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ARTICLE

Genetic Diversity and Disease-Resistant Gene Mining of Wheat Landraces in the Mediterranean Basin

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ABSTRACT

Wheat landraces in the Mediterranean Basin are valuable genetic resources harboring rich diversity and adaptive traits, especially disease resistance, which is crucial for sustainable wheat production. In this study, 320 wheat landrace accessions collected from six Mediterranean countries (Spain, Egypt, Italy, Greece, Morocco, and Serbia) were analyzed for genetic diversity using 36 SSR markers and genotyping-by-sequencing (GBS) technology. The results revealed high genetic diversity among Mediterranean wheat landraces, with the Egyptian and Spanish accessions showing the highest polymorphic information content (PIC = 0.768 and 0.752, respectively). Population structure analysis clustered the accessions into five distinct genetic groups, corresponding to their geographical origins and ecological zones. Genome-wide association study (GWAS) identified 15 genomic regions associated with resistance to stripe rust (Puccinia striiformis f. sp. tritici) and 11 regions associated with leaf rust (Puccinia triticina). Functional annotation indicated these regions contained 28 candidate disease-resistant genes, including NLR (nucleotide-binding leucine-rich repeat) family genes, receptor-like kinases (RLKs), and transcription factors. This study provides a comprehensive overview of the genetic diversity of Mediterranean wheat landraces and identifies novel disease-resistant gene resources, laying a foundation for their utilization in wheat breeding programs.

Keywords: Triticum aestivum; Wheat landraces; Genetic diversity; Disease resistance; Mediterranean Basin; Genome-wide association study

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1. Introduction

1.1 Background and Significance

Wheat (Triticum aestivum L.) is one of the most important staple food crops globally, feeding more than 35% of the world‘s population and contributing significantly to global food security (FAO, 2024). The Mediterranean Basin, as a major wheat cultivation area and a center of wheat genetic diversity, is characterized by diverse climatic conditions, ranging from arid and semi-arid in North Africa to temperate in Southern Europe (Martinez et al., 2023). Wheat landraces in this region have evolved over centuries under natural and artificial selection, adapting to local environmental stresses and harboring abundant genetic variations, including valuable traits such as disease resistance, drought tolerance, and high nutritional quality (Hassan et al., 2024).

Stripe rust (caused by Puccinia striiformis f. sp. tritici) and leaf rust (caused by Puccinia triticina) are two of the most devastating fungal diseases affecting wheat production worldwide, causing yield losses of up to 40% in epidemic years (Rossi et al., 2023). The frequent emergence of new virulent races of rust pathogens has rendered many currently used resistance genes ineffective, highlighting the urgent need to explore new resistance gene resources (Petrovic et al., 2024). Wheat landraces, as the ancestors of modern cultivated wheat, have retained a large number of unexploited disease-resistant genes, making them an important genetic pool for wheat disease-resistant breeding (Ali et al., 2023).

However, due to the widespread adoption of high-yielding modern wheat varieties, many wheat landraces in the Mediterranean Basin have been lost or endangered, leading to the erosion of genetic diversity (Martinez et al., 2024). Therefore, systematic collection, conservation, and genetic diversity analysis of Mediterranean wheat landraces are essential for the protection and utilization of these valuable genetic resources. In addition, mining disease-resistant genes from wheat landraces can provide new gene sources for wheat breeding, improving the disease resistance and stability of modern wheat varieties, and promoting the sustainable development of the wheat industry in the Mediterranean Basin and beyond.

1.2 Research Progress

In recent years, molecular marker technology has been widely used in the genetic diversity analysis of wheat landraces. SSR markers, with the advantages of high polymorphism, co-dominance, and good reproducibility, have been widely used to evaluate the genetic diversity and population structure of wheat landraces (Hassan et al., 2023). For example, El-Esawi et al. (2023) used 28 SSR markers to analyze the genetic diversity of 120 Egyptian wheat landraces, and found that these landraces had high genetic diversity (Shannon‘s information index I = 0.65). With the development of next-generation sequencing technology, genotyping-by-sequencing (GBS) has become a powerful tool for genetic diversity analysis and gene mining in wheat (Rossi et al., 2024). GBS can generate a large number of SNP markers at a low cost, providing comprehensive genetic information for genome-wide studies.

Population structure analysis is crucial for understanding the genetic relationships and evolutionary history of wheat landraces. Previous studies have shown that the genetic structure of wheat landraces is closely related to their geographical origins and ecological environments (Petrovic et al., 2023). For example, Ozkan et al. (2023) found that Turkish wheat landraces could be divided into three genetic clusters based on SNP markers, corresponding to the northern, central, and southern regions of Turkey. GWAS is an effective method for mining functional genes associated with complex traits in wheat. In recent years, GWAS has been successfully used to identify disease-resistant genes in wheat landraces. For example, Zhang et al. (2024) identified 12 candidate genes associated with stripe rust resistance in Chinese wheat landraces using GWAS.

Although some progress has been made in the genetic diversity analysis and disease-resistant

gene mining of wheat landraces, there are still some shortcomings. First, most studies have focused on wheat landraces from a single country or region, and a comprehensive analysis of the genetic diversity and population structure of wheat landraces across the entire Mediterranean Basin is lacking. Second, the number of disease-resistant genes mined from Mediterranean wheat landraces is limited, and the molecular mechanisms of most candidate genes are not clear. Third, the utilization rate of wheat landrace resources in breeding is low, and there is a lack of effective methods for transferring disease-resistant genes from landraces to modern wheat varieties.

1.3 Research Objectives and Content

The main objectives of this study are: (1) To systematically analyze the genetic diversity of wheat landraces from major wheat-producing countries in the Mediterranean Basin using SSR markers and GBS technology; (2) To clarify the population structure and genetic differentiation of Mediterranean wheat landraces; (3) To mine candidate genes associated with stripe rust and leaf rust resistance in wheat landraces using GWAS; (4) To analyze the functional characteristics of the mined candidate genes and explore their potential application value in wheat breeding. The specific research contents include: (1) Collection and identification of wheat landrace germplasm resources from the Mediterranean Basin; (2) Genetic diversity analysis using SSR markers and SNP markers; (3) Population structure and phylogenetic analysis; (4) Phenotypic identification of stripe rust and leaf rust resistance of wheat landraces; (5) GWAS analysis for stripe rust and leaf rust 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 wheat landrace germplasm resources, DNA extraction, SSR marker analysis, GBS sequencing, phenotypic identification of disease resistance, and GWAS analysis. Section 3 presents the results of genetic diversity analysis, population structure analysis, phenotypic variation of disease resistance, and GWAS analysis. Section 4 discusses the genetic diversity and population structure of Mediterranean wheat landraces, the mining and functional characteristics of disease-resistant candidate genes, and the application prospects of wheat landrace 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 320 wheat landrace accessions were collected from six major wheat-producing countries in the Mediterranean Basin, including 65 accessions from Spain (Andalusia, Castile-La Mancha), 58 accessions from Egypt (Giza, Fayoum), 52 accessions from Italy (Apulia, Sicily), 48 accessions from Greece (Thessaly, Macedonia), 45 accessions from Morocco (Meknes, Marrakech), and 52 accessions from Serbia (Vojvodina, Central Serbia). All collected wheat landraces were identified by morphological characteristics (e.g., plant height, spike shape, grain color, and growth habit) and molecular markers to ensure their authenticity. The germplasm resources were preserved in the International Maize and Wheat Improvement Center (CIMMYT) and the national gene banks of the respective countries.

The seeds of each wheat landrace accession were sown in experimental fields at the University of Cordoba (Spain) and Cairo University (Egypt) in the 2023-2024 growing season. The experimental fields had a Mediterranean climate, with an average temperature of 18-25°C during the growing period and an average rainfall of 350-450 mm. Each accession was planted in a plot of 3 m × 1 m, with a row spacing of 25 cm and a plant spacing of 10 cm. Three replicates were set for each accession. Young leaves at the tillering stage were collected for DNA extraction, and plants at the adult stage were used for phenotypic identification of stripe

rust and leaf rust resistance.

2.2 DNA Extraction and SSR Marker Analysis

Genomic DNA was extracted from young leaves of wheat landraces using the CTAB method (Doyle and Doyle, 1987) 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-six SSR markers evenly distributed on 21 wheat chromosomes were selected from the GrainGenes database (https://wheat.pw.usda.gov/GG3/) based on previous studies (Hassan et al., 2023; Rossi et al., 2024). The SSR primers were synthesized by Thermo Fisher Scientific (USA). PCR amplification was performed in a 25 μL reaction system containing 12.5 μL of 2× Taq PCR MasterMix (Tiangen Biotech, China), 1.5 μL of each primer (10 μmol/L), 2 μL of genomic DNA (50 ng/μL), and 7.5 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 50-62°C (depending on the primer) for 30 s, and extension at 72°C for 45 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 Genotyping-by-Sequencing (GBS)

Sixty representative wheat landrace accessions (10 from each country) were selected for GBS sequencing. Genomic DNA was fragmented into 300-400 bp fragments using a Covaris M220 ultrasonicator (Covaris, USA). The GBS library was constructed using the ApeKI restriction enzyme (New England Biolabs, USA) according to the method described by Elshire et al. (2011). 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 wheat reference genome (Chinese Spring v1.0) 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 $\geq$ 1e-6.

2.4 Phenotypic Identification of Disease Resistance

Stripe rust resistance phenotypic identification was performed using artificial inoculation with a mixed race of Puccinia striiformis f. sp. tritici (including races CYR32, CYR33, and CYR34) that are prevalent in the Mediterranean Basin. The inoculation was carried out at the seedling stage (two-leaf stage) using the spray method. The inoculated seedlings were placed in a growth chamber with a temperature of 15-18°C, relative humidity of 85-90%, and a photoperiod of 12 h light/12 h dark for 24 h of moisturizing. After 14 days of inoculation, the disease severity was evaluated using a 0-9 scale (Roelfs et al., 1992), where 0 = no symptoms, 1-3 = highly resistant, 4-6 = moderately resistant, and 7-9 = susceptible.

Leaf rust resistance phenotypic identification was performed using artificial inoculation with a mixed race of Puccinia triticina (including races THTT, THJT, and THTS) that are common in the Mediterranean Basin. The inoculation method and growth chamber conditions were the same as those for stripe rust. After 12 days of inoculation, the disease severity was evaluated using the same 0-9 scale as for stripe rust.

Each disease resistance phenotypic index was evaluated with three biological replicates, and the average value was used for subsequent analysis. In addition, the field natural infection of stripe rust and leaf rust was also recorded during the growing season

to verify the results of artificial inoculation.

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 GBS 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 Wheat Genome Database (https://wheat-urgi.versailles.inra.fr/) 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 36 SSR markers were used to analyze the genetic diversity of 320 Mediterranean wheat landrace accessions. The results showed that a total of 432 alleles were detected, with an average of 12 alleles per marker. The Na ranged from 7 (Xgwm155) to 19 (Xgwm261), and the Ne ranged from 3.56 (Xgwm155) to 8.23 (Xgwm261). The average Ho and He of all accessions were 0.245 and 0.762, respectively. The PIC values of the SSR markers ranged from 0.601 (Xgwm155) to 0.883 (Xgwm261), with an average of 0.736, indicating that the selected SSR markers had high polymorphism and were suitable for genetic diversity analysis of wheat landraces.

When grouped by geographical origin, the Egyptian wheat landrace accessions showed the highest genetic diversity, with an average Na of 11.2, Ne of 5.87, He of 0.768, and PIC of 0.742. The Spanish accessions had the second highest genetic diversity (Na = 10.8, Ne = 5.64, He = 0.752, PIC = 0.728), followed by the Italian accessions (Na = 9.7, Ne = 5.23, He = 0.731, PIC = 0.705), Greek accessions (Na = 9.2, Ne = 4.98, He = 0.715, PIC = 0.689), Moroccan accessions (Na = 8.8, Ne = 4.76, He = 0.698, PIC = 0.673), and Serbian accessions (Na = 8.5, Ne = 4.52, He = 0.682, PIC = 0.656). The genetic distance analysis showed that the genetic distance between the Egyptian and Spanish accessions was the smallest (0.245), while the genetic distance between the Moroccan and Serbian accessions was the largest (0.398).

The NJ phylogenetic tree constructed based on SSR markers divided the 320 wheat landrace accessions into five main clusters. Cluster I mainly included accessions from Egypt and Morocco. Cluster

II mainly included accessions from Spain and Italy. Cluster III mainly included accessions from Greece. Cluster IV mainly included accessions from Serbia. Cluster V was a mixed cluster, including a small number of accessions from all six countries. The results of population structure analysis with STRUCTURE software showed that the optimal K value was 5, which was consistent with the phylogenetic tree analysis. Each cluster corresponded to a specific geographical region or ecological zone, indicating that the genetic structure of Mediterranean wheat landrace accessions was closely related to their geographical origin and ecological environment.

3.2 Genetic Diversity Analysis Based on GBS Data

GBS sequencing of 60 representative wheat landrace accessions generated a total of 1.42 Tb of raw sequencing data, with an average of 23.7 Gb per accession. After filtering, 1.35 Tb of clean data was obtained, with an average mapping rate of 97.8% to the wheat reference genome. A total of 6,234,578 high-quality SNP markers were identified, with an average density of 1 SNP per 486 bp. The nucleotide diversity (π) of the Egyptian accessions was the highest (π = 0.0035), followed by the Spanish accessions (π = 0.0033), Italian accessions (π = 0.0030), Greek accessions (π = 0.0028), Moroccan accessions (π = 0.0026), and Serbian accessions (π = 0.0024). The haplotype diversity (Hd) showed a similar trend, with the Egyptian accessions having the highest Hd (0.882) and the Serbian accessions having the lowest (0.795). Tajima‘s D values of all populations were negative (-0.92 to -0.35), indicating that the Mediterranean wheat landrace accessions 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 Egyptian and Spanish accessions was 0.132, between the Egyptian and Italian accessions was 0.165, between the Egyptian and Greek accessions was 0.187, between the Egyptian and Moroccan accessions was 0.143, between the Egyptian and Serbian accessions was 0.225, between the Spanish and Italian accessions was 0.118, between the Spanish and Greek accessions was 0.156, between the Spanish and Moroccan accessions was 0.135, between the Spanish and Serbian accessions was 0.203, between the Italian and Greek accessions was 0.124, between the Italian and Moroccan accessions was 0.148, between the Italian and Serbian accessions was 0.189, between the Greek and Moroccan accessions was 0.131, between the Greek and Serbian accessions was 0.178, and between the Moroccan and Serbian accessions was 0.196. These results indicated that there was moderate genetic differentiation between Mediterranean wheat landrace populations, and the genetic differentiation between the Egyptian and Serbian accessions was the highest.

Principal component analysis (PCA) based on SNP markers showed that the first two principal components explained 24.8% and 16.5% of the total genetic variation, respectively. The PCA plot clearly separated the wheat landrace accessions into five groups, corresponding to their geographical origins. The Egyptian and Moroccan accessions were distributed in the upper left of the plot, the Spanish and Italian accessions in the upper right, the Greek accessions in the middle, and the Serbian accessions in the lower part of the plot. This result was consistent with the population structure analysis based on SSR markers, further confirming that the Mediterranean wheat landrace accessions had obvious geographical genetic structure.

3.3 Phenotypic Variation of Disease Resistance

The phenotypic variation of stripe rust and leaf rust resistance of 320 Mediterranean wheat landrace accessions was analyzed. For stripe rust resistance, the disease severity score ranged from 0 to 8, with an average of 4.2. Among the accessions, 68 accessions (21.25%) were highly resistant (score 1-3), 124 accessions (38.75%) were moderately resistant (score 4-6), and 128 accessions (40.00%) were susceptible (score 7-9). For leaf rust resistance, the disease

severity score ranged from 0 to 9, with an average of 4.5. Among the accessions, 56 accessions (17.50%) were highly resistant, 118 accessions (36.87%) were moderately resistant, and 146 accessions (45.63%) were susceptible.

Statistical analysis showed that there were significant differences in disease resistance phenotypic indices between different geographical populations ($P < 0.05$). The Spanish wheat landrace accessions had the highest stripe rust resistance, with an average disease severity score of 3.2. The Egyptian accessions had the highest leaf rust resistance, with an average disease severity score of 3.5. The Serbian accessions had the lowest stripe rust and leaf rust resistance, with average disease severity scores of 5.8 and 6.2, respectively. Correlation analysis showed that there was a significant positive correlation between stripe rust resistance and leaf rust resistance ($r = 0.63$, $P < 0.01$), indicating that some wheat landrace accessions might have broad-spectrum disease resistance.

3.4 GWAS Analysis for Disease Resistance Traits

GWAS analysis was performed using the mixed linear model (MLM) to identify genomic regions associated with stripe rust and leaf rust resistance in wheat landraces. For stripe rust resistance, a total of 15 significant SNP markers were identified, distributed on 9 chromosomes (Chr. 1A, 2B, 3A, 4B, 5A, 5B, 6A, 7A, and 7B). These SNP markers defined 15 genomic regions associated with stripe rust resistance, with the physical distance ranging from 100 kb to 350 kb. For leaf rust resistance, 11 significant SNP markers were identified, distributed on 7 chromosomes (Chr. 1B, 2A, 3B, 4A, 5D, 6B, and 7D), defining 11 genomic regions associated with leaf rust resistance.

Candidate genes were identified within the genomic regions 50 kb upstream and downstream of the significant SNP markers. A total of 28 candidate disease-resistant genes were identified, including 16 genes associated with stripe rust resistance and 12 genes associated with leaf rust resistance. Among these candidate genes, 8 were NLR family genes (e.g., TraesCS2B02G187600, TraesCS3A02G213400), 6 were receptor-like kinase (RLK) genes (e.g., TraesCS4B02G198700, TraesCS5A02G201200), and 4 were transcription factor genes (e.g., TraesCS5B02G156700, TraesCS6A02G234500). Other candidate genes included genes involved in plant hormone signal transduction (e.g., TraesCS1A02G167800, encoding an ABA receptor) and genes involved in reactive oxygen species (ROS) scavenging (e.g., TraesCS1B02G123400, encoding a peroxidase).

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

4. Discussion

4.1 Genetic Diversity and Population Structure of Mediterranean Wheat Landraces

This study systematically analyzed the genetic diversity of 320 wheat landrace accessions from six major wheat-producing countries in the Mediterranean Basin using SSR markers and GBS technology. The results showed that Mediterranean wheat landraces had high genetic diversity, which was consistent with previous studies (Martinez et al., 2023; Hassan et al., 2024). The Egyptian and Spanish wheat landrace accessions showed the highest genetic diversity, which might be due to the fact that Egypt and Spain are important centers of wheat domestication and cultivation in the Mediterranean Basin, with a long history of wheat cultivation and rich ecological environments, promoting the formation of rich genetic variations (El-Esawi et al., 2023; Rossi et al., 2023). The Serbian accessions had the lowest genetic diversity, which might be related to the relatively narrow

distribution area and single ecological environment of wheat landraces in this region, leading to genetic drift and population bottlenecks (Petrovic et al., 2023).

Population structure analysis based on SSR markers and SNP markers showed that Mediterranean wheat landrace accessions could be divided into five distinct genetic clusters, corresponding to their geographical origins and ecological zones. This indicated that the genetic structure of wheat landraces was closely related to their geographical origin and ecological environment, which was consistent with the results of previous studies (Ozkan et al., 2023; Zhang et al., 2024). The genetic distance between the Egyptian and Spanish accessions 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 frequent cultural and trade exchanges between the two regions in history, which promoted the exchange of wheat germplasm resources (Martinez et al., 2024). The genetic distance between the Moroccan and Serbian accessions 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 North Africa and the Balkans (e.g., the Mediterranean Sea and mountain ranges), which hindered gene flow between populations.

The negative Tajima's D values of all Mediterranean wheat landrace populations indicated that these populations might have experienced a recent population expansion. This might be related to the expansion of wheat cultivation areas in the Mediterranean Basin during the Neolithic Age and the subsequent agricultural development. With the improvement of agricultural techniques, the distribution area of wheat landraces expanded, leading to population expansion (Ali et al., 2023). This result provides important clues for understanding the evolutionary history and domestication process of wheat in the Mediterranean Basin.

4.2 Mining and Functional Characteristics of Disease-Resistant Candidate Genes

GWAS analysis identified 15 genomic regions associated with stripe rust resistance and 11 regions associated with leaf rust resistance in Mediterranean wheat landraces. A total of 28 candidate disease-resistant genes were identified, including NLR family genes, RLK genes, and transcription factor genes. NLR family genes are the largest class of disease-resistant genes in plants, playing a crucial role in recognizing pathogen effectors and triggering immune responses (Rossi et al., 2024). The NLR genes identified in this study, such as TraesCS2B02G187600 and TraesCS3A02G213400, are homologous to known disease-resistant genes in wheat, suggesting that they might have similar functions in regulating stripe rust and leaf rust resistance.

RLK genes play an important role in plant disease resistance by recognizing pathogen-associated molecular patterns (PAMPs) and activating PAMP-triggered immunity (PTI) (Hassan et al., 2023). The RLK genes identified in this study, such as TraesCS4B02G198700 and TraesCS5A02G201200, might be involved in the recognition of rust pathogen PAMPs and the activation of downstream immune response pathways. Transcription factor genes, such as the NAC and MYB family genes identified in this study, can regulate the expression of a series of downstream disease-resistant genes, thereby enhancing plant disease resistance (Petrovic et al., 2024). For example, overexpression of the NAC transcription factor gene in wheat can significantly improve its resistance to stripe rust by regulating the expression of genes involved in cell wall reinforcement and ROS scavenging (Martinez et al., 2023).

Functional annotation and pathway analysis showed that the candidate disease-resistant genes were enriched in plant-pathogen interaction, MAPK signaling pathway, and plant hormone signal transduction. These pathways are closely related to plant disease resistance. The plant-pathogen interaction pathway is the first line of defense for plants against pathogens, involving the recognition of pathogens and the activation of immune responses (Ali et al., 2023). The MAPK signaling pathway transmits disease resistance signals to the

nucleus, activating the expression of disease-resistant genes (Zhang et al., 2024). The plant hormone signal transduction pathway regulates plant disease resistance through hormones such as salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA) (El-Esawi et al., 2023). These results indicate that the disease resistance of wheat landraces is a complex quantitative trait regulated by multiple genes and pathways.

4.3 Application Prospects of Wheat Landrace Germplasm Resources in Breeding

Wheat landraces in the Mediterranean Basin harbor abundant genetic diversity and valuable disease-resistant gene resources, which are important genetic materials for wheat breeding. The results of this study show that Mediterranean wheat landraces have high genetic diversity, and there are significant differences in disease resistance between different geographical populations. The Spanish wheat landraces have high stripe rust resistance, and the Egyptian landraces have high leaf rust resistance. These germplasm resources can be used as parents in wheat breeding programs to improve the disease resistance of modern wheat varieties.

The candidate disease-resistant genes identified in this study can be used as molecular markers for marker-assisted selection (MAS) in wheat breeding. For example, the significant SNP markers associated with stripe rust and leaf rust resistance can be used to screen disease-resistant wheat varieties at the seedling stage, improving breeding efficiency. In addition, the candidate genes can be cloned and transferred into modern wheat varieties through genetic engineering technology to obtain disease-resistant transgenic wheat varieties. For example, overexpression of the NLR family gene TraesCS2B02G187600 in wheat may improve its resistance to stripe rust.

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

5. Conclusion

This study systematically analyzed the genetic diversity and population structure of 320 wheat landrace accessions from six major wheat-producing countries in the Mediterranean Basin using SSR markers and GBS technology. The results showed that Mediterranean wheat landraces had high genetic diversity, with the Egyptian and Spanish accessions showing the highest genetic diversity. Population structure analysis divided the tested accessions into five distinct genetic clusters, corresponding to their geographical origins and ecological zones. We identified 15 genomic regions associated with stripe rust resistance and 11 regions associated with leaf rust resistance through GWAS, and 28 candidate disease-resistant genes were identified within these regions. These candidate genes are mainly involved in plant-pathogen interaction, MAPK signaling pathway, and plant hormone signal transduction, which are closely related to plant disease resistance.

This study provides a comprehensive overview of the genetic diversity of Mediterranean wheat landraces and identifies novel disease-resistant gene resources, laying a foundation for the utilization of wheat landraces in wheat breeding programs. The results also provide important clues for understanding the evolutionary history and adaptive mechanisms of wheat in the Mediterranean Basin. Future research should focus on the functional verification of candidate disease-resistant genes, the development of molecular

markers for MAS, and the utilization of wheat landrace germplasm resources in wheat breeding to improve the disease resistance of modern wheat varieties and promote the sustainable development of the wheat industry in the Mediterranean Basin and worldwide.

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