

RESEARCH

Open Access



Identification and fine mapping of blast resistance gene *Rpg3(t)* in foxtail millet (*Setaria italica* L.)

Xinge Xia^{1,2†}, Muhammad Sohaib Shafique^{2†}, Weiqing Huang², Yapei Liu², Hui Zhi², Chunlian Wang², Yi Zhou^{1,2*} and Zhiyuan Ji^{2*} 

Abstract

Foxtail millet (*Setaria italica* L.) is an important cereal crop, resilient to abiotic stresses, but suffers significant yield losses from blast disease, caused by *Pyricularia grisea*. Understanding the genetic mechanisms underlying blast resistance is essential for developing resistant cultivars. This study employed bulk segregant analysis sequencing and gene expression analysis to uncover resistance in the Yugu1 cultivar, which is known for its innate immunity. Our analysis identified three genomic regions associated with resistance, and fine mapping pinpointed a critical quantitative trait locus, *qLB-03.1*, within a 56.8 kb interval on chromosome 3. Eight genes were annotated in this region, and time series expression analysis revealed that *Seita.3G119300*, which encodes a cysteine-rich secretory protein, was significantly upregulated following inoculation. This upregulation suggests a crucial role in the resistance phenotype. Intriguingly, a significant 4592 bp deletion in the promoter region of *Seita.3G119300* in the susceptible Ci846 cultivar appears to affect its expression and susceptibility to the disease. This deletion highlights *Seita.3G119300* (tentatively named *Rpg3(t)*) as a candidate resistance gene, warranting functional validation and underscoring its potential for inclusion in breeding programs aimed at enhancing blast resistance in foxtail millet. Continued research is needed to fully validate the functional role of this gene and harness its potential for enhancing crop resilience.

Keywords Blast disease, *Pyricularia grisea*, QTL mapping, BSA-seq, Resistance

Background

Foxtail millet (*Setaria italica* L.), also known as small millet, is an important cereal crop noted for its resilience and nutritional value across Asia and Europe. Originally

domesticated in China, foxtail millet plays a critical role in agricultural systems, particularly in regions with challenging growing conditions (Li et al. 2021a). It is a fundamental food source and supports the livelihoods of farmers on marginal lands, where its cultivation covers extensive areas and contributes significantly to local economies (Nadeem et al. 2020). Millets are proposed to play a crucial role in combating global food insecurity (Li et al. 2021a; Wilson and VanBuren 2022). Additionally, foxtail millet has emerged as a model C4 crop due to its compact plant structure, short growth cycle, readily accessible reproductive organs, small genome size, and few genomic repetitions (Huang et al. 2014; Xu et al. 2022). Despite its resilience to various insect pests and abiotic stresses, foxtail millet production remains

[†]Xinge Xia and Muhammad Sohaib Shafique have contributed equally to this work.

*Correspondence:

Yi Zhou
zhouyi@yangtzeu.edu.cn

Zhiyuan Ji
jizhiyuan@caas.cn

¹ College of Agriculture, Yangtze University, Jingzhou 434025, China

² State Key Laboratory of Crop Gene Resources and Breeding/ National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China



threatened by biotic stresses, with blast disease being a significant constraint, particularly in northern China (Diao 2017; Islam et al. 2020). Severe outbreaks have been observed in Hebei, Henan, Shandong, Inner Mongolia, Shaanxi, and Heilongjiang provinces, severely impacting local millet cultivation.

Leaf blast of foxtail millet is a fungal disease caused by the pathogen *Pyricularia grisea* Sacc. (anamorphic form: *Magnaporthe grisea* (T.T. Herbert) M.E. Barr) (Klaubauf et al. 2014). This filamentous fungus, capable of airborne transmission, can cause diseases in various gramineous plants (Paul et al. 2022). It infects nearly all aerial parts of millet plants, leading to disease development at all growth stages. The disease manifests as circular spots with straw-colored centers on the leaf blades, typically 2–5 mm in diameter. These small, scattered spots are bordered by a dark brown margin (Pordel et al. 2018). In severe instances, particularly under humid conditions and high plant density, affected leaves exhibit wilting and subsequent drying (Zhang et al. 2016). The infection affects all stages of plant growth and is a key factor limiting yields in millet-producing regions, especially during the spring and summer seasons. Yield losses caused by blast disease are significant, frequently surpassing 20%–30%. In some cases, sensitive cultivars can suffer grain losses exceeding 40% (Ramakrishnan et al. 2016). Historically restricted to the North China Plain because of its favorable humid conditions, blast disease has recently spread to Northeast China and the Northwest Loess Plateau (Xu et al. 2022). The spread of blast disease to new regions may be attributed to climate change, as previously unsuitable environments now present conditions favorable for the disease's development.

Genetic resistance provides a sustainable and cost-effective solution for disease control, particularly beneficial for subsistence farmers who may lack the resources for expensive and often less effective chemical management methods (Mbinda and Masaki 2020). Despite the significant impact of this disease, research into genetic resistance to blast in foxtail millet is still limited. In contrast, extensive research on rice has clarified the interactions between fungal pathogens and host plants, leading to the identification of numerous resistance (R) genes (Wang et al. 2019). Many of the R genes identified share significant structural similarities, predominantly encoding proteins that contain the nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains (Zhai et al. 2022). The blast pathogen has a broad host range, affecting over 50 different cereal grain crops, each showing varying rates of infestation (Liu et al. 2014). Despite its broad host range, the pathogen can specifically adapt to its hosts and effectively infect an individual host (Li et al. 2020). Moreover, the concept of non-host resistance

has not yet been successfully reported for millet crops (Dida et al. 2021). Leveraging plant innate immunity and genetic resistance offers a sustainable and effective approach to disease control (Ji et al. 2018). However, only a limited number of studies have reported on the localization of genes or QTL related to blast resistance in millet crops, particularly in foxtail millet (Andersen and Nepal 2017).

Screening a core collection of foxtail millet in fields and greenhouses revealed various blast-resistant accessions, highlighting candidates for cultivation with improved agronomic and nutritional qualities (Sharma et al. 2014). Bioinformatic analyses have identified 269 NBS-encoding resistance genes in the Yugu1 genome, with chromosome 8 containing the highest density of these disease-resistance candidates (Zhu et al. 2014). Another study found that integrating DArT and SSR markers identified a key rust resistance QTL on LG1 in pearl millet, explaining 58% of the phenotypic variation and indicating substantial innate immunity (Ambawat et al. 2016). Recently, a genome-wide association study identified and mapped the linkage of two genomic loci on chromosomes 2 and 9 associated with leaf blast disease resistance in China (Li et al. 2021b). Bulk segregant analysis (BSA) has been applied to identify genetic loci controlling plant height and blast resistance in millet species, including foxtail millet (Tian et al. 2021; Gao et al. 2022). BSA-Seq integrates traditional bulked segregant analysis with next-generation sequencing (NGS) to quickly identify blast-resistant genomic regions/QTLs with high resolution and accuracy (Majeed et al. 2022). Three novel QTLs on chromosomes 1, 2, and 8 in foxtail millet were identified, which explain 9%–20% of the phenotypic variations for leaf blast resistance, marking a significant advance in the genetic understanding and breeding for disease resistance (Tian et al. 2021). Therefore, BSA-seq is an excellent tool for identifying genetic regions that govern resistance in foxtail millet.

In this study, we initially screened a collection of *P. grisea* isolates on the native cultivar Yugu1, known for its superior traits. Yugu1 showed strong resistance to four different pathogenic isolates collected from field conditions. To explore the genetic basis of this resistance, we employed BSA-seq analysis on a crossbred population of Yugu1 and Ci846. Whole-genome resequencing of both resistant and susceptible plant pools revealed three candidate regions associated with resistance. Fine mapping further pinpointed a resistance locus on chromosome 3, located between markers W-132 and W-192, spanning 56.8 kb. Eight genes were identified and annotated within this interval based on the genomic data from Yugu1. Time-series qRT-PCR analyses indicated significant upregulation of the gene *Seita.3G119300* in the Yugu1

cultivar following fungal inoculation at 8-, 24-, and 48-h post-inoculation, suggesting its key role in conferring resistance. This gene has been tentatively named *Resistance to P. grisea3* (*Rpg3*).

Results

Yugu1 confers resistance to prevalent isolates of *Pyricularia grisea*

In initial screenings, the native inbred line Yugu1 demonstrated robust resistance against four distinct *P. grisea* isolates, unlike the Ci846 line, which was susceptible to all tested isolates (Fig. 1a). Notably, Yugu1 showed significantly strong resistance particularly to the GW20-1-1 strain (Fig. 1b), prompting its use for inoculating the mapping population (Fig. 2a). We assessed a total of 382 F_2 plants from the cross between Yugu1 and Ci846 for resistance. Based on lesion severity, plants were classified into grades 1 and 2, indicating resistance, and grades 3 through 5, indicating susceptibility. Among these, 131 plants were classified as resistant and 252 as susceptible. The number of plants for each grade is shown in Fig. 2b. The resulting distribution suggests a segregation ratio of approximately 2:1, indicating that resistance in Yugu1 may be governed by more than one gene or that the effect of a single gene is masked or modified by other genetic or environmental factors. This complexity in inheritance could suggest incomplete dominance, epistasis, or a significant influence of environmental factors on the expression of resistance. The chi-square test returned a value of 0.56 with a $P < 0.05$, which does not fit a simple Mendelian 3:1 ratio, further supporting the hypothesis of

complex genetic controls influencing the resistance trait in the Yugu1 cultivar (Table 1).

Genomic profiling of resistant and susceptible pools in the foxtail millet F_2 population

To identify genetic regions associated with resistance in the Yugu1 cultivar, we conducted a BSA-seq analysis using high-throughput whole-genome sequencing of the parental lines, along with pools of resistant and susceptible plants. Sequencing of the four samples—two parental and two pooled (Resistant and Susceptible)—generated a total of 61.36 Gb of data. With read mapping rates exceeding 99%, the data reliability was confirmed, ensuring robust coverage depth and a normal GC distribution (Table 2). These parameters confirm the suitability of the data for detecting genetic variations and identifying loci related to resistance. SNP analysis identified 1,939,580 SNPs between the parental lines. These results, depicted in Fig. 3a, illustrate the SNP distribution across the chromosomes of Yugu1 and Ci846, highlighting the genomic regions potentially involved in resistance.

BSA-seq identifies three candidate regions for blast resistance

To accurately represent the distribution of the SNP index across the chromosomes, we constructed SNP index graphs for both the resistant (R-pool) and susceptible (S-pool) pools, utilizing a resolution of 1 Mb as the unit window and a step size of 10 Kb. The Δ SNP index for each single nucleotide polymorphism (SNP) was calculated by subtracting the SNP index value of

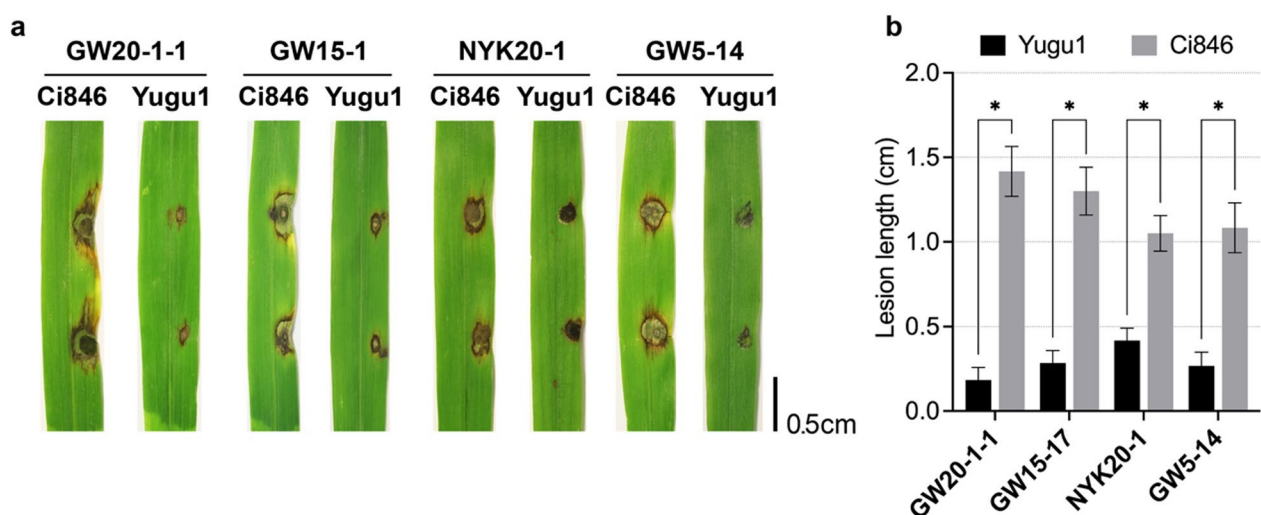


Fig. 1 Disease assay of leaf blast resistance for Yugu1. **a** Yugu1 reveals robust resistance against four representative *P. grisea* isolates (GW20-1-1, GW15-1, NYK20-1, and GW5-14), contrasting with the susceptibility observed in Ci846 (scale 0.5cm). Photographs were taken five days post-inoculation using the leaf detachment method. **b** Statistically significant differences in lesion length were observed between the Yugu1 and Ci846 cultivars, as determined by a Student's *t*-test, following point inoculation

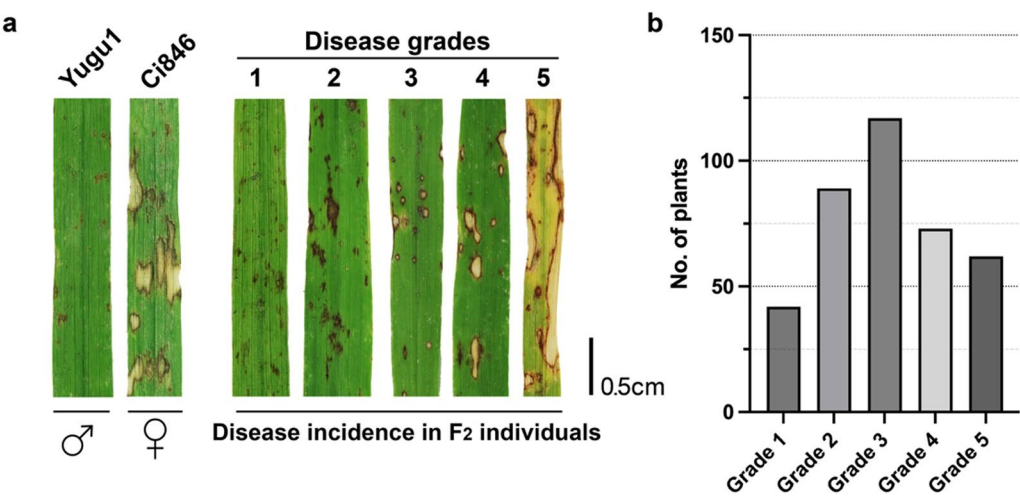


Fig. 2 Phenotypic characterization of blast resistance in parental lines and F₂ progeny. **a** The contrasting responses of the susceptible female parent Yugu1 (used as male) and Ci846 (female parent) to inoculation with *P. grisea* strain GW20-1-1, with a 0.5 cm scale bar provided for reference. **b** illustrates the segregation pattern in the F₂ population (n=383), showing both representative leaf images demonstrating the 1–5 disease grading scale (where grades 1–2 indicate resistance and grades 3–5 indicate susceptibility) and the corresponding frequency distribution of these grades across the population

Table 1 Number of plants in the F₂ population showing infection response to GW20-1-1 inoculation. The segregation ratio indicates a 2:1 ratio, suggesting polygenic inheritance

Combination	No. of F ₂ individual			$\chi^2(2 : 1)$	P value
	Susceptible	Resistant	Total		
Ci846/Yugu1	252	131	383	0.56	0.718

Table 2 Various sequencing parameters of Yugu1, Ci846, resistant pool (CY-4R), and susceptible pool (CY-4S). Quality scores are measured as Q20 and Q30, and the mapping rate is calculated as a percentage

Samples	Total reads	Total bases	Q20	Q30	Mapping rate (%)
Yugu1	68,664,146	10,299,621,900	97.32	92.60	99.52
Ci846	69,187,534	10,378,130,100	97.27	92.44	99.16
CY-4R	132,389,140	19,858,371,000	97.73	93.56	99.24
CY-4S	138,871,344	20,830,701,600	97.66	93.45	99.30

the S-pool from that of the R-pool. This method enabled us to develop a comprehensive Δ SNP index map that covers all nine chromosomes, as illustrated in Fig. 3b. Our analysis identified three regions where the average Δ SNP index was equal to or greater than the established confidence value ($p < 0.1$), suggesting significant genetic differentiation between the resistant and susceptible pools in these areas. Specifically, two of these critical regions, each extending 1.96 Mb, were located on chromosome 3, while a third significant region, covering 4.48 Mb, was found on chromosome 5. These identified regions are considered promising candidates for containing genes responsible for resistance to leaf blast in foxtail millet. As such, we have tentatively designated these regions as candidate resistance loci. Following this identification, we used the sequencing data to conduct linkage mapping studies aimed at fine-mapping the specific loci associated with resistance within the Yugu1 cultivar.

(See figure on next page.)

Fig. 3 BSA-seq analysis identifies candidate regions associated with blast resistance in foxtail millet. **a** BSA-seq analysis of dof SNPs between Yugu1 and Ci846 across foxtail millet chromosomes (Chr1–Chr9). The y-axis shows SNP density (number of SNPs per 200 kb window) with million base pairs (Mbp) length across chromosomes, with blue color highlighting high-density regions (> 50 SNPs/window). Red boxes indicate candidate QTL intervals (*qLB-03.1*, *qLB-03.2*, *qLB-05.1*). **b** Data analyzed from comparative genome sequencing analysis using 0.2 Mb window size and Δ SNP index plot derived from alignment to the Yugu1 v2.2 genome. Gray dashed lines indicate 95% confidence intervals from permutation tests (1,000 iterations). Candidate regions are highlighted with red and blue boxes on the Y-C Δ SNP-index. *qLB-03.1* is associated with resistance, shown in the red box, while other regions showed a non-significant association (ns), shown in the blue boxes

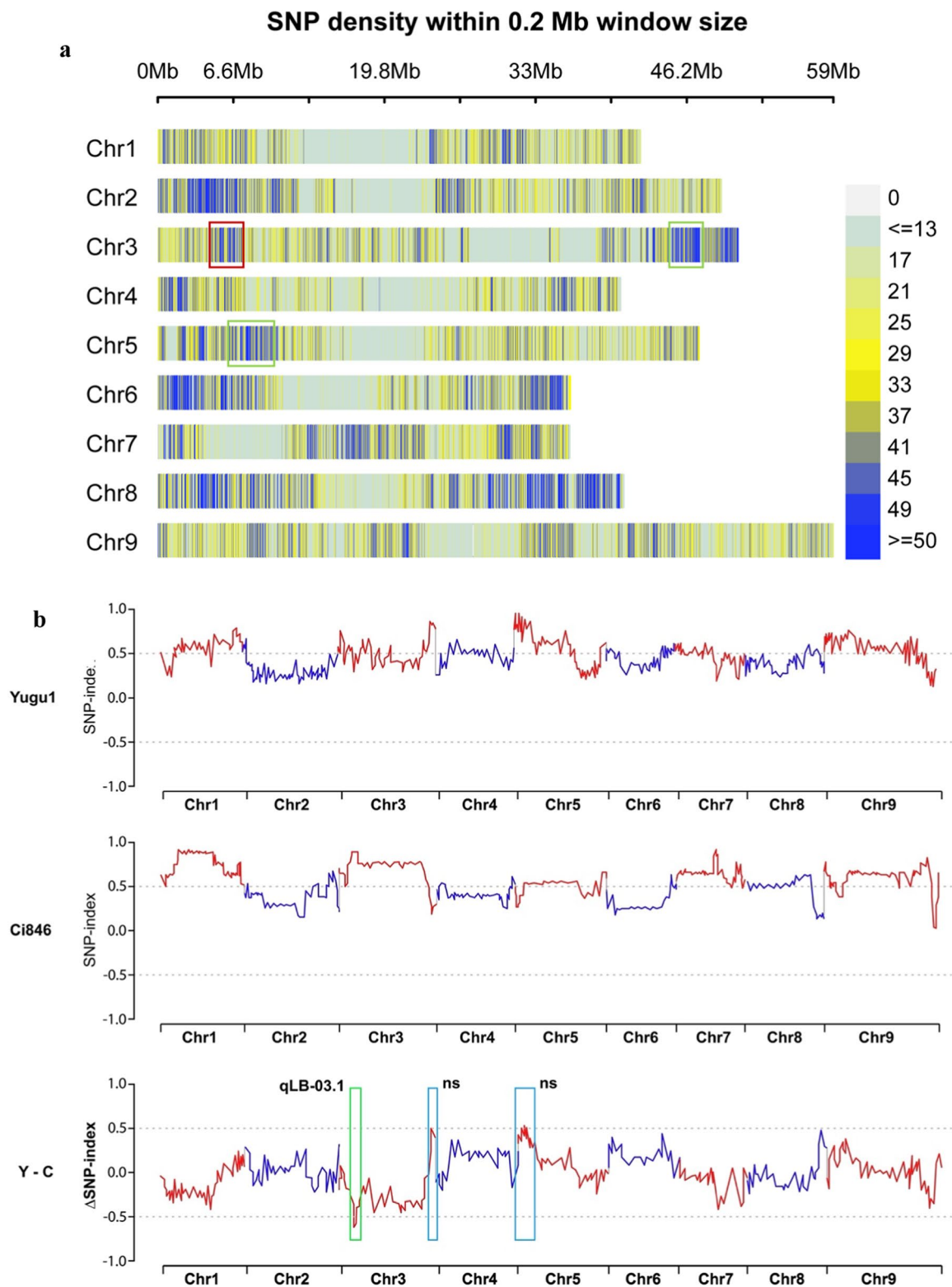


Fig. 3 (See legend on previous page.)

Linkage analysis identifies chromosome 3 *qLB-03.1* linked to resistance

To fine-map the loci responsible for resistance to millet blast disease, we utilized highly resistant plants from our mapping population and employed Indel molecular markers. From the BSA-seq analysis, three quantitative trait loci (QTL) intervals were identified: *qLB-03.1* and *qLB-03.2* on chromosome 3, and *qLB-05.1* on chromosome 5. We designed Indel molecular markers to precisely define and narrow down these regions (Table 3). The *qLB-03.1* interval was associated with Indel markers W-69 and W-74 on the short arm of chromosome 3, whereas *qLB-03.2* was linked with W-53 and W-55 on the long arm. For *qLB-05.1*, polymorphic Indel markers did not align with the phenotypes observed, suggesting no association with the resistance phenotype (data not shown). Subsequent efforts focused on chromosome 3, where linkage mapping helped further refine the intervals. The *qLB-03.2* locus was closely linked to markers W-53 and W-54, and a co-segregating marker, W-97, was identified between them, spanning a physical distance of approximately 1.86 Mb. This close linkage indicated a lack of recombinants in this interval, presenting challenges in further narrowing down the genetic region. The physical and genetic positions of all markers are detailed in Fig. 4a and Table 3. In the *qLB-03.1* region, we identified nine Indel markers between W-69 and W-74, with markers W-38 and W-67 positioned on the same side as the target loci and markers W-192, W-3, W-10, W-48, W-87, W-85, and W-84 on the opposite side (Fig. 4a, Table 3). Ultimately, we pinpointed the resistance loci between markers W-132 and W-192, covering a distance of 56.8 kb (Fig. 4b).

QTL *qLB-03.1* region encompasses eight annotated genes

We annotated eight genes within the 56.8kb span of the *qLB-03.1* locus on chromosome 3 using the foxtail millet functional genomics database (You et al. 2015). These genes are *Seita.3G118700* (Aspartyl proteases), *Seita.3G118800* (Leucine rich repeat, LRR_1), *Seita.3G118900* (PH domain, PH), *Seita.3G119000* (Rhamnogalacturonan endolyase/Rhamno-galacturonase-B), *Seita.3G119100* (Nucleoside diphosphate kinase-related), *Seita.3G119200* (PTCCT motif family protein), *Seita.3G119300* (Cysteine-rich secretory protein-related), and *Seita.3G119400* (NAD dependent epimerase/dehydratase). Notably, *Seita.3G119300* and *Seita.3G119400* are of particular interest due to their potential roles in plant defense mechanisms (Table 4). This annotation provides a foundation for further investigation into the specific functions of these genes in conferring resistance to blast disease in foxtail millet.

Expression analysis of candidate genes revealed candidate genes for *qLB-03.1*

To evaluate the expression profiles of candidate genes within the *qLB-03.1* locus, we initially consulted the RNA-seq database to determine their basal expression levels, which are generally minimal under normal conditions. Subsequent qRT-PCR analysis at five time points revealed that two genes, *Seita.3G119000* and *Seita.3G119300*, exhibited significant upregulation at all time points after post-inoculation in the Yugu1 cultivar, suggesting their involvement in early defense mechanisms against blast disease (Fig. 5d, g). Specifically, *Seita.3G119300* showed the highest expression levels, underscoring its potential key role in conferring resistance. In contrast, the other genes within the *qLB-03.1* locus displayed expression changes in the Ci846 cultivar, which may suggest a negative role in blast disease

Table 3 Polymorphic InDel marker name, their position, and primer sequence used to narrow down the genetic region of resistance for chromosome 3 of Yugu1 cultivar

Marker	Position	Forward sequence (5'–3')	Reverse sequence (5'–3')
W-69	6,703,621	GTAGCCTTGATATCATCGT	CCTTTCCTCGGGACTATAAC
W-38	7,393,902	GCATGGGTCGATGAGTTTTA	CTCTTTGTCAAGATGTGCTT
W-67	7,594,713	CAGATCAATCTGTCCAGAAC	AGTTTTCTACCAAAACCATTT
W-192	8,040,456	GTCACCAGACCAGGTGTTTC	GTACAAATGTCGCCTTGAGAC
W-132	8,119,248	GATCTGCTGTTATTCGGAAAGT	TGAATGACACTCGGTGTTTCT
W-10	8,752,322	TAGTCTCGTAGAGTAGCCTG	CGTCTGTGTTGAGAAACAAC
W-48	11,018,586	GACCCTTTCAAACAATCCAG	GCACCAATTTGCCAAGTTAT
W-87	12,589,941	CCAAATACACAGTTGCTGTTG	TTGATAGACTCGTAAGTCTTTATTT
W-85	14,083,977	CTCCTATACAACACTCTGGC	CAAGTATCTGGAAGGTCTCG
W-84	14,976,008	GTCATTTTCGGTCACGTATAA	CTACCTACCTTTGACAGGC
W-74	17,456,859	AGGTGTGTGGTGTGGTC	CTCCTCCATTTGCAGCTTAT

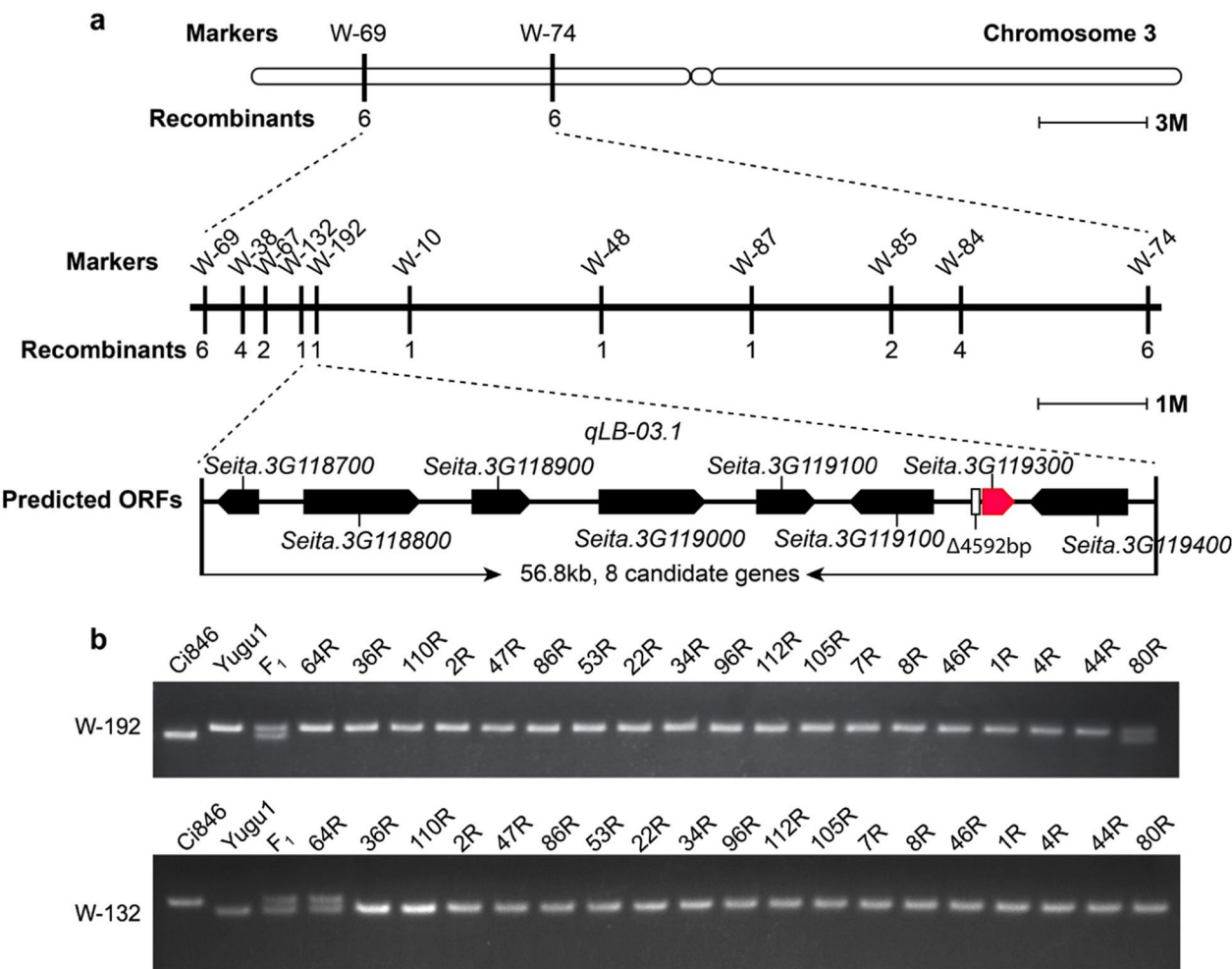


Fig. 4 Map-based cloning of *qLB-03.1* using linkage mapping. **a** Marker Distribution along chromosome 3 and recombination frequencies (cM/Mb) are indicated below the chromosome. The *qLB-03.1* finally mapped to a 56.7kb interval between markers W-132 and W-192 with the least recombination. Predicted genes within a 56.7 kb interval are shown, while candidate genes for *qLB-03.1* are shown in red. $\Delta 4592$ bp insertion in Yugu1 genome shown as a box in upstream on *Seita.3G119300*. **b** Linkage mapping using indel markers W-132 and W-192 shows its association with the resistant locus *qLB-03.1*. The gel pictures were cropped from the original images of gels

Table 4 List of genes and their encoded proteins investigated for expression induction in Yugu1 and Ci846 cultivars from Phytozome and NCBI

	Phytozome v13	NCBI
Seita.3G118700	PTHR13683//PTHR13683:SF306—ASPARTYL PROTEASES	<i>Setaria italica</i> aspartic proteinase CDR1
Seita.3G118800	PF00069//PF00560//PF08263//PF13855—Protein kinase domain (Pkinase)//Leucine Rich Repeat (LRR_1)//Leucine rich repeat N-terminal domain (LRRNT_2)//Leucine rich repeat (LRR_8)	<i>Setaria italica</i> leucine-rich repeat receptor-like serine/threonine-protein kinase BAM3
Seita.3G118900	PF00169—PH domain (PH)	<i>Setaria italica</i> uncharacterized
Seita.3G119000	Rhamnogalacturonan endolyase/Rhamnogalacturonase B	<i>Setaria italica</i> probable Rhamno-galacturonase lyase B
Seita.3G119100	PTHR11349:SF53—Nucleoside Diphosphate Kinase-Related	Nucleoside diphosphate kinase 3
Seita.3G119200	PTHR31717:SF2—CCT Motif Family Protein	<i>Setaria italica</i> uncharacterized
Seita.3G119300	PTHR10334//PTHR10334:SF235—Cysteine-Rich Secretory Protein-Related	pathogenesis-related protein PRB1-3
Seita.3G119400	PTHR10366//PTHR10366:SF308—NAD Dependent Epimerase/Dehydratase	UDP-glucose 4-epimerase 1

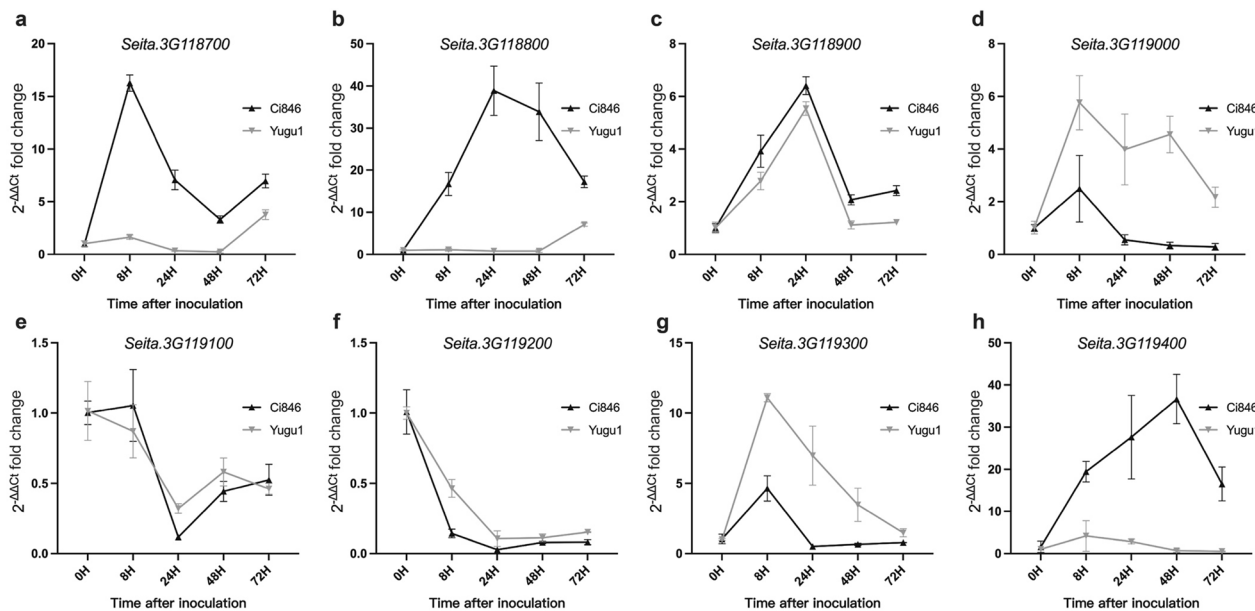


Fig. 5 Gene expression analysis of Ci846 and Yugu1 after 0-, 8-, 24-, 48- and 72-h post-inoculation. Relative expression is shown for Ci846 and Yugu1 cultivars as a fold change in transcript levels after inoculation of the GW20-1-1 isolate of *P. grisea* in a time series experiment. The relative expression level of genes was calculated using $2^{-\Delta\Delta C_t}$ method, while 0 h (0H) was used as a control for measurement of relative expression levels, while actin was used as an internal control

defense. These observations lead us to hypothesize that *Seita.3G119000* and *Seita.3G119300* are likely candidates for the resistance locus in *qLB-03.1*. This hypothesis is further supported by genomic analysis, which shows that while *Seita.3G119000* does not exhibit genomic changes between Yugu1 and Ci846, *Seita.3G119300* has an upstream deletion of 4592 bp (Fig. 4a), leading to structural variation potentially linked to resistance in Yugu1. Bioinformatically, no gene ORF is predicted in this region. Based on these findings, *Seita.3G119300* on chromosome 3 of the Yugu1 cultivar is tentatively identified as a potential candidate for *qLB-03.1* and is tentatively named *Rpg3(t)*, warranting further investigation into its functional roles and regulatory mechanisms in resistance to blast disease in foxtail millet.

Discussion

Originally domesticated in China, foxtail millet is increasingly recognized globally for its adaptability and nutritional value. Leaf blast resistance remains a key breeding objective, emphasizing the need to identify and utilize genetic regions conferring resistance for cloning and marker-assisted breeding (Yang et al. 2012; Babu et al. 2014; Dida et al. 2021; Li et al. 2021a). Numerous studies have identified and cloned resistance genes and QTLs in foxtail millet through BSA, GWAS, and genome resequencing (Babu et al. 2012; Sharma et al. 2014; Rajesh et al. 2019; Li et al. 2021b).

The ongoing evolution of pathogens and the emergence of new strains highlight the critical need for developing resistant cultivars, particularly in China (Mbinda and Masaki 2020; Cheng et al. 2023).

Yugu1 has been pivotal in genomic studies and breeding programs aimed at developing disease-resistant cultivars. For instance, the Zhonggu2 and Yugu5 cultivars, which exhibit resistance to leaf blast, are derived from Yugu1, indicating its valuable genetic contributions (Tian et al. 2021; Zhang et al. 2022). Resistance in millet, including in closely related species such as pearl and finger millet, tends to follow both qualitative and quantitative inheritance patterns (Ramakrishnan et al. 2016).

Use of BSA-Seq has proven effective in fine mapping resistance traits, reinforcing its utility in trait mapping across millet varieties (Masumoto et al. 2016; Tian et al. 2021; Gao et al. 2022). Although resistance loci have been mapped on chromosomes 1, 2, and 8 in foxtail millet, precise mapping remains sparse, especially for native Chinese cultivars (Li et al. 2021b; Tian et al. 2021). In this study, we isolated prevalent isolates of *P. grisea* from Heilongjiang and utilized Yugu1 for pathotyping, which displayed robust resistance against multiple virulent isolates. Crossing Yugu1 with the susceptible Ci846 resulted in a mapping population that showed a polygenic mode of inheritance, deviating from simple Mendelian expectations and suggesting the

influence of multiple genes and environmental factors on resistance traits (Zschocke et al. 2023).

Our BSA-seq analysis, supported by high-quality sequencing data with a mapping rate exceeding 99%, confirmed the reliability of our genetic screening. The comprehensive coverage essential for detecting polymorphisms enabled us to identify critical genomic regions associated with resistance traits (Huang et al. 2022). Numerous SNPs and InDels were detected between parental lines and within pools. Notably, non-synonymous SNPs (nsSNPs) could affect gene function. BSA analysis pinpointed three genomic regions with significant Δ SNP index values, refining our search for resistance genes. We identified three potential regions on chromosomes 3 and 5. Using InDel molecular markers, we closely examined the genomic region *qLB-03.1*, which demonstrated a strong linkage with the resistant loci in the F₂ population. The region on chromosome 5 displayed inconsistency between genotyping and phenotyping when utilizing markers. As for *qLB-03.2*, no single recombinant plant was identified, maintaining a range of 1.86Mb without narrowing down the interval. We focused on *qLB-03.1* and fine-mapped this QTL to a 56.8 kb region, within which 8 genes were annotated on the Yugu1 genome. To our knowledge, no resistance gene has been reported in this region. Therefore, we determined that this novel QTL represents a new locus conferring resistance against emerging strains of the pathogen in foxtail millet. We utilized a wealth of genomic data of Yugu1 for the annotation of 8 genes found in the *qLB-03.1* region. The *qLB-03.1* locus on chromosome 3 represents a novel source of blast resistance in foxtail millet, distinct from known loci on chromosomes 1, 2, and 8. Unlike the NBS-LRR clusters in *qLB-8.2*, *qLB-03.1* contains a CRISP gene (*Seita.3G119300*) and shows strong efficacy against Chinese blast isolates. Its large effect size (complete susceptibility in promoter deletion mutants) contrasts with the partial resistance of *qLB-1* (20% PVE) and *qLB-2.3* (15% PVE). This discovery enables new pyramiding strategies for durable resistance.

We then used gene expression analysis after inoculating the fungal isolate GW20-1-1, employing time series analyses. Previously, defense-related genes were identified using expression analysis (Du et al. 2024). Our focused study on two particular genes, *Seita.3G119000* and *Seita.3G119300*, revealed significant insights into their roles in disease resistance. *Seita.3G119000*, despite showing no genetic changes between the resistant and susceptible lines, displayed increased expression levels following pathogen inoculation. This suggests that its role in resistance may predominantly be linked to its transcriptional response rather than genetic variations.

Conversely, *Seita.3G119300* not only showed heightened expression in response to pathogen challenge but also exhibited a significant genetic variation that correlates with resistance. Specifically, a 4592 bp deletion in the promoter region of *Seita.3G119300*, found in the susceptible Ci846 cultivar but absent in the resistant Yugu1, likely affects the gene's expression level and functionality. This deletion may reduce the promoter's activity or disrupt regulatory interactions necessary for mounting effective defense responses, thus contributing to susceptibility. *Seita.3G119300* encodes a Cysteine-Rich Secretary Protein-Related (CRISP) protein, known for its role in modulating ion channels and signaling pathways crucial for plant defense. The presence of CRISP proteins facilitates the direct interaction with pathogen-derived molecules, triggering essential defense mechanisms. This protein's function, coupled with the observed gene expression and promoter activity influenced by the genetic deletion, positions *Seita.3G119300* as a critical component of the resistance phenotype in foxtail millet.

The *Seita.3G119300* (*Rpg3(t)*) CRISP protein shares functional parallels with resistance genes in other cereals, including rice's *OsRLK1* (ROS-mediated blast resistance) and wheat's *Lr67* (nutrient limitation-based resistance), but lacks their kinase/transporter domains, suggesting a novel resistance mechanism. All three genes contain conserved cysteine-rich motifs and pathogen-responsive promoter elements, indicating the convergent evolution of CRISP-mediated defense strategies in cereals. This comparison highlights *Rpg3(t)* potential as both a unique resistance source and part of a conserved defense network in grasses. These findings led us to tentatively map *Seita.3G119300* as a novel resistance locus, highlighting its potential as a prime target for breeding efforts aimed at developing leaf blast-resistant foxtail millet cultivars. The distinct contributions of *Seita.3G119300* through both its genetic setup and protein function underscore the complex nature of resistance mechanisms in foxtail millet. Moreover, developing transgenic plants and gene knockout plants in *S. italica* remains a challenge, hence limiting gene functional studies.

Conclusions

This study identified and finely mapped *qLB-03.1*, a novel blast resistance locus on chromosome 3 of foxtail millet, *Seita.3G119300* (encoding a CRISP protein), as its candidate gene based on multiple lines of evidence. The 4592 bp promoter deletion in susceptible cultivars, coupled with strong pathogen-responsive expression patterns, suggests this gene plays a crucial role in defense responses. These findings significantly expand our understanding of blast resistance genetics in foxtail millet, providing both a valuable molecular marker for breeding

programs and new insights into non-NBS-LRR resistance mechanisms in cereals. The discovery holds particular promise for developing blast-resistant cultivars adapted to Chinese growing regions, where current isolates show heightened virulence. Future research should prioritize: (1) functional validation of *Seita.3G119300* through genetic transformation and gene editing, (2) pyramiding with other resistance QTLs (e.g., *qLB-8.2*) to enhance durability, and (3) comparative studies to assess CRISPR-mediated resistance across related cereal crops. This work establishes a foundation for both applied breeding solutions and fundamental research into alternative plant immunity pathways.

Methods

Plant material, growth conditions, and mapping population development

The seeds of resistant cultivar Yugu1 (accession No. Ci309) and susceptible line Ci846 (accession No. SSR57) were obtained from the national foxtail millet germplasm repository maintained by the Institute of Crop Sciences, CAAS, a key center for millet genetic resource conservation and breeding programs in China (He et al. 2024). Yugu1, noted for its high resistance to *P. grisea* isolates, was used as the male parent, while the susceptible Ci846 served as the female parent. Both cultivars were cultivated either in a net house or a greenhouse under natural or controlled conditions in Beijing (40.21N, 116.59E). Parental lines (Yugu1 and Ci846) and F_1 hybrids were grown in isolated greenhouse compartments. F_1 plants were verified using 16 polymorphic SSR markers (Additional file 1: Table S1) and showed intermediate phenotypes. F_1 plants were self-pollinated to produce F_2 seeds. The F_2 population, comprising 383 plants, was sown in seedling pots measuring $8 \times 8 \times 8$ cm³, spaced approximately 2 cm apart. The growth chamber conditions were maintained at 28°C with alternating 12-h light and darkness cycles. A film covering was applied three days post-budding, with evaluations conducted 17–20 days after transplanting. The F_2 population underwent screening for resistance or susceptibility using artificial inoculation methods (Thakur et al. 2009).

Preparation of inoculations and phenotyping

P. grisea isolates were obtained from naturally infected foxtail millet leaves collected from fields affected by blast disease in Heilongjiang Province, China. Single-spore isolates were cultured on PDA medium and maintained at 28°C for 7 days prior to inoculation. The inoculation protocol was modified from (Thakur et al. 2009) for foxtail millet. Briefly, the fungal inoculum was prepared by culturing *P. grisea* hyphae on filter paper placed in PDA plates (200g/L boiled potatoes, 20g/L glucose, 15g/L

agar) and incubating at 28°C in the dark for 3 days. The culture was then transferred to sterilized sorghum grains in Erlenmeyer flasks and kept in the dark for 7 days to promote growth. The mycelia were subsequently transferred to a sterilized, moist Petri dish, covered, and subjected to a 6–12-h dark treatment at 28°C. After drying for 5–7 days, the mycelia were stored at –20°C.

Two inoculation methods were utilized: detached leaf and spray inoculation. For detached leaf inoculation, 7 cm long leaves from millet plants at the 5–6 leaf stage were placed on sterilized filter paper in square Petri dishes. Cotton balls soaked in 0.2% 6-Benzylaminopurine were placed at leaf ends, and 10 µl of spore suspension were applied to wounded sites. After a 24-h dark treatment at 28°C, 12-h light–dark cycles were maintained for 4 days. Lesion size was measured to the nearest millimeter.

For spray inoculation, seedlings at the four-leaf stage were sprayed with a suspension of approximately 1×10^5 spores/mL (optimized for foxtail millet) to ensure uniform leaf coverage. Spore germination (>90%) was verified microscopically before inoculation. Following inoculation, conditions of 90% humidity and 28°C–30°C were maintained. Plants were kept in darkness for 24 h, followed by alternating 12-h light and dark cycles. Disease incidence was evaluated 5–7 days post-inoculation using a grading scale by two independent researchers. Disease scoring was performed, with <5% discrepancies resolved via consensus, and the inter-observer correlation was 0.93. Grade 1 for needle-tip-sized brown spots, grade 2 for small fusiform brown lesions, grade 3 for round or spindle-shaped lesions with grayish-white centers about 1mm in diameter, grade 4 for larger spots 3mm or more in diameter, and grade 5 for extensive diseased spots or leaf yellowing and curling. Grades 1–2 were classified as resistant and grades 3–5 as susceptible responses (Fig. 2a). Disease severity scores (Grades 1–5) were analyzed using Student's t-test for parental line comparisons (Yugu1 vs. Ci846) and chi-square (χ^2) tests for segregation ratios in the F_2 population. Each genotype-inoculation combination included three biological replicates (individual plants), with each replicate evaluated on 5–6 leaves.

DNA extraction and BSA mixed pool sequencing

Genomic DNA was extracted from leaf samples using the CTAB method (Allen et al. 2006). The quality and quantity of DNA were assessed via 1.0% agarose gel electrophoresis and a Nanodrop 2000 spectrophotometer (Eppendorf Inc., Germany). Resistant (CY-4R) and susceptible (CY-4S) DNA pools were created by sampling 50 leaves each from distinctly resistant and highly susceptible plants in the F_2 population, respectively. DNA

extraction involved punching holes in each sampled leaf. DNA from the parental lines, Yugu1 and Ci846, was also isolated. DNA libraries were prepared using the Illumina TruSeq DNA Nano Kit (Illumina, USA), with 350 bp insert sizes selected via bead-based normalization. Genomic DNA was fragmented to ~350 bp using a Covaris ultrasonicator (Covaris, USA), followed by end repair, A-tailing, and adapter ligation according to the manufacturer's protocol. Pooled libraries were sequenced on an Illumina platform (150 bp paired-end) to an average depth of 50× coverage. Sequencing quality was validated using Illumina's SAV software, with all samples meeting thresholds of Q20 > 97% and Q30 > 92%. The high consistency of quality scores (SD < 0.5% across samples) and mapping rates (> 99%) supported the reliability of the single-run data.

BSA-seq analysis

To analyze the BSA data, we first used BWA (Burrows-Wheeler Aligner) and GATK (Genome Analysis Toolkit) for variant calling. We then aligned the sequence reads from the resistant and susceptible bulks to the reference Yugu1 v2.2 genome (Bennetzen et al. 2012) using BWA (Li and Durbin). The aligned reads were then subjected to pre-processing steps, including duplicate removal, base quality score recalibration, and local realignment around indels using GATK (McKenna et al. 2010). Next, variant calling was performed using GATK to identify SNPs and indels between the two bulks. Following variant calling, variant filtration was applied to obtain high-quality variants. NGS-based BSA was conducted using QTLseqr (Mansfeld and Grumet 2018), an R package designed for bulked segregant analysis combined with next-generation sequencing. This software is derived from the original analysis pipeline of QTLseq. SNPs were retained if they passed: depth ≥ 10×, Phred ≥ 30, MAF ≥ 0.05, and < 20% missing data (GATK/QTLseqr). We utilized the ΔSNP index method to identify candidate regions containing the target gene. High-quality SNPs obtained from the detection and screening of mutant loci were employed to calculate the SNP index, which represents the ratio of mutant base depth to total depth in the mixed pool sequencing (Takagi et al. 2013). To mitigate the impact of sequencing and comparison errors, parental polymorphic loci were filtered based on predefined criteria (Zhong et al. 2017). Parental genotypes with heterozygous mutations, loci lacking coverage or with low sequencing depth, SNP index values below 0.3 or above 0.7, and mutations on non-major chromosomes were excluded. The ΔSNP index = (ΔSNP-indexR - ΔSNP-indexS), with candidate regions defined by permutation-based 95% confidence intervals (CIs) ($p < 0.05$), ΔSNP > ± 0.3 with the exclusion of regions with low read depth (< 10×). Subsequently,

with a window size of 1 Mb and a step length of 10 Kb, the average ΔSNP index of each window was determined. A ΔSNP index map was constructed using the physical location of each chromosome on the x-axis and the ΔSNP index values on the y-axis. To mitigate false-positive loci, the ΔSNP index values of markers on the same chromosome were fitted using the DISTANCE method (Fekih et al. 2013). Statistical confidence intervals for Δ(SNP index) at all SNP positions were calculated based on read depth under the null hypothesis of no QTLs. Additionally, 95% confidence intervals for the ΔSNP index were generated for each read depth as described previously (Fekih et al. 2013; Wang et al. 2018).

Development of molecular markers

Initially, we utilized whole-genome sequencing data from Yugu1 and Ci846 foxtail millet genotypes to identify potential InDel and SSR regions (Liu et al. 2012). Bioinformatic tools, including Primer3 and BLASTn, were employed to analyze whole-genome sequencing data for InDel polymorphisms (≥ 5 bp size difference) and conserved flanking sequences (≥ 50 bp) between the parental lines Yugu1 and Ci846. Candidate markers were selected based on their proximity to the identified QTL regions (≤ 1 Mb), amplicon size (100–300 bp) for optimal resolution via agarose gel electrophoresis, and absence of repetitive motifs. Polymorphic markers were validated through PCR amplification using standardized conditions (94°C denaturation, 55°C–58°C annealing, 72°C extension) followed by 3%–5% agarose gel electrophoresis (120 V, 45 min) as described by (Lv et al. 2013). Markers exhibiting inconsistent segregation patterns or amplification failures were excluded, ensuring robust linkage analysis.

RNA isolation and real-time quantitative PCR (qRT-PCR)

We conducted a time-series qRT-PCR to assess transcriptional changes following inoculation with GW20-1-1. Leaves of both Yugu1 and Ci846 cultivars were sprayed with the pathogen and sampled at 0, 8-, 24-, 48-, and 72-h post-inoculation. Total RNA was extracted using the RNAiso Plus reagent, and cDNA synthesis was performed with the First-Strand cDNA Synthesis SuperMix (Tiangen Biotech, China) according to the manufacturer's instructions. qRT-PCR was performed on an ABI7500 system using SYBR Green PCR Master Mix. Expression levels were quantified using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001), with the Actin gene from *S. italica* serving as an internal control, and three technical replicates per biological sample were analyzed using ANOVA with Duncan's post-hoc test ($P < 0.05$). Primers were designed based on the *S. italica* v2.2 genome from

Phytozome v13 using Primer3 software, and details are listed in Additional file 1: Table S2.

Abbreviations

BSA-seq	Bulk segregant analysis sequencing
CRISP	Cysteine-rich secretory protein
InDel	Insertion or deletion
MAF	Minor allele frequency
nsSNP	Nonsynonymous single nucleotide polymorphism
PVE	Phenotypic variation explained
QTLseqr	R package for QTL detection using BSA-seq data
qLB-03	Quantitative trait locus leaf blast on chromosome 3

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42483-025-00372-y>.

Additional file1: Table S1. Linkage table of markers segregating with the resistant or susceptible parent, with the final row displaying the number of recombinants per marker. **Table S2.** qPCR specific primers designed according to the CDS region of the gene. Forward and reverse primers are given.

Acknowledgements

We are very grateful to Xianmin Diao and Hui Zhi from Institute of Crop Sciences, CAAS, for providing the germplasm.

Author contributions

XX conducted the investigation, curated the data, and contributed to validation. MSS performed formal analysis, wrote the original draft, and participated in validation. WH carried out the experiments and contributed to validation. YL contributed to formal analysis and methodology development. HZ was involved in methodology design and software development. CW provided project administration and resources. YZ supervised the project and assisted with administration. ZJ conceived the study, supervised the research, acquired funding and resources, and revised the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by National Key R&D Program of China (2023YFD1202700, and 2023YFD1202703), and Basic Research Center, Innovation Program of Chinese Academy of Agricultural Sciences (CAAS-BRC-CS-2025-02).

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 17 November 2024 Accepted: 14 July 2025

Published online: 21 October 2025

References

- Allen GC, Flores-Vergara MA, Krasynanski S, Kumar S, Thompson WF. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nat Protoc*. 2006;1(5):2320–5. <https://doi.org/10.1038/nprot.2006.384>.
- Ambawat S, Senthilvel S, Hash CT, Nepolean T, Rajaram V, Eshwar K, et al. QTL mapping of pearl millet rust resistance using an integrated DArT- and SSR-based linkage map. *Euphytica*. 2016;209(2):461–76. <https://doi.org/10.1007/s10681-016-1671-9>.
- Andersen EJ, Nepal MP. Genetic diversity of disease resistance genes in foxtail millet (*Setaria italica* L.). *Plant Gene*. 2017;10:8–16. <https://doi.org/10.1016/j.plgene.2017.03.002>.
- Babu TK, Thakur RP, Upadhyaya HD, Reddy PN, Sharma R, Girish AG, et al. Resistance to blast (*Magnaporthe oryzae*) in a mini-core collection of finger millet germplasm. *Eur J Plant Pathol*. 2012;135(2):299–311. <https://doi.org/10.1007/s10658-012-0086-2>.
- Babu BK, Dinesh P, Agrawal PK, Sood S, Chandrashekar C, Bhatt JC, et al. Comparative genomics and association mapping approaches for blast resistant genes in finger millet using SSRs. *PLoS ONE*. 2014;9(6):e99182. <https://doi.org/10.1371/journal.pone.0099182>.
- Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J, Pontaroli AC, et al. Reference genome sequence of the model plant setaria. *Nat Biotechnol*. 2012;30(6):555–61. <https://doi.org/10.1038/nbt.2196>.
- Cheng X, Wang Q, Zhi H, Man X, Liu J, Diao X. Differentiating the pathogenicity of millet blast races and establishing a stable genetic transformation system. *Sci Sin Vitae*. 2023;53(4):519–28. <https://doi.org/10.1360/SSV-2022-0014>.
- Diao XM. Production and genetic improvement of minor cereals in China. *Crop J*. 2017;5(2):103–14. <https://doi.org/10.1016/j.cj.2016.06.004>.
- Dida MM, Oduori CA, Manthi SJ, Avosa MO, Mikwa EO, Ojulong HF, et al. Novel sources of resistance to blast disease in finger millet. *Crop Sci*. 2021;61(1):250–62. <https://doi.org/10.1002/csc2.20378>.
- Du Y, Liang D, Qi Z, Yu J, Zhang R, Song T, et al. Transcriptome and differential expression analysis revealed the pathogenic-related genes in *Magnaporthe oryzae* during leaf and panicle infection. *Phytopathol Res*. 2024. <https://doi.org/10.1186/s42483-024-00248-7>.
- Fekih R, Takagi H, Tamiru M, Abe A, Natsume S, Yaegashi H, et al. Mutmap+: genetic mapping and mutant identification without crossing in rice. *PLoS ONE*. 2013;8(7):e68529. <https://doi.org/10.1371/journal.pone.0068529>.
- Gao Y, Yuan Y, Zhang X, Song H, Yang Q, Yang P, et al. Conuping BSA-Seq and RNA-Seq reveal the molecular pathway and genes associated with the plant height of foxtail millet (*Setaria italica*). *Int J Mol Sci*. 2022. <https://doi.org/10.3390/ijms231911824>.
- He Q, Wang C, He Q, Zhang J, Liang H, Lu Z, et al. A complete reference genome assembly for foxtail millet and *Setaria*-db, a comprehensive database for *Setaria*. *Mol Plant*. 2024;17(2):219–22. <https://doi.org/10.1016/j.molp.2023.12.017>.
- Huang P, Feldman M, Schroder S, Bahri BA, Diao X, Zhi H, et al. Population genetics of *Setaria viridis*, a new model system. *Mol Ecol*. 2014;23(20):4912–25. <https://doi.org/10.1111/mec.12907>.
- Huang L, Tang W, Wu W. Optimization of BSA-seq experiment for QTL mapping. *G3 Genes|Genomes|Genetics*. 2022;12(1):jkab370. <https://doi.org/10.1093/g3journal/jkab370>.
- Islam MT, Gupta DR, Hossain A, Roy KK, He X, Kabir MR, et al. Wheat blast: a new threat to food security. *Phytopathol Res*. 2020;2(1):28. <https://doi.org/10.1186/s42483-020-00067-6>.
- Ji Z, Wang C, Zhao K. Rice routes of counteracting xanthomonas oryzae. *Int J Mol Sci*. 2018;19(10):3008. <https://doi.org/10.3390/ijms19103008>.
- Klaubauf S, Thareau D, Fournier E, Groenewald JZ, Crous PW, de Vries RP, et al. Resolving the polyphyletic nature of *Pyricularia* (*Pyriculariaceae*). *Stud Mycol*. 2014;79:85–120. <https://doi.org/10.1016/j.simyco.2014.09.004>.
- Li J, Cornelissen B, Rep M. Host-specificity factors in plant pathogenic fungi. *Fungal Genet Biol*. 2020;144:103447. <https://doi.org/10.1016/j.fgb.2020.103447>.
- Li S, Liu F, Liu M, Cheng R, Xia E, Diao X. Current status and future prospective of foxtail millet production and seed industry in China. *Sci Agric Sin*. 2021a;54(3):459–70. <https://doi.org/10.3864/j.issn.0578-1752.2021.03.001>.
- Li Z-J, Jia G-Q, Li X-Y, Li Y-C, Zhi H, Tang S, et al. Identification of blast-resistance loci through genome-wide association analysis in foxtail millet (*Setaria italica* (L.) Beauv.). *J Integr Agr*. 2021b;20(8):2056–64. [https://doi.org/10.1016/s2095-3119\(20\)63196-3](https://doi.org/10.1016/s2095-3119(20)63196-3).

- Liu B, Wang Y, Zhai W, Deng J, Wang H, Cui Y, et al. Development of InDel markers for *Brassica rapa* based on whole-genome re-sequencing. *Theor Appl Genet*. 2012;126(1):231–9. <https://doi.org/10.1007/s00122-012-1976-6>.
- Liu W, Liu J, Triplett L, Leach JE, Wang GL. Novel insights into rice innate immunity against bacterial and fungal pathogens. *Annu Rev Phytopathol*. 2014;52:213–41. <https://doi.org/10.1146/annurev-phyto-102313-045926>.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*. 2001;25(4):402–8. <https://doi.org/10.1006/meth.2001.1262>.
- Lv H-h, Yang L-m, Kang J-g, Wang Q-b, Wang X-w, Fang Z-y, et al. Development of InDel markers linked to Fusarium wilt resistance in cabbage. *Mol Breed*. 2013;32(4):961–7. <https://doi.org/10.1007/s11032-013-9925-x>.
- Majeed A, Johar P, Raina A, Salgotra RK, Feng X, Bhat JA. Harnessing the potential of bulk segregant analysis sequencing and its related approaches in crop breeding. *Front Genet*. 2022;13:944501. <https://doi.org/10.3389/fgene.2022.944501>.
- Mansfeld BN, Grumet R. QTLseqr: an R package for bulk segregant analysis with next-generation sequencing. *Plant Genome*. 2018. <https://doi.org/10.3835/plantgenome2018.01.0006>.
- Masumoto H, Takagi H, Mukainari Y, Terauchi R, Fukunaga K. Genetic analysis of NEKODE1 gene involved in panicle branching of foxtail millet, *Setaria italica* (L.) P. Beauv., and mapping by using QTL-seq. *Mol Breed*. 2016;36(5):59. <https://doi.org/10.1007/s11032-016-0481-z>.
- Mbinda W, Masaki H. Breeding strategies and challenges in the improvement of blast disease resistance in finger millet. *Curr Rev Front Plant Sci*. 2020;11:602882. <https://doi.org/10.3389/fpls.2020.602882>.
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The genome analysis toolkit: a mapreduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297–303. <https://doi.org/10.1101/gr.107524.110>.
- Nadeem F, Ahmad Z, Ul Hassan M, Wang R, Diao X, Li X. Adaptation of foxtail millet (*Setaria italica* L.) to abiotic stresses: a special perspective of responses to nitrogen and phosphate limitations. *Front Plant Sci*. 2020;11:187. <https://doi.org/10.3389/fpls.2020.00187>.
- Paul SK, Mahmud NU, Gupta DR, Rani K, Kang H, Wang G-L, et al. *Oryzae* pathotype of *Magnaporthe oryzae* can cause typical blast disease symptoms on both leaves and spikes of wheat under a growth room condition. *Phytopathol Res*. 2022. <https://doi.org/10.1186/s42483-022-00114-4>.
- Pordel A, Tharreau D, Cros-Arteil S, Shams E, Moumeni A, Mirzadi Gohari A, et al. *Pyricularia oryzae* causing blast on foxtail millet in Iran. *Plant Dis*. 2018;102(9):1853. <https://doi.org/10.1094/pdis-01-18-0091-pdn>.
- Rajesh M, Sudha A, Nirmalakumari A, Parasuraman P. Identification of resistant sources for blast and rust in foxtail millet incited by *Pyricularia setariae* and *Uromyces setariae-italica*. *Int J Curr Microbiol Appl Sci*. 2019;8(03):1796–800. <https://doi.org/10.20546/ijcmas.2019.803.210>.
- Ramakrishnan M, Antony Ceasar S, Duraipandiyar V, Vinod KK, Kalpana K, Al-Dhabi NA, et al. Tracing QTLs for leaf blast resistance and agronomic performance of finger millet (*Eleusine coracana* (L.) Gaertn.) genotypes through association mapping and in silico comparative genomics analyses. *PLoS One*. 2016;11(7):e0159264. <https://doi.org/10.1371/journal.pone.0159264>.
- Sharma R, Girish AG, Upadhyaya HD, Humayun P, Babu TK, Rao VP, et al. Identification of blast resistance in a core collection of foxtail millet germplasm. *Plant Dis*. 2014;98(4):519–24. <https://doi.org/10.1094/PDIS-06-13-0593-RE>.
- Takagi H, Abe A, Yoshida K, Kosugi S, Natsume S, Mitsuoka C, et al. QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome re-sequencing of DNA from two bulked populations. *Plant J*. 2013;74(1):174–83. <https://doi.org/10.1111/tpj.12105>.
- Thakur R, Sharma R, Rai K, Gupta S, Rao V. Screening techniques and resistance sources for foliar blast in pearl millet. *J SAT Agric Res*. 2009;7:1–5.
- Tian B, Zhang L, Liu Y, Wu P, Wang W, Zhang Y, et al. Identification of QTL for resistance to leaf blast in foxtail millet by genome re-sequencing analysis. *Theor Appl Genet*. 2021;134(2):743–54. <https://doi.org/10.1007/s00122-020-03730-w>.
- Wang N, Liu Z, Zhang Y, Li C, Feng H. Identification and fine mapping of a stay-green gene (Brnye1) in pakchoi (*Brassica campestris* L. ssp. *chinensis*). *Theor Appl Genet*. 2018;131(3):673–84. <https://doi.org/10.1007/s00122-017-3028-8>.
- Wang L, Zhao L, Zhang X, Zhang Q, Jia Y, Wang G, et al. Large-scale identification and functional analysis of NLR genes in blast resistance in the Tetep rice genome sequence. *Proc Natl Acad Sci U S A*. 2019;116(37):18479–87. <https://doi.org/10.1073/pnas.1910229116>.
- Wilson ML, VanBuren R. Leveraging millets for developing climate resilient agriculture. *Curr Opin Biotechnol*. 2022;75:102683. <https://doi.org/10.1016/j.copbio.2022.102683>.
- Xu J, Kong F, Zhang H, Zhang W, Wu H, Chen G. First report of root rot caused by *Fusarium nygamai* on foxtail millet (*Setaria italica*) in China. *Plant Dis*. 2022. <https://doi.org/10.1094/PDIS-06-22-1388-PDN>.
- Yang X, Wan Z, Perry L, Lu H, Wang Q, Zhao C, et al. Early millet use in northern China. *Proc Natl Acad Sci USA*. 2012;109(10):3726–30. <https://doi.org/10.1073/pnas.1115430109>.
- You Q, Zhang L, Yi X, Zhang Z, Xu W, Su Z. SIFGD: *Setaria italica* functional genomics database. *Mol Plant*. 2015;8(6):967–70. <https://doi.org/10.1016/j.molp.2015.02.001>.
- Zhai K, Liang D, Li H, Jiao F, Yan B, Liu J, et al. NLRs guard metabolism to coordinate pattern- and effector-triggered immunity. *Nature*. 2022;601(7892):245–51. <https://doi.org/10.1038/s41586-021-04219-2>.
- Zhang H, Zheng X, Zhang Z. The *Magnaporthe grisea* species complex and plant pathogenesis. *Mol Plant Pathol*. 2016;17(6):796–804. <https://doi.org/10.1111/mpp.12342>.
- Zhang W, Wang B, Liu B, Chen Z, Lu G, Ge Y, et al. Trait selection for yield improvement in foxtail millet (*Setaria italica* Beauv.) under climate change in the North China Plain. *Agronomy*. 2022. <https://doi.org/10.3390/agronomy12071500>.
- Zhong C, Sun S, Li Y, Duan C, Zhu Z. Next-generation sequencing to identify candidate genes and develop diagnostic markers for a novel *Phytophthora* resistance gene, RpsHC18, in soybean. *Theor Appl Genet*. 2017;131(3):525–38. <https://doi.org/10.1007/s00122-017-3016-z>.
- Zhu YB, Xie XQ, Li ZY, Bai H, Dong L, Dong ZP, et al. Bioinformatic analysis of the nucleotide binding site-encoding disease-resistance genes in foxtail millet (*Setaria italica* (L.) Beauv.). *Genet Mol Res*. 2014;13(3):6602–9. <https://doi.org/10.4238/2014.August.28.5>.
- Zschocke J, Byers PH, Wilkie AOM. Mendelian inheritance revisited: dominance and recessiveness in medical genetics. *Nat Rev Genet*. 2023;24(7):442–63. <https://doi.org/10.1038/s41576-023-00574-0>.