

Research Insight

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High-Throughput Phenotyping Coupled with GWAS for Fusarium Head Blight Resistance in Wheat

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Abstract Fusarium head blight (FHB) of wheat is a fungal disease that seriously affects global wheat yield and food safety. Its resistance breeding has always been limited by inaccurate phenotypic evaluation and low efficiency of resistance gene localization. This study introduced the prevalence mechanism of FHB and the resistance types of wheat, and analyzed the biological basis of its main phenotypic indicators. By constructing a GWAS model based on SNP chips and resequencing data, and conducting joint analysis in combination with multi-dimensional phenotypic information, multiple stably expressed resistance QTLs and candidate genes were identified. It further revealed the regulatory pathways related to the infection, spread and toxin accumulation of scab. In the practical case section, this study reviewed the resistance research practices of representative wheat groups in the United States, China and other places, and verified the application value of combined phenotypic-genotype analysis in the discovery of new resistance resources. This study demonstrates the significant role of high-throughput phenotypes and GWAS integration strategies in enhancing the efficiency of resistance gene mining and phenotypic accuracy, and is expected to achieve new breakthroughs in precise breeding of FHB resistance, providing technical support for ensuring global wheat production security.

Keywords Wheat scab; High-throughput phenotype; Genome-wide association analysis (GWAS); Resistant QTL; Candidate gene

1 Introduction

Wheat scab (FHB) has long been a headache for breeders, not only because of the yield loss it causes, but also because of the food safety risks it triggers. *Fusarium graminearum* is the "culprit" of this disease. It causes grains to accumulate a large amount of mycotoxins, such as trichothecene compounds. Contaminated grains may be inedible at all and even affect the health of humans and animals (Song et al., 2025; Wang et al., 2025). If it were merely a decrease in output, the problem might still be manageable, but unfortunately, this disease is very likely to break out. The climate has become increasingly unstable, and coupled with some changes in agricultural management methods, wheat scab has become more frequent and severe in many areas in recent years.

Many people think that as long as they find disease-resistant genes, the problem can be solved. But the actual situation is much more complicated. Wheat resistance to scab is a typical quantitative trait regulated by multiple genes, and the contribution of each locus is usually not significant (Syed et al., 2025). What is even more difficult is that there are not many truly stable resistant germplasm resources. Even the resistance genes that have been cloned, such as Fhb1 and Fhb7, cannot be used at will in actual breeding projects. Most commercial varieties still lack resistance. Furthermore, the expression of resistance traits is also complex influenced by the environment, host and pathogenic bacteria, making phenotypic assessment difficult to standardize. This makes large-scale and precise screening extremely challenging (Buerstmayr et al., 2020). Of course, there are still breakthroughs. In recent years, the development of molecular marker technology and the wide application of GWAS have indeed made the identification of resistance sites much more efficient (Jiang et al., 2025). The problem lies in that whether the technological achievements in the laboratory can truly be implemented in the fields still needs to overcome a complete set of adaptation thresholds for the breeding system. Whether it is high-throughput assessment or genomic tools, if they cannot be integrated into the existing processes, they will be difficult to play a substantive role.

This study reviews the current threats and challenges faced by scab, elaborates on the methods and results of high-throughput phenotypic analysis and genome-wide association analysis, and finally explores its impact on future wheat breeding strategies and food security. This study aims to accelerate the identification and application of wheat scab resistance gene loci by combining high-throughput phenotypic analysis with genome-wide association analysis (GWAS), thereby enhancing breeding efficiency and food security. By integrating advanced phenotypic analysis techniques with genomic tools, this study aims to overcome the bottlenecks of traditional resistance evaluation and gene mapping, providing new ideas and resources for resistance breeding.

2 Pathogenesis and Resistance Mechanisms of Fusarium Head Blight in Wheat

2.1 Pathogenic characteristics and epidemic conditions of *Fusarium graminearum*

Once wheat encounters high humidity and high temperature weather during the flowering period, it is very likely to be affected by scab. The "culprit" behind it - *Fusarium graminearum* - is no new face. This pathogen not only leads to reduced yields but also leaves toxins such as deoxynivalenol (DON) in grains, which are not very friendly to human and animal health. Its spread is not entirely a natural accident. Climate change has contributed to it, and some farming methods have also unintentionally provided it with a "breeding ground", such as no ploughing and corn-to-wheat rotation (Fernando et al., 2020). Once it invades, it gradually breaks through the defense line of wheat by secreting enzymes and effectors. The production of DON further accelerates the progression of the disease.

2.2 Types of resistance in wheat

Not all wheat gives up without a fight. Some varieties can resist pathogenic bacteria when they first come into contact, which is called type I resistance. While others, even if infected, can limit the spread of the pathogen within the spike, which belongs to type II resistance (Wu et al., 2022). Of course, there is also the accumulation resistance to toxins such as DON, which is particularly crucial for ensuring food safety. It is worth noting that a single resistance is often insufficient. The varieties that truly stand out are mostly the "result" of the superposition of several resistances.

2.3 Biological basis of resistance traits and phenotypic indicators

Ultimately, the resistance display of wheat is still the "collaborative result" of genes and molecules. Some key signaling pathways are activated at the early stage of pathogen invasion. For example, genes involved in pathogen recognition, cell wall reinforcement or toxin detoxification are up-regulated in expression (Dong et al., 2023; Wang et al., 2025; Yang et al., 2025). At the same time, mechanisms such as phenylalanine metabolism, the glutathione cycle, and those related to reactive oxygen species will also be involved (Figure 1). We can observe these resistance manifestations in various ways. Besides the traditional disease grade scoring, molecular-level and metabolomics data also provide a considerable amount of quantifiable evidence. In terms of resistance, "appearance" and "core" are not two separate levels, but rather a whole that reflects each other.

3 High-Throughput Phenotyping Technologies and Their Application in FHB Evaluation

3.1 High-throughput platforms: image analysis, near-infrared spectroscopy (NIRS), and thermal imaging

Nowadays, relying on manual visual inspection to assess the resistance to Fusarium head blight (FHB) can no longer keep up with the pace. Image analysis (such as RGB, multispectral, hyperspectral), near-infrared spectroscopy (NIRS), and thermal imaging techniques have gradually become mainstream tools for tracking plant health and identifying disease manifestations (Leiva-Sandoval, 2023). Whether in greenhouses or directly in fields, these systems can be deployed and put into use. Some even incorporate devices like Phenocave, which are low-cost and not complicated to operate, making it easier for breeders and research teams to access these methods that were originally only available in high-end laboratories (Yang et al., 2020).

3.2 Automated phenotypic data collection and disease severity detection algorithms

In the past, collecting lesion data was time-consuming and relied on human eye judgment. Now, with the use of automatic acquisition systems and image recognition algorithms, the process has become fast and accurate (Xu & Li, 2022; Jin et al., 2025). Deep learning has been able to identify the subtle changes in grain morphology, and the size of the lesion area can also be quantified very clearly. The most crucial point is that the difference between the

output result and the manual scoring by experts is not significant, but it saves a lot of manpower. It is precisely for this reason that the acquisition of phenotypic data can finally be more standardized and consistent.

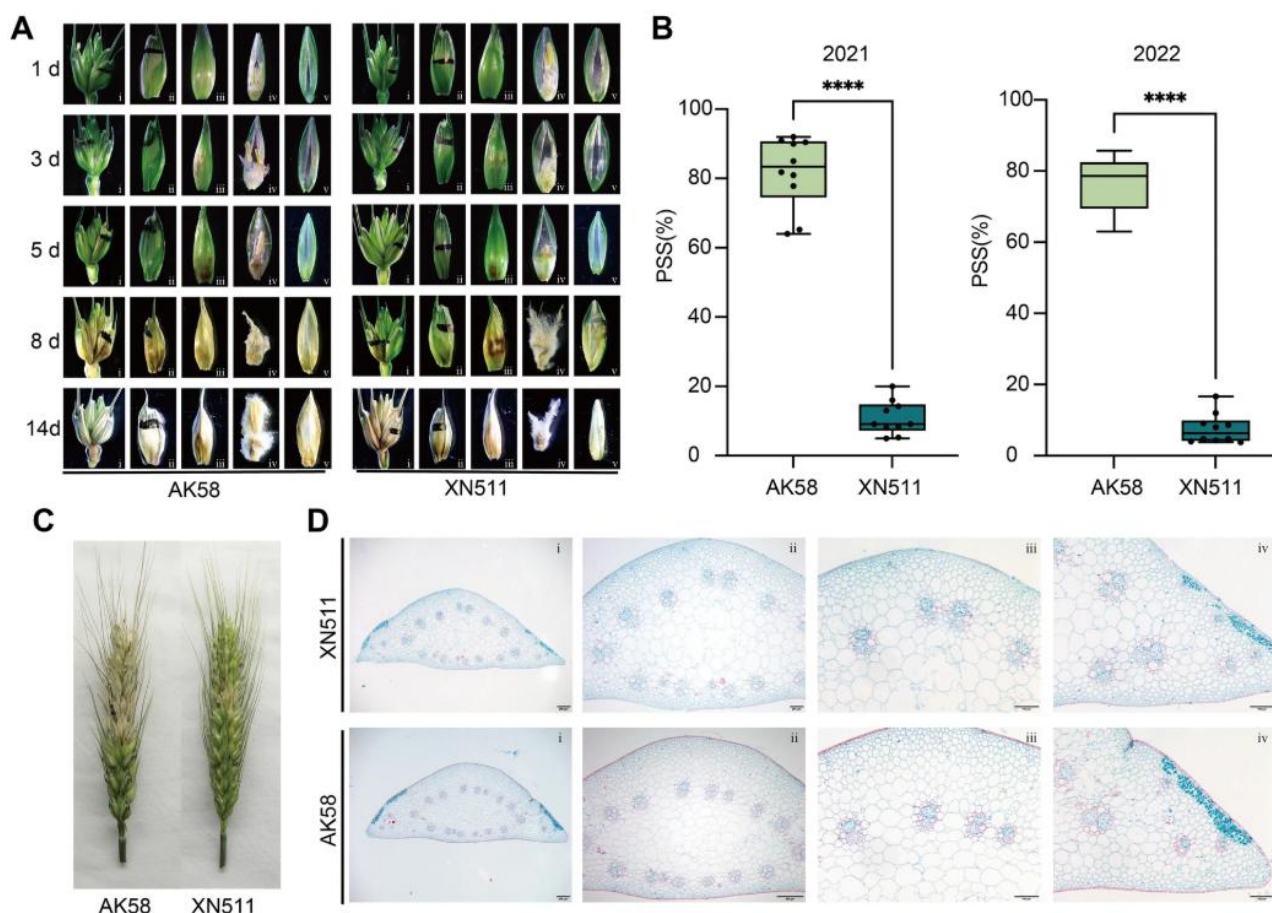


Figure 1 *Fusarium* head blight resistance in Xinong 511 and Aikang 58 (Adopted from Yang et al., 2025)

3.3 Multi-timepoint, multi-trait dynamic monitoring and data standardization

Fusarium head blight does not end as soon as it appears. It will continue to develop throughout the entire growth period. Therefore, data at a single time point is inevitably one-sided. The advantage of the new generation phenotypic platform lies in its ability to monitor multiple traits in real time and continuously, including various detailed reactions during the disease development process. Although data collection has become easier, standardization processing is still necessary in the later stage to ensure that data from different batches and locations match. This step still cannot be ignored. Especially when it comes to linking phenotypic results with GWAS, the accuracy of data integration becomes even more important (Yang et al., 2020; Leiva-Sandoval, 2023). Judging from the current trend, these technologies are gradually shortening the path between genotype screening and resistance breeding.

4 Overview of Genome-Wide Association Study (GWAS) Methods

4.1 Principles and application processes of GWAS in plants

To identify the genes behind complex traits like *Fusarium* head blight resistance, GWAS has become a mainstream tool. This type of analysis usually focuses on SNPs (Single nucleotide polymorphisms), but in fact, the entire process is more complicated than imagined. From the very beginning of designing the experiment, to obtaining phenotypic and genotypic data, and then to the later statistical analysis, no step can be taken lightly. For plant research, the appeal of GWAS lies in its ability to identify the connections between traits and genetic variations in natural populations, thereby helping to identify potential breeding candidate genes (Uffelmann et al., 2021). However, the reliability of the result depends on whether each link is solid, especially the rigor of the statistical part and the consistency of the data.

4.2 SNP array and resequencing data selection and processing for GWAS

Not all genetic markers are suitable for GWAS, especially when the data quality is substandard. At present, there are two commonly used methods: one is SNP chips, and the other is high-throughput resequencing. The former is cheap and efficient, and is suitable for large samples. Although the latter is a bit more expensive, it has more comprehensive information and can also capture rare mutations (Yin et al., 2020). No matter which way it is, data cleaning is the first step: low-quality markers need to be removed and missing genotypes need to be filled. These may seem like technical details, but in fact, they directly affect whether reliable conclusions can be drawn in the subsequent analysis.

4.3 Common statistical models and control strategies in FHB resistance GWAS

Once the phenotypic and genotypic data are in place, the next challenge lies in modeling. The initial GWAS method only measured each SNP one by one, but in plant research, the problem of too many false positives often troubles researchers. Thus, the mixed linear model (MLM) has become the mainstream choice because it can simultaneously consider the group structure and the kinship among individuals (Huang et al., 2025). In some studies with large sample sizes and complex backgrounds, more advanced methods are also employed, such as MLMM (Multilocus Mixed Model) or meta-analysis. Controlling confounding effects, conducting multiple tests and corrections reasonably, and combining them with clear result visualization all determine whether GWAS results are truly useful, especially when it comes to the breeding of Fusarium head blight resistance, which cannot be taken lightly.

5 Integrated Analysis of High-Throughput Phenotyping and GWAS

5.1 Enhancing mapping accuracy with multidimensional phenotypic data

To precisely identify the genes related to FHB resistance, relying solely on traditional methods is often insufficient. Nowadays, many studies tend to combine high-throughput phenotypes with GWAS. Because this method can obtain various types of data - such as morphological, physiological and biochemical traits, and can also dynamically track plant changes (Merida-Garcia et al., 2024). Compared with traditional measurements, this type of phenotypic data contains much more information and can also capture some response details that would otherwise be easily overlooked. With these more "abundant" data, the accuracy of detecting marker-trait associations naturally improves, especially when studying complex traits like FHB that are controlled by multiple genes, the advantages are more obvious.

5.2 Principal component extraction and mixed model construction in joint analysis

The "abundance" of multi-dimensional data does not mean that analysis is easy. Too much data and too many variables often lead researchers into an "information overload". In order to reduce interference and highlight key features, principal component analysis (PCA) is generally used for a round of dimensionality reduction first to extract the main variations (Zhang et al., 2020). These principal components are then incorporated into the mixed linear model to correct background noise and enhance the stability of the analysis. In this way, even if the research involves multiple traits and multiple time points, some gene loci with less obvious effects can be screened out (Wu et al., 2021). Especially for genes with pleiotropy or temporal dynamic effects, this method can capture signals more effectively.

5.3 Stability assessment of QTLs through multi-environment and temporal-spatial data integration

No matter how well a QTL performs at a certain experimental point, if it becomes ineffective in a different year or environment, it is clearly not suitable for breeding. This situation is not uncommon. Therefore, before evaluating whether the QTLs related to FHB resistance are "reliable", multi-point validation across environments and years is very necessary. With the help of high-throughput phenotypic platforms, researchers can now repeatedly collect data under different field conditions, with more standardized operations and smaller errors. By combining these phenotypic data with GWAS results for analysis, it is possible to more clearly see which QTLs can be stably expressed in various environments (Xiao et al., 2021; Merida-Garcia et al., 2024). The ultimately selected "stable performance" genetic markers are more suitable for practical breeding applications and can also help us understand exactly how genes and the environment interact with each other.

6 Case Studies

6.1 GWAS of fusarium head blight resistance in U.S. winter wheat core germplasm

In the United States, researchers did not start from scratch to search for the fusarium head blight resistance gene. Over the past few years, they have conducted high-density SNP genotyping on breeding populations of hard and soft winter wheat, combined with multi-locus GWAS methods. Many loci related to FHB resistance have been identified on multiple chromosomes - such as 2A, 3B, 4A, 4B, 5A, 6A, 7A and 7D - (Arruda et al., 2016; Larkin et al., 2020). Some resistance sites are familiar faces, while others are newly discovered. Research has found that as long as certain alleles are present in varieties, the incidence of FHB and the level of mycotoxins (DON) can be significantly reduced (Figure 2). However, it is not the case that multiple beneficial alleles combined together are very common. In reality, strains with such an "ideal combination" are quite rare, which also indicates that the path of disease-resistant breeding is not simple (Ghimire et al., 2022; Zhang et al., 2022).

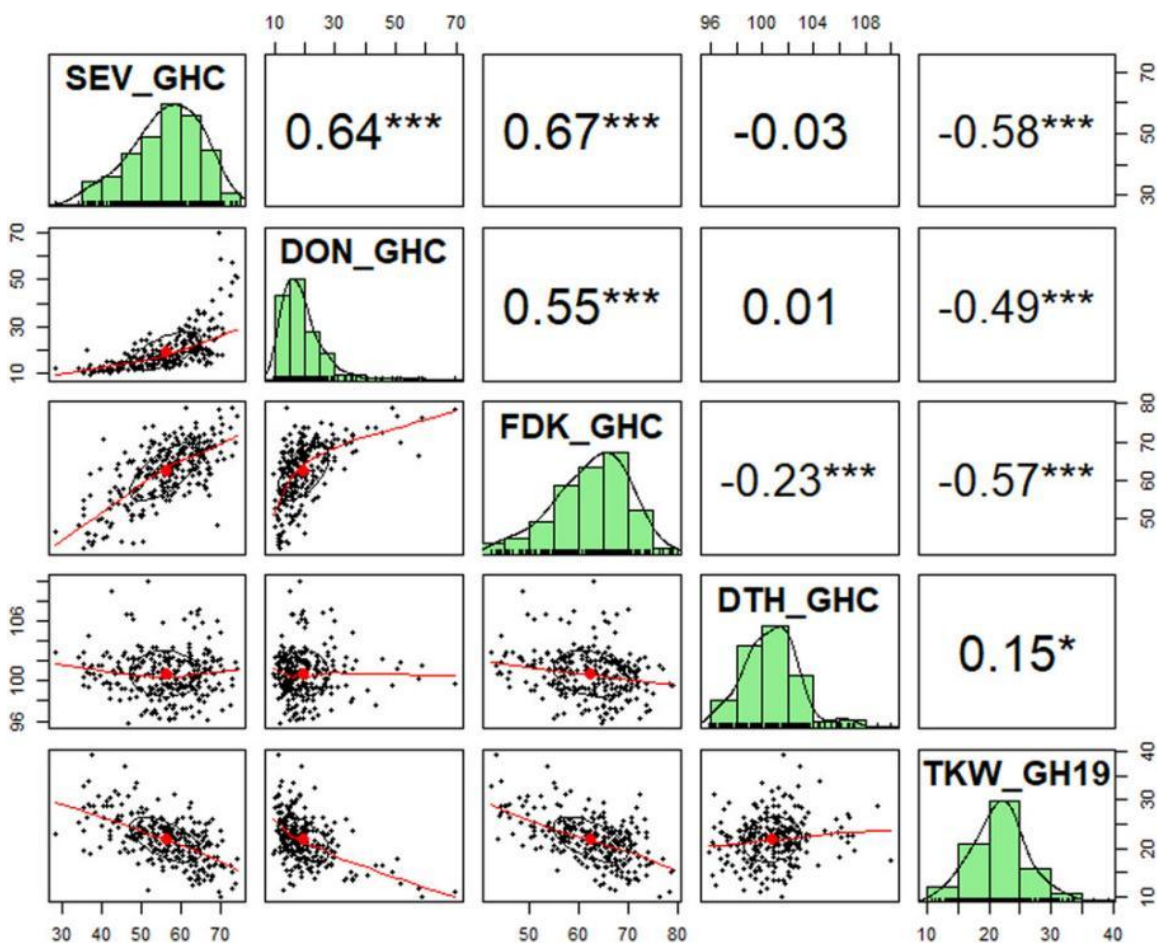


Figure 2 Pearson's correlation matrix among three Fusarium head blight (FHB) and two morphological traits of 278 soft red winter wheat lines on combined data in the greenhouse across 2 yr (Adoted from Ghimire et al., 2022)

6.2 Multi-platform phenotyping and QTL mapping in wheat varieties from the Huang-Huai region of China

In the Huanghuai wheel-growing region, GWAS is also a major tool, but in terms of approach, it focuses more on integrating multiple phenotypic platforms and environmental data. For example, by using 90K SNP chips and in combination with multi-year and multi-site field trials, researchers discovered QTL loci related to FHB resistance stability on multiple chromosomes (Wang et al., 2023). Some sites have been able to stably appear in different environments for several consecutive years, which gives people more confidence in their reliability. More importantly, such results can directly support molecular marker-assisted selection and provide a breeding direction for regionally adapted disease-resistant wheat varieties.

6.3 Application of integrated image data and genetic maps for identifying novel resistance genes

Some studies have taken a different approach, simply combining image data and genetic maps for analysis. By obtaining phenotypic characteristics through high-throughput imaging and combining GWAS and QTL localization, researchers identified new resistance loci and candidate genes on chromosomes such as 4A, 5D, 6B and 7A (Hu et al., 2019; Song et al., 2025). On this basis, KASP markers were also developed specifically for screening genotypes with stronger disease resistance. Although this method involves many steps, it has a high accuracy rate and good efficiency, and is especially suitable for the current breeding rhythm that emphasizes "fast, accurate and decisive".

7 Functional Validation and Breeding Applications of Resistance Genes

7.1 Expression profiling and mutant validation of candidate genes

Sometimes, whether a gene is a "disease-resistant gene" or not cannot be determined merely by prediction; it also needs to be verified through experiments. In soybean research, people first use GWAS to screen out candidate genes and then observe whether the expression of these genes changes after pathogen infection. Immediately after that, it was time to "knock" - CRISPR/Cas9 was used to knock out the target gene, and as a result, the mutant was more prone to disease infection. However, after overexpressing this gene, the plant became more resistant to diseases instead (Dai et al., 2025). These results indicate that this gene does indeed play a key role in the defense mechanism. Although the above example is from soybeans, when it comes to wheat, corn, or even rice, a similar process is also used.

7.2 Potential of gene editing (e.g., CRISPR/Cas9) in functional studies of resistance

The application of CRISPR/Cas9 goes far beyond "verifying individual genes". It is more like a precise tool that can achieve rapid and targeted genetic modification in crop breeding. Compared with traditional methods, this technology enables people to directly observe the performance of a certain gene after modification, whether it is more disease-resistant and whether it affects other traits. In practical research, this approach can not only knock out "problem genes", but also design new superior alleles (Khadgi et al., 2025; Pedrozo et al., 2025). Therefore, CRISPR/Cas9 is no longer merely a research tool but an indispensable weapon in the modern breeding system.

7.3 Strategies for introgressing favorable alleles into commercial cultivars through molecular breeding

However, even if it is determined which gene is good and has strong resistance, it cannot just remain in the laboratory. The ultimate goal is still to bring these superior alleles into the varieties grown by farmers. At this point, molecular breeding comes in handy. Methods such as marker-assisted selection and gene aggregation have been repeatedly verified to efficiently superimpose multiple disease-resistant genes and breed varieties with more stable and broad-spectrum resistance (Li et al., 2020; Hafeez et al., 2021; Li et al., 2025). Of course, what is needed behind this is a complete genetic map and functional marker system. After all, in the face of increasingly "intelligent" pathogens, relying on just one gene is not sufficient. Multiple resistance sites need to work together to ensure stable crop yields without failure (Hafeez et al., 2021; Li et al., 2020).

8 Conclusion and Perspectives

In recent years, the localization of genes related to wheat scab (FHB) resistance has made considerable progress through high-throughput phenotypic techniques and GWAS. This type of method may seem complex, but its core is actually to match the phenotypic expression with the genotype data, and then identify the gene loci related to resistance through association analysis. With the support of such large-scale data, the speed of finding genes has increased, the accuracy has also improved, and the corresponding molecular markers have become more and more numerous, providing a lot of convenience for subsequent breeding work. Especially in the area of identifying resistant traits, it used to rely on experience, but now it is more dependent on algorithms.

However, it's not necessarily easy to say so. When the volume of data increases, problems follow. Phenotypic data from different platforms and environments often have deviations, and just organizing these data is a headache. Coupled with the interweaving of multiple omics information, it is simply impossible to handle the analysis without some technical expertise and interdisciplinary collaboration. Moreover, there is still a lack of a unified

standard procedure at present. Even the same set of methods may yield different results when the experimental conditions are changed. Such instability to some extent restricts the promotion of related achievements in resistance breeding.

Looking ahead, the situation might change. Tools such as artificial intelligence and machine learning are gradually being introduced into the breeding process. In the future, it will no longer be a fantasy to screen massive amounts of data through algorithms, predict resistance performance, or even participate in the design of breeding strategies. New models like federated learning may be able to train accurate models without concentrating data, and they are also more efficient and secure. Once these technologies are truly implemented, they will not only increase the success rate of breeding disease-resistant varieties, but may also fundamentally change the way of breeding. However, this path cannot do without continuous cooperation and technological updates in different fields. If one hopes to no longer "chase after problems" when facing complex diseases but to make early plans, an intelligent, high-throughput and multi-disciplinary integrated breeding system will undoubtedly become the mainstream direction in the future.

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Conflict of Interest Disclosure

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

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