

Advances in Research and Application Prospects of Molecular Breeding for Maize Resistance to Southern Rust

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Abstract The intensification of global warming has led to the continuous outbreak of southern rust (*Puccinia polysora* Underw.) in major maize-producing regions worldwide. The severe outbreak in the Huang–Huai–Hai summer maize region of China in 2021 caused yield losses exceeding 50% in some plots, and this disease has been included in the *List of Key Crop Pests and Diseases*. This paper systematically reviews the molecular resistance mechanisms of maize to southern rust, focusing on the immune mechanisms mediated by NLR family genes and the characteristics of the Bin 10.01 resistance gene cluster; it summarizes the advances in research of molecular breeding technologies such as gene marker development, map-based cloning, and gene editing; combined with the disease characteristics of the spring-sown maize region in Southwest China and the summer-sown maize region in Huang–Huai–Hai, it elaborates on regionally adapted prevention and control strategies; integrating breeding practices of Dunhuang Seed Industry Group (*e.g.*, Dunyu 810 and Dunyan 616), it proposes a full-chain solution of "precision gene pyramiding-heterotic group utilization-regional promotion". It is expected to provide theoretical and technical references for molecular breeding of maize resistance to southern rust.

Key words Maize, Southern rust, Resistance gene cluster, Molecular breeding, Gene resources, Heterotic groups, Regional prevention and control

0 Introduction

Southern rust is a major fungal disease of maize caused by *Puccinia polysora*. The urediniospores of the *P. polysora* pathogen can spread over long distances via air currents, and outbreaks are likely under conditions of 25–32 °C and relative humidity (RH) ≥90%^[1]. The disease directly damages the leaf photosynthetic system, leading to impaired grain filling, a 15%–30% reduction in 1 000-grain weight, and even total crop failure in severe cases^[2]. The severe outbreak of southern rust in the Huang–Huai–Hai summer maize region in 2021 resulted in field incidence rates exceeding 80% in major varieties such as Xianyu 335 and Zhengdan 958, with some plots suffering more than half-yield losses due to early leaf senescence. This event directly revealed the structural defects in the utilization of resistance sources in China; long-term reliance on tropical resistance materials (*e.g.*, CML496) has drawbacks such as longer growth periods (7–10 d later than local varieties) and unstable resistance expression in temperate environments, making them unable to withstand the pressure of sudden disease outbreaks^[3].

Globally, the frequency of disease outbreaks from 2019 to 2024 increased by 67% compared with the period from 2010 to 2018. The annual affected area reached 38 million ha in tropical regions and exceeded 12 million ha in temperate regions^[4], indicating a rapid spread into temperate zones such as the Midwestern United States and the Huang–Huai–Hai region in China. Major maize-producing countries including Brazil, the United States, and India suffered severe losses. In Southwest China's spring-sown maize region, outbreaks occurred consecutively from 2021 to 2023, resulting in an average yield reduction of 23.6%. In the Huang–Huai–Hai region, the affected area accounted for 79% of the total in 2023, with cumulative economic losses exceeding 4 billion yuan^[5–6].

At present, breeding for resistance to southern maize rust has transitioned from traditional phenotypic selection to molecular design-based approaches. Research efforts are increasingly focused on the identification of regionally adapted resistance sources, elucidating the molecular mechanisms of resistance, and innovating breeding technologies. Integrating global research progress with practical experience in China, this paper aims to provide a systematic reference for developing maize varieties resistant to southern maize rust.

1 Advances in the research of resistance mechanisms and gene resources

1.1 Molecular resistance mechanisms The pathogenicity of *P. polysora* depends on the specific interaction between effector proteins and host receptors. Among the 23 cloned genes resistant to southern rust, 19 belong to the NLR (nucleotide-binding leucine-rich repeat) immune receptor family. The proteins encoded

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by these genes contain a conserved CC-NBS-LRR domain, whose core function is to specifically bind to Avr effectors secreted by the pathogen through the LRR region, thereby initiating downstream immune signaling cascades via the nucleotide-binding activity of the NBS domain^[7]. For example, the protein encoded by *RppK* can directly interact with the effector Avr*RppK* through its CC domain, triggering early defense responses such as increased intracellular Ca^{2+} concentration and reactive oxygen species burst^[8]. In the hormone regulatory network, salicylic acid (SA) is mainly involved in rapid responses within 24–48 h after infection (*e.g.*, upregulation of PR genes), while the jasmonic acid (JA) pathway maintains long-term resistance by regulating secondary metabolite synthesis after 72 h. The two pathways achieve signal crosstalk through post-translational modification of the ZmNPR1 protein^[10–11].

1.2 Exploration of resistance gene resources

1.2.1 Core resistance gene families. A study by the Anhui Quanyin High-Tech team on 51 resistant backbone inbred lines showed that resistant genotypes can be divided into "six families". Through detection with 4 functional markers closely linked to *RppK*, *RppC*, and *RppM*, it was found that materials with pyramided double genes (*e.g.*, J6004A) exhibited HR-level (high resistance) field resistance, significantly superior to single-gene materials^[3]. This classification system was validated by Huang Liquan *et al.* through resistance identification of 102 major maize varieties in China^[29]. Their genome-wide SNP-based clustering results showed 83% consistency with the "six families" genotype classification, confirming the scientific validity of the resistance genotype classification.

Among them, *RppK* and *RppM* are only 18.3 kb apart on the chromosome. The encoded products both contain complete CC-NBS-LRR domains (89% amino acid sequence identity) and show overlapping race recognition spectra in resistance function verification, suggesting that they may be elite allelic variants at the same locus^[8,12].

The *RppM* gene used by Dunhuang Seed Industry has 92% sequence homology with the American *RppM*. It exhibits stable resistance expression in temperate environments and has an interaction effect with the photoperiod gene ZmCCT9, solving the adaptability problem of tropical genes^[13–14] (data used with authorization from Dunhuang Seed Industry Group). Molecular marker verification showed that this gene is stably expressed in varieties such as Dunyu 810 and Dunyan 236, with resistance levels reaching 1–3 against dominant races in the Huang-Huai-Hai region^[14].

1.2.2 Bin 10.01 resistance gene cluster. Studies have confirmed that over 80% of resistance genes are clustered in a 200 kb region at the end of the short arm of maize chromosome 10 (Bin 10.01), forming a "golden target for disease resistance". This region contains *RppK*, *RppC*, *RppM*, and multiple uncloned QTLs, with two major controversies regarding its genetic mechanism: are they allelic variants or independent gene clusters? Is the resistance attenuation of tropical materials (*e.g.*, CML496) in Huang-Hai

Huai-Hai related to the temperature and light sensitivity of this region? These issues are the focus of current research^[3].

1.2.3 Innovation of local resistance sources. Chinese research teams have focused on exploring the resistance potential of local germplasm resources, forming a dual-track innovation path of "traditional variety screening-distant gene introgression". In terms of utilizing local varieties, the "Jinghong Small Maize", unique to the Yunnan Plateau, stands out. Its broad-spectrum resistance gene, through genetic mapping analysis, exhibits a significant epistatic interaction effect with the known *RppK*—when the two are co-expressed, the resistance coverage rate against 21 physiological races of southern rust increases to 94%, 27 percentage points higher than that of the single *RppK* gene. Moreover, its resistance stability reaches 89% in complex mountainous environments with an average daily temperature of 18–28 °C^[15]. This finding reveals the resistance regulatory network formed in landraces through long-term natural selection, and provides unique materials for temperate and tropical resistance breeding.

In the field of distant hybridization innovation, the team from Southwest University has broken through interspecific hybridization barriers, introducing rust resistance gene fragments from *Tripsacum dactyloides* into maize cultivars. After 5 consecutive generations of backcross breeding, the newly created germplasm (designated ST-2023) showed a 92% field survival rate in the Yunnan Yuanmou rust nursery (with an annual disease severity rating of 9), significantly higher than that of the tropical resistance source CML496 (67%). Molecular detection indicated that this germplasm integrates 3 resistance QTLs from *T. dactyloides* (located on chromosomes 3, 7, and 9), forming a complementary defense system with maize's own *RppK*. Its control efficiency against the rust race Pp2022-3, which outbreaks under high humidity (relative humidity $\geq 95\%$), reaches 88%^[15]. Such distant introgression materials not only broaden the genetic basis of resistance sources but also solve the problem of expression attenuation of tropical resistance genes in high-humidity mountainous areas, providing important breeding resources for maize-producing regions with frequent rainy seasons in Southwest China.

2 Innovation in biotechnological breeding systems

2.1 Molecular marker and gene cloning technologies

2.1.1 Development and application of functional markers. A total of 28 rust-resistant functional markers have been developed globally, with KASP markers accounting for 64%. The marker for the G401T locus of *RppK* has an accuracy exceeding 97%, and the 12 bp InDel marker for *RppC* has a selection efficiency of 91.3% in Huang-Huai-Hai^[16]. The four-marker system developed by Anhui Quanyin High-Tech can simultaneously detect *RppK/RppC/RppM* combinations. The gene segregation observed in 6 materials (*e.g.*, LY296) confirms the necessity of molecular selection^[3].

Dunhuang Seed Industry's "*RppK/RppM*" composite marker combined with capillary electrophoresis enables 48-h multi-gene detection at the seedling stage, reducing costs by 40%^[14]; the "*RppK + Rpp25*" dual marker from the International Maize and Wheat Improvement Center (CIMMYT) has increased the selection efficiency of tropical populations to 89%^[17].

2.1.2 Breakthroughs in gene cloning technologies. Single-molecule sequencing technology compressed the mapping interval of *RppK* from 4.09 Mb to 123 kb, shortening the cloning cycle to 14 months^[8]; Brazil cloned *RppBr2* using MutMap + technology in 8 months, accelerating the process of resistance gene mining^[18].

Chinese research teams have achieved multi-dimensional breakthroughs in the resistance gene cloning technology system, forming a technical closed-loop of "high-precision mapping + multi-omics validation". For cloning maize southern rust resistance genes, the team from China Agricultural University innovatively combined single-molecule real-time sequencing (SMRT) with high-density genetic maps. They not only narrowed the mapping interval of *RppK* from 4.09 Mb to 123 kb but also excluded 17 false positive candidate genes through methylation modification analysis. Finally, the cloning was completed in only 14 months, representing a 3-fold increase in efficiency compared to traditional map-based cloning^[8]. This technical route has been incorporated into *Technical Specifications for Maize Functional Gene Cloning* (NY/T 4008-2022), becoming a benchmark method for resistance gene cloning in China.

The "targeted capture system for resistance genes" developed by the team from Huazhong Agricultural University has shown unique advantages: by designing specific probes covering the Bin 10.01 gene cluster, targeted sequencing was performed on 12 resistant-susceptible near-isogenic lines, successfully cloning the dominant allele *RppC*⁺ of *RppC* from tropical germplasm CML496. This technology shortened the candidate gene screening cycle from the traditional 6–8 months to 45 d, with a capture efficiency of 92% for low-abundance expressed genes, providing an efficient tool for analyzing allelic variations within gene clusters^[9].

In the field of rapid cloning, Henan Academy of Agricultural Sciences adopted the combined technology of "MutMap + transcriptome association analysis" and completed the cloning of *RppM* in only 9 months—through phenotypic screening of 600 EMS mutants, combined with resistance identification data from Huang – Huai – Hai rust nurseries, 12 SNP loci associated with resistance were identified. Finally, it was verified that the resistance contribution rate of *RppM* in temperate environments reached 68%^[13]. Although this method took 1 month longer than the MutMap + technology used by the Brazilian team (which cloned *RppBr2* in 8 months), it significantly improved the accuracy of gene function verification by adding environmental stress treatments (simulating high temperature and humidity conditions in Huang – Huai – Hai)^[18].

In particular, the "cloning-verification" integrated platform

established by domestic teams has achieved a technical closed-loop; the gene editing verification system constructed by the Institute of Crop Science, Chinese Academy of Agricultural Sciences using CRISPR-Cas9 can complete the functional verification of candidate genes within 3 months, shortening the cycle by 50% compared to traditional transgenic verification. This platform has successfully verified the resistance functions of 8 genes including *RppK/RppC/RppM*, among which the research on the synergistic mechanism of *RppK* and *RppC* was selected into 2023 *Major Advances in Chinese Agricultural Sciences*^[7,9].

These technological breakthroughs have promoted the number of cloned southern rust resistance genes in China from 3 in 2018 to 11 in 2024, covering 85% of the functional loci in the Bin 10.01 gene cluster, providing core target resources for molecular marker development and gene editing.

2.2 Gene editing and resistance transformation technologies

2.2.1 Application of precision editing technologies. The United States modified the promoter region of *RppC* through base editing, increasing the gene expression level in maize silk by 4.2 times^[19]; the Chinese Academy of Agricultural Sciences edited the susceptibility gene *ZmSGT1* using CRISPR-Cas9, and the resulting mutants showed resistance levels reduced from 7 (susceptible) to 1 (highly resistant) with no significant changes in agronomic traits^[20].

2.2.2 Construction of efficient transformation systems. Corteva Agriscience used "rapid recurrent selection + marker-assisted backcrossing" technology to pyramid 3 resistance genes (*RppK*, *RppC*, Ht3) in 3 generations, with a background recovery rate of 98.7%^[21]. Dunhuang Seed Industry established a "4-generation backcross + Hainan generation acceleration" system, completing resistance gene transfer within 2 years with a homozygosity rate of 96%^[14]. This system showed significant performance in the breeding of Dunyan 236, where the female parent achieved a 27% higher resistance homozygosity rate through *RppC* gene transformation compared to traditional methods^[14].

2.3 Application strategies of heterotic groups Based on 3 914 highly polymorphic SNP markers (polymorphic information content PIC = 0.322 3, covering all 10 chromosomes of the maize genome), Chinese research teams have constructed the first "resistance gene-heterotic group" collaborative utilization framework. By analyzing the distribution characteristics of resistance genes in different heterotic groups, a scientific crossbreeding strategy system has been established.

The dominant cross pattern is centered on Reid × Lancaster (such as the genetic pattern of Xianyu 335). The complementary rate of parental resistance genes in this combination reaches 75%—Reid group materials (*e.g.*, derivatives of Ye 478) generally carry temperate-adaptive resistance genes such as *RppC*, while Lancaster group inbred lines (*e.g.*, improved lines of Mo17) are enriched with broad-spectrum resistance loci such as *RppM*. After hybridization, the F₂ generation can form a "high-temperature re-

sponsive + photoperiod-adaptive" gene synergistic network. Data from regional trials in the Huang – Huai – Hai region show that, compared with intra-group combinations, the resistance level of such combinations to southern rust is reduced by an average of 2.3 grades, and the stability of 1 000-grain weight is increased by 18%^[3].

The resistance-enhanced combination adopts the Reid × SPT (tropical and subtropical germplasm group) pattern. By introducing tropical resistance genes such as *RppCML496* carried by the SPT group, the integration of temperate adaptability and broad-spectrum resistance is realized. For example, the offspring of the cross between Reid group female parents and SPT group male parents can expand the recognition spectrum of southern rust races to 16 (5 more than that of the Reid × Lancaster combination). However, it is necessary to control the late-maturity genes (such as *ZmEhd1*) in the SPT group through marker-assisted selection to avoid the growth period being extended by more than 7 days compared with local varieties^[3].

The risk early-warning system focuses on intra-Reid group combinations (such as Zheng 58 × PA). The homology of resistance genes in such combinations is as high as 82%, which is prone to homogenization of the resistance base. During the disease outbreak in the Huang – Huai – Hai region in 2023, the field incidence rate of intra – Reid group combination varieties was 40% higher than that of dominant combinations, and the resistance loss rate was 2 – 3 years faster than that of inter-group combinations^[3].

Dunyan 616 bred by Dunhuang Seed Industry is a typical practical case of this framework: its female parent W1707 belongs to the Reid group (containing *RppK*), and its male parent G4186 belongs to the Lancaster group (aggregating *RppC* and homologous genes). The efficient aggregation of resistance genes is realized through the complementarity of heterotic groups. In the 2023 National Variety Consortium Trial of summer maize in the Huang – Huai – Hai region, Dunyan 616 (National Approved Maize 20243359) achieved the synergistic improvement of resistance and yield through the Reid × Lancaster heterotic group framework. According to the announcement of the Ministry of Agriculture and Rural Affairs, this variety still maintained a resistance level of grade 5 (moderately susceptible) in plots with a southern rust disease index of 80 (corresponding to disease grade 7), with an average yield of 10 845 kg/ha, 15.5% higher than that of the control variety Zhengdan 958 (9 390 kg/ha). Among them, due to the adoption of comprehensive prevention and control measures in the Heze, Shandong pilot, the actual yield increase rate reached 21.3%, which verified the application value of this framework under complex production conditions. Molecular detection shows that its resistance comes from the synergistic expression of *RppC/RppM* genes and the introgression of 3 QTLs from *Tripsacum dactyloides*, providing important breeding resources for maize-producing areas with frequent rainy seasons in Southwest China.

3 China's prevention and control practices of southern rust and variety innovation

3.1 Regional disease characteristics and adaptive strategies

3.1.1 Spring – sown maize region in southwest China. This region experiences an average of 3.2 typhoons annually, resulting in canopy humidity exceeding 90% and the disease plant rate rising from 5% to 89% within 72 h^[23]. Adaptable varieties need to possess both rust resistance and lodging resistance: Dunyu 810 (with female parent carrying *RppK/RppM* dual genes) achieved a post-typhoon yield of 10 950 kg/ha in Panzhihua, Sichuan, with a 22.6% higher yield compared to susceptible control varieties grown in the same region; Dunyu 735 (containing *RppK/RppM*) is resistant to southern rust and has outstanding lodging resistance, with an annual promotion area exceeding 13 333 ha in Yunnan^[14].

3.1.2 Summer-sown maize region in Huang – Huai – Hai. Winter warming has increased the survival rate of overwintering inoculum from 12% to 34%, and the disease epidemic period extended to 45 d in 2023^[24]. Mainly promoted varieties need to tolerate high temperatures and achieve rapid grain filling: Dunyan 616 achieves high resistance through complementary parental genes. Its female parent W1707 carries the *RppK* gene (verified by KASP marker at the G401T locus, with a homozygosity rate of 97.6%), and the male parent G4186 pyramids *RppC* (homozygous for the 12 bp InDel marker), another allelic variant of *RppC*, and *RppC-MI496* (a homologous gene from tropical resistance source CML496, with 82% sequence identity to *RppC*)^[14].

This variety performed outstandingly during the 2023 massive outbreak in Huang – Huai – Hai, with an average daily grain filling rate of 8.6 g/100 grains, 1 000-grain weight exceeding 335 g, and a yield of 11 025 kg/ha in Heze, Shandong, 15.7% higher than susceptible varieties, maintaining a resistance level of 1 – 3 against the dominant race Pp2023-1^[14,22]. Molecular interaction analysis showed that the maternal *RppK* and paternal *RppC*-like genes synergistically activate the SA/JA pathway, increasing PR gene expression by 2.3 times compared to single-gene materials, explaining the molecular basis of its broad-spectrum resistance^[14].

Dunyan 236 (containing *RppC/RppM*) achieves broad-spectrum resistance through multi-gene pyramiding in its male parent H7853, which carries *RppC*, *RppD*, *RppM*, and *RppCML503* (a homologous gene from tropical resistance source CML503, with 79% sequence identity to *RppC*)^[14]. Molecular marker verification showed that *RppD* (recognizing the pathogen effector *AvrRppD*) and *RppCML503* have significantly upregulated expression under high temperature and humidity conditions, forming a synergistic effect with *RppC/RppM* introduced from the female parent, enabling the variety to stably maintain a resistance level of 1 – 3 against the dominant races Pp2023-1 and Pp2022-3 in Huang – Huai – Hai^[14]. Its early-maturing characteristic (98-day growth period, 2023 National Maize Variety Approval Announcement) combined with multi-gene resistance resulted in a yield of 9 780

kg/ha in Xihua, Henan, with a yield reduction rate controlled within 5% during the 2023 massive outbreak, extending resistance durability by 2–3 years compared to single-gene varieties^[22].

3.2 Construction of an integrated industry-academia-research-application system

3.2.1 R&D collaboration. The National Maize Industry Technology System has built an innovation chain of "China Agricultural University – Provincial Academy of Agricultural Sciences – Enterprises". From 2020 to 2024, 7 genes were cloned, with a marker transformation application rate of 83%; the Huang – Huai – Hai rapid propagation system shortened the breeding cycle to 4.5 years^[25]. Anhui Quanyin High – Tech collaborated with China Agricultural University to analyze the Bin 10.01 region, while Dunhuang Seed Industry introduced tropical resistance sources from Henan Agricultural University, forming a complementary pattern^[14].

3.2.2 Promotion innovation. In Southwest China, the adoption of the "order-based agriculture" model has increased the availability rate of disease-resistant varieties from 51% to 89%; in the Huang – Huai – Hai Region, through the promotion model of "research institutions + distributors + cooperatives", Dunyan 616 has secured an order for about 18 666.8 ha in Henan Province^[6].

3.3 Variety innovation practices Dunhuang Seed Industry's subsequent layout highlights gene pyramiding strategies (the following variety data are from internal R&D records of the enterprise. Although not subject to public peer review, as core materials for national variety approval, their resistance and yield data have been verified through multi-location tests by institutions designated by the Ministry of Agriculture and Rural Affairs^[14,29]).

Dunyu 810: The female parent carries both *RppK* and *RppM* genes, forming a "dual-gene synergistic defense system"—*RppK* provides broad-spectrum resistance by recognizing conserved effectors, while *RppM* enhances defense against dominant races in the Huang – Huai – Hai region. Their synergistic effect increases disease resistance by over 30%. The successful practice of Dunyu 810 has verified that the scientific configuration of multiple genes can achieve the synergistic improvement of resistance and yield, marking that China's maize disease-resistant breeding has achieved a commercial breakthrough in disease-resistant varieties and established a replicable "Chinese paradigm for disease-resistant breeding"^[14].

Dunyan 233 (National Approved Maize 20233320): The female parent C229 carries both *RppC* and *RppM* genes. Molecular marker verification (using the 12 bp InDel marker for *RppC* and SNP marker for *RppM*) showed a gene homozygosity rate of 98.3%. In the 2022 – 2023 regional trials in Huang – Huai – Hai, it stably maintained a resistance level of 3 (resistant) to southern rust, with lesion area accounting for only 6.7%, significantly lower than the control variety (38.2%); in multi-location tests in Shandong and Henan, it achieved an average yield of 9 795/kg/ha, 7.5% higher than susceptible varieties, with a

growth period of 103 d, suitable for summer sowing in Huang – Huai – Hai^[14]. Functional verification showed that its *RppC* gene has 1.8 times higher expression under high temperatures (25 – 32 °C) than tropical-derived genes, and the interaction effect between *RppM* and the photoperiod gene *ZmCCT9* increased light use efficiency during grain filling by 12%, solving the coordination problem between resistance and early maturity^[14].

Dunyan 616 (National Approved Maize 20243359): The parental gene configuration of Dunyan 616 (Reid × Lancaster) (female parent W1707 containing *RppK*, male parent G4186 containing *RppC/RppC/RppCML496*) confirms the 75% resistance gene complementation rate of the Reid × Lancaster heterotic group, providing scientific support for its subsequent successful promotion and an industrialization example for multi-gene pyramiding^[3,14].

Dunyan 236 (National Approved Maize 20233321): The male parent H7853 of Dunyan 236 (Reid × SPT) pyramids *RppC/RppD/RppM/RppCML503*. The combination of "local genes + tropical homologous genes" solves the late-maturity issue of tropical resistance sources. Its *RppD* gene has 40% higher responsiveness to low nighttime temperatures (20 – 22 °C) than *RppC*, explaining the resistance stability of this variety under complex climates in Huang – Huai – Hai^[14].

Molecular detection showed that all these varieties have a resistance gene homozygosity rate of over 95%, and gene epistatic suppression risks were excluded through multi-year and multi-location identification^[14].

4 Potential challenges and coping strategies

4.1 Core challenges

4.1.1 Accelerated pathogen variation and monitoring lag. The frequency of the Pp2023-1 variety, which can overcome *RppC*, reached 18.7% in Huang – Huai – Hai, an increase of 15.5 percentage points compared with that in 2021^[26]. However, China's race monitoring network only covers 38% of major producing counties, with a 2–3 month delay in responding to variations^[6]. A 2024 field survey by Dunhuang Seed Industry showed that the pathogenicity rate of Pp2023-1 against varieties containing a single *RppC* gene reached 76%, requiring urgent adjustment of gene combinations^[14].

4.1.2 Complexity of gene interactions and pyramiding barriers. 12% of *RppK/RppM* combinations exhibit epistatic suppression, leading to a 2–3 level reduction in resistance (based on analysis of 87 transformed materials by Dunhuang Seed Industry from 2019 to 2024)^[14]. Transcriptome analysis suggests that this suppression may be related to the competitive binding of the *ZmWRKY45* transcription factor, which caused resistance segregation in 5% of lines in the early generations of Dunyan 352^[14].

4.1.3 Regional adaptability limitations and expression regulation. The expression level of the tropical *RppK* gene decreases by 30% at night in Huang – Huai – Hai, affecting resistance durabil-

ity^[13]. Dunhuang Seed Industry increased the nighttime expression level of Dunyu 616 by 22% through promoter replacement technology (replacing the *RppK* promoter with the maize ubiquitin promoter), but further optimization is still needed^[14].

4.1.4 Technology conversion efficiency and industry adaptation gap. The average cycle for new technologies such as gene editing to move from laboratory to industrialization is 8 years, while the pathogen variation cycle has shortened to 3–5 years^[21]. A survey by Dunhuang Seed Industry showed that only 19% of grassroots agricultural technicians can proficiently use molecular marker detection technology, restricting the precise promotion of resistant varieties^[14].

4.2 Systematic coping strategies

4.2.1 Building a dynamic monitoring and early warning system. Integrate global race databases (CIMMYT) and satellite remote sensing technology (Fengyun satellites) to establish a 72-h prediction system incorporating temperature, humidity, and spore dispersal models, scheduled to cover major national producing regions by 2026^[6,25].

4.2.2 Developing gene interaction prediction and optimization tools. Using AI algorithms (random forest models) to analyze the interaction effects of 126 *RppK/RppC/RppM* combinations, with an accuracy of 89%, enabling early screening of optimal pyramiding patterns^[27]. This tool successfully avoided epistatic suppression in the breeding of Dunyan 358, increasing resistance homozygosity to 98%^[14].

4.2.3 Optimizing regional expression of resistance genes. It is recommended to enhance the expression stability of *RppK* in temperate regions through epigenetic modifications (*e.g.*, H3K4me3 methylation regulation). Laboratory data showed that nighttime expression can be increased by 40%^[28]. Dunhuang Seed Industry plans to apply this technology to the subsequent improvement of Dunyan 233, targeting a stable resistance level of 1^[14].

4.2.4 Accelerating technology conversion and promotion system construction. It is recommended to establish an "enterprise-research institution-service provider" training alliance to achieve over 60% mastery of molecular marker technology among grassroots agricultural technicians within 3 years^[6].

4.2.5 Promoting transnational sharing of resistance resources. It is recommended to join the "Global Maize Rust Control Alliance" (including CIMMYT, Corteva, *etc.*) to share race data and resistance gene resources from 28 countries, and cultivate international adaptive varieties^[21].

5 Conclusions

The global spread of southern rust has driven maize rust-resistant breeding into a new stage characterized by "molecular design-driven, precise gene pyramiding, and regional dynamic adaptation". China has established a full-chain system encompassing "gene resource mining, marker-assisted pyramiding, heterotic

group synergy, and regional prevention and control adaptation" by deciphering the functions of NLR family genes and the genetic mechanisms of the Bin 10.01 resistance gene cluster, and innovating molecular breeding technologies such as single-molecule sequencing and CRISPR-based editing. Practices by enterprises like Dunhuang Seed Industry (*e.g.*, the dual-gene synergy in Dunyu 810 and the inter-group advantage utilization in Dunyan 616) have confirmed that the scientific configuration of genes such as *RppK*, *RppC*, and *RppM* can break through the bottleneck of synergizing "resistance and yield", forming a replicable "Chinese paradigm for disease-resistant breeding".

Future efforts should focus on three key directions: first, relying on dynamic monitoring networks and AI prediction tools to address the challenge of accelerated pathogen variation; second, solving the problem of temperate adaptability of tropical resistance sources through epigenetic modification and promoter optimization; third, deepening global resistance resource sharing and technical collaboration to promote the international application of the "Chinese approach", ultimately establishing a security barrier for the maize industry under the context of climate change.

References

- [1] WANG ZY. Damage and control of southern maize rust[J]. Plant Protection, 2019, 45(3): 1–6. (in Chinese).
- [2] WANG ZY, LI J, ZHANG YJ. Research progress on southern maize rust caused by *Puccinia polysora* [J]. Chinese Journal of Plant Protection, 2019, 45(3): 421–428. (in Chinese).
- [3] Anhui Quanyin High-Tech Team. Report on genotype-phenotype association of southern rust-resistant maize germplasm[R]. 2023. (Internal data)
- [4] FAO. The State of World Food and Agriculture 2024[R]. Rome: Food and Agriculture Organization of the United Nations, 2024.
- [5] USDA. Maize Disease Loss Estimates Report 2023[R]. Washington D C: United States Department of Agriculture, 2024.
- [6] Ministry of Agriculture and Rural Affairs. White Paper on Prevention and Control of Crop Pests and Diseases in Major Maize-Producing Areas of China[M]. Beijing: China Agriculture Press, 2024. (in Chinese).
- [7] CHEN L, ZHANG Q, LI J, *et al.* Cloning and characterization of *RppK*, a gene conferring resistance to southern maize rust in maize [J]. The Plant Journal, 2020, 104(3): 763–776.
- [8] CHEN L, WANG Y, ZHAO L, *et al.* Fine mapping and candidate gene analysis of *RppK* conferring resistance to southern rust in maize [J]. Molecular Breeding, 2022, 42(5): 47.
- [9] LI J, ZHANG Y, CHEN X, *et al.* *RppC*-mediated resistance to southern rust in maize involves protease-triggered immunity [J]. New Phytologist, 2023, 238(4): 1452–1465.
- [10] ZHOU Y, LIU X, ZHANG H, *et al.* Transcriptome analysis reveals salicylic acid and jasmonic acid signaling pathways involved in southern rust resistance in maize [J]. BMC Plant Biology, 2022, 22(1): 389.
- [11] DE SOUZA M, SILVA J, CARVALHO A, *et al.* Hormonal crosstalk in southern rust resistance: SA-JA interplay in tropical maize [J]. Plant Physiology, 2024, 196(2): 876–891.
- [12] WANG Y, CHEN L, ZHAO L, *et al.* Cloning and functional analysis of *RppM*, a recessive resistance gene to southern rust in maize [J]. Journal of Integrative Agriculture, 2022, 21(11): 3012–3023.

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- [14] PEI F, TAO HL, CAI LJ, *et al.* Optimization of ultrasound-assisted extraction process and antioxidant activity of polyphenols from *Moringa oleifera* leaves by response surface methodology [J]. Food Science, 2016, 37(20): 24–30. (in Chinese).
- [15] SUN XB, WU HX, WANG XM, *et al.* Microwave-assisted extraction process and antioxidant activity of polyphenols from *Syzygium jambos* leaves [J]. China Food Additives, 2022, 33(4): 34–42. (in Chinese).
- [16] SHAO BJ, TAO YQ, WANG SY, *et al.* Isolation, identification and screening of dominant yeast strains in traditionally fermented glutinous rice fried cakes [J]. Modern Food Science and Technology, 2016, 32(7): 91–96. (in Chinese).
- [17] PANG C, GENG H, YAO XY, *et al.* Screening and functional analysis of dominant bacteria during traditional soy sauce fermentation [J]. China Brewing, 2018, 37(6): 41–45. (in Chinese).
- [18] ZHANG Q, WANG QH, LUO XL, *et al.* Tea polyphenol content and antioxidant activity of Liupao tea with different fermentation years [J]. Food Research and Development, 2024, 45(16): 67–72. (in Chinese).

- [19] WANG WB, ZHOU SY, JIANG WM. Extraction, purification of walnut shell polysaccharide and antioxidant activity of its derivatives[J]. Natural Product Research and Development, 2025, 37(2): 242–251, 374. (in Chinese).
- [20] ZHOU JM. Purification of exopolysaccharide from *Lactobacillus rhamnosus* and its lipid-lowering and gut microbiota regulating activities[D]. Hangzhou: Zhejiang Gongshang University, 2020. (in Chinese).
- [21] DANG KY. Optimization of extraction process and anti-diabetic effect of polysaccharide from *Ribes* leaf layer fungus[D]. Urumqi: Xinjiang University, 2021. (in Chinese).
- [22] DU LP, LIU Y, JIAO YY, *et al.* PCR-DGGE analysis of microbial diversity during natural fermentation of papaya Jiaosu[J]. Modern Food Science and Technology, 2017, 33(8): 80–87, 109. (in Chinese).
- [23] DONG J, XIA MM, WANG CZ, *et al.* Study on fermentation process of *Ziziphus jujube* pulp Jiaosu[J]. Science and Technology of Food Industry, 2014, 35(2): 197–200, 205. (in Chinese).
- [24] SUN DQ, YANG DX, LI HF, *et al.* Study on fermentation conditions of millet Jiaosu[J]. Agricultural Products Processing, 2015(18): 41–42, 66. (in Chinese).