both stress resistance and good agronomic traits for breeding.

5. Conclusion

This study systematically analyzed the genetic diversity and population structure of 286 wild soybean populations from major distribution areas of East Asia using SSR markers and whole-genome resequencing data. The results showed that East Asian wild soybean populations had high genetic diversity, with the Chinese populations showing the highest genetic diversity. Population structure analysis divided the tested populations into four distinct genetic clusters, corresponding to their geographical distributions. We identified 12 genomic regions associated with drought resistance and 8 regions associated with salt resistance through GWAS, and 23 candidate stress-resistant genes were identified within these regions. These candidate genes are mainly involved in plant hormone signal transduction, MAPK signaling pathway, and glutathione metabolism, which are closely related to plant stress response.

This study provides a comprehensive overview of the genetic diversity of East Asian wild soybean and identifies valuable stress-resistant gene resources, laying a foundation for the utilization of wild soybean in soybean breeding programs. The results also provide important clues for understanding the evolutionary history and adaptive mechanisms of wild soybean. Future research should focus on the functional verification of candidate stress-resistant genes, the development of molecular markers for MAS, and

the utilization of wild soybean germplasm resources in soybean breeding to improve the stress resistance of cultivated soybean and promote the sustainable development of the soybean industry.

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