Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 5 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Breeding and Molecular Characterization of Rice Varieties Resistant to Bacterial Leaf Blight and False Smut in Pakistan

Nadia Jabeen (Corresponding)
Department of Agriculture, Hazara University Mansehra.
nadia_khan909090@yahoo.com

Ayesha Jahangir
Department plant sciences (botany), Agriculture University Faisalabad.
awanayehii@gmail.com

Bai-Bureh O'Bai Kamara
Sierra Leone Agricultural Research Institute (SLARI).
kamarabaibureobai@gmail.com

Sarnaz basheer
Department of botany, university of makran Panjgur. Sark10131@gmail.com

Talha
University of makran. talha@uomp.edu.pk

ABSTRACT

Bacterial leaf blight (BLB), caused by Xanthomonas oryzae pv. oryzae (Xoo), and rice false smut (RFS), caused by Ustilaginoidea virens, are among the most destructive diseases affecting rice production in Pakistan, leading to yield losses of 20–80% for BLB and up to 75% for RFS, along with mycotoxin contamination risks from RFS. BLB manifests as vascular clogging and necrotic lesions, while RFS replaces grains with smut balls containing ustiloxins that pose food safety hazards. Epidemiological surveys highlight high prevalence in Punjab (e.g., Gujranwala 55%) and Sindh (Badin up to 46% for RFS), exacerbated by high humidity, excessive nitrogen, and susceptible Basmati varieties. Molecular characterization has identified key resistance genes including Xa4, xa5, xa13, Xa21, and Xa23 for BLB, with successful pyramiding in Basmati lines (BILs carrying Xa4 + xa5 + Xa21) via marker-assisted backcrossing (MABB), achieving >90% recurrent genome recovery. False smut resistance is more complex, governed by multiple QTLs, with ongoing screening of IRRI germplasm. Breeding programs at RRI Kala Shah Kaku and NARC have released resistant varieties like Super Gold and advanced Green Super Rice (GSR) lines. Integrated management combines resistant cultivars with cultural practices, seed treatment, and targeted chemical/biological controls. Future priorities include novel R-gene deployment,

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

CRISPR editing of susceptibility genes, pathotype monitoring, and climate-resilient strategies to safeguard Pakistan's rice sector and ensure sustainable production.

Keywords—bacterial leaf blight, rice false smut, Xanthomonas oryzae pv. oryzae, Ustilaginoidea virens, gene pyramiding, marker-assisted selection, Basmati rice, disease resistance breeding, Pakistan rice pathology, sustainable rice production

1. Introduction

The agricultural landscape of Pakistan is inextricably linked to the production of rice (Oryza sativa L.), which stands as the second most vital food staple and a premier export commodity after cotton and wheat (Khalid Bashir & Mehmood, 2010). Beyond its status as a source of high-quality carbohydrates, rice provides essential micronutrients, dietary fiber, and amino acids crucial for the nutritional security of a burgeoning population (Das et al., 2023). As the world's 12th largest producer and 4th largest exporter, Pakistan's economy derives approximately 3.5 percent of its agricultural value-added and a significant portion of its gross domestic product from rice cultivation (Zia & Khan, 2021). Despite this prominence, the sector faces a perennial struggle against low per-acre yields, which frequently fall below global averages due to an array of biotic stresses (Sarma et al., 2023). Among these, bacterial leaf blight (BLB) and the increasingly prevalent rice false smut (RFS) have emerged as the most devastating challenges, capable of causing catastrophic yield losses and compromising the safety of the food chain (Khanal et al., 2023).

2. The Phyto pathological Crisis: Bacterial Leaf Blight and False Smut

The stability of rice production in Pakistan is increasingly threatened by pathogens that thrive in the specific agro-climatic conditions of the Indus Basin. Bacterial leaf blight, caused by the gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a systemic vascular disease that remains a primary constraint on achieving yield targets (Varanasi et al., 2023). Concurrently, rice false smut, an ascomycete fungal disease caused by *Ustilaginoidea virens*, has transitioned from a sporadic minor concern to an epidemic threat (Fan et al., 2020). The dual pressure of these diseases necessitates a sophisticated understanding of their etiology, the molecular basis of host resistance, and the deployment of advanced breeding strategies (Reddy et al., 2025).

2.1 Etiology and Pathogenesis of Bacterial Leaf Blight

The pathogen Xoo enters the rice plant primarily through natural openings like hydathodes or mechanical wounds. Once inside the host, it colonizes the xylem vessels, multiplying rapidly and producing extensive extracellular polysaccharides (EPS) that physically obstruct the transport of water and nutrients (Yadeta & J. Thomma, 2013). This vascular clogging manifests as characteristic water-soaked stripes on leaf margins, which eventually coalesce into large, necrotic blighted areas. The disease progresses through two distinct symptomatic phases: the leaf blight phase, common in maturing plants, and the "Kresek" phase, which involves the acute wilting and mortality of young seedlings (Dewangan et al., 2023).

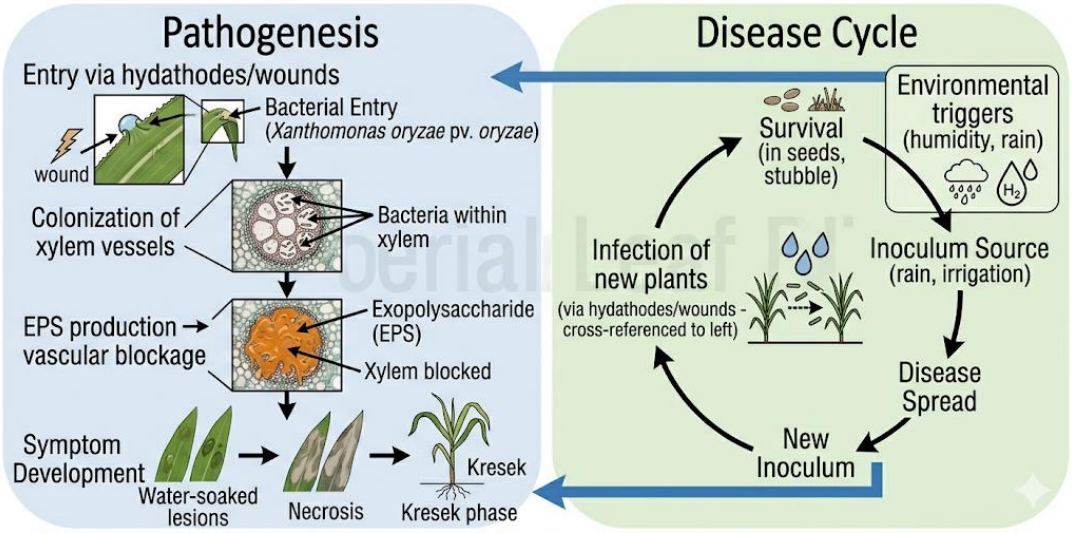
The economic impact of BLB in Pakistan is profound. Yield losses are generally estimated between 20% and 50%, but under favorable conditions for the pathogen such as high humidity and heavy monsoon rains losses can escalate to 80% or even 100%

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crop failure in susceptible Basmati varieties if infection occurs at the tillering stage (Ahmad et al., 2023). The "Kaller" belt of Punjab, famous for its premium aromatic rice, has reported disease incidence rates as high as 30-55% in recent years, highlighting the vulnerability of the country's most valuable germplasm (Ejaz et al., 2025). The infection cycle and systemic progression of BLB within the host vascular tissues are illustrated in Figure 1. This vascular colonization explains the rapid spread of symptoms under favorable environmental conditions.

Figure 1. Disease Cycle and Pathogenesis of Bacterial Leaf Blight (BLB)



2.2 Pathogenesis and Mycotoxin Risks of Rice False Smut

In contrast to the systemic nature of BLB, rice false smut is a floral disease that specifically targets the reproductive organs of the rice plant. The infection process begins at the late booting stage, where U. virens spores germinate on the spikelet surface and enter the inner floral space through the gap between the lemma and the palea (Song et al., 2024). Research has elucidated that the presence of rice stamens is essential for the formation of the "smut ball," a velvety fungal structure that replaces the grain (Wang et al., 2025). These smut balls, initially covered by a thin membrane that later ruptures to release yellow to greenish-black chlamydospores, represent the most visible symptom of the disease (Guo et al., 2025).

The implications of RFS extend beyond direct yield reductions, which can reach 75% in severe cases. The pathogen produces hazardous secondary metabolites, including ustiloxins and ustilaginoidins (Li et al., 2025). Ustiloxins are particularly concerning due to their antimitotic properties, which inhibit microtubule assembly and can cause significant liver and kidney damage in humans and livestock (Chen et al., 2024). This toxicity raises critical food safety concerns, particularly as RFS incidence increases alongside the adoption of high-yield hybrids and excessive nitrogen fertilization (Guo et al., 2025).

Table 1: Key Pathological Features of Bacterial Leaf Blight (BLB) and Rice False Smut (RFS)

Disease Feature	Bacterial Leaf Blight (BLB)	Rice False Smut (RFS)

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Causal Agent	*Xanthomonas oryzae* pv. *oryzae*	*Ustilaginoidea virens*
Infection Site	Vascular system (Xylem)	Floral organs (Stamen/Pistil)
Primary Symptoms	Necrotic leaf stripes, Kresek	Greenish-black smut balls
Peak Infection Stage	Tillering to Maturity	Booting to Heading
Yield Loss Potential	20% to 80% (Varanasi et al., 2023)	Up to 75% (Zhou et al., 2024)
Major Risk Factors	High humidity, High Nitrogen	Late planting, High Nitrogen, Hybrids
Safety Concern	Reduced grain filling	Mycotoxin contamination (Zhou et al., 2024)

3. Epidemiology and Distribution in Pakistan

The spatial distribution of these diseases in Pakistan is influenced by regional microclimates and traditional farming practices. BLB is prevalent across the major rice-growing provinces, with Punjab exhibiting the highest prevalence due to its intensive Basmati cultivation (Ullah et al., 2020). Surveys conducted in the 2019-20 period revealed that districts such as Gujranwala and Sialkot are hotspots for BLB, with prevalence rates reaching 55% and 29%, respectively (Khan et al., 2021). Conversely, the Sindh province has historically shown lower BLB incidence, though recent years have seen an uptick in areas like Badin (Latif et al., 2021).

False smut incidence has also shown a geographical bias. In southern Pakistan, specifically the Badin district of Sindh, a survey identified disease incidence ranging from 24% in city surroundings to a staggering 46% in the Khorwah region. The frequency of *U. virens* in these samples was measured at 45.12%, confirming its status as the primary threat to the region's floral health (Ahmed et al., 2023). The epidemiology of both diseases is further complicated by the use of infected seeds, volunteer plants, and the survival of spores in soil and crop debris over several years (Shahid et al., 2022).

Table 2: Regional Prevalence and Incidence of Rice Diseases in Pakistan (2019-2025 Surveys)

Province	**Region/District**	**BLB Prevalence (%)**	**RFS Incidence (%)**	**Key Pathogen**
Punjab	Gujranwala	55	ND	Xoo Pathotypes (Arshad et al., 2021)
Punjab	Sialkot	29	ND	Xoo Pathotypes (Arshad et al., 2021)
Punjab	Narowal	27	ND	Xoo Pathotypes (Arshad et al., 2021)

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Sindh	Badin (Khorwah)	13	46	*U. virens* (Mubeen et al., 2025)
Sindh	Shikarpur	10	ND	Xoo (Arshad et al., 2021)
KP	Mansehra	37 (Incidence)	ND	Xoo (Arshad et al., 2021)

4. Molecular Characterization of Resistance Genes

The cornerstone of modern rice improvement in Pakistan is the molecular characterization of the existing germplasm to identify and utilize resistance (R) genes. This process allows for a departure from traditional selection, which is often confounded by environmental factors and the masking effects of gene epistasis (Zubair et al., 2022).

4.1 Genomic Dissection of BLB Resistance

To date, approximately 45 R genes against BLB have been identified and mapped globally. In Pakistan, molecular screening has focused on a subset of these genes that have proven effective against local Xoo pathotypes. The most frequently utilized genes include *Xa4*, *xa5*, *Xa7*, *xa13*, and *Xa21* (Arshad et al., 2016).

- ***Xa4***: A dominant gene that confers resistance by strengthening the cell walls of the host. While widely deployed, its effectiveness has diminished in some regions as Xoo populations have evolved virulence against it (Varanasi et al., 2023).
- ***xa5***: A recessive gene that modulates the host's transcription machinery. It is highly prevalent in Pakistani GSR (Green Super Rice) germplasm, with frequencies as high as 91.8% in some collections (Naheed et al., 2024).
- ***xa13***: A recessive gene that targets the copper transporter (SWEET) genes. By preventing the pathogen from hijacking host nutrients, *xa13* provides highly durable resistance (Eom et al., 2019).
- ***Xa21***: Derived from wild rice (*Oryza longistaminata*), this gene encodes a receptor-like kinase that recognizes the pathogen's secretion systems. It remains one of the most effective broad-spectrum genes in Pakistani breeding (Hsu et al., 2020).
- ***Xa23***: An executor R gene from *Oryza rufipogon* that confers high resistance at all growth stages. It is increasingly being integrated into new varieties to ensure broad-spectrum protection (Varanasi et al., 2023).

A molecular analysis of 110 GSR lines at the National Agricultural Research Centre (NARC) identified diverse resistance gene profiles, with some lines possessing up to eight distinct markers (Arshad et al., 2021).

Table 3: Distribution and Frequency of Resistance Genes in Green Super Rice (GSR) Germplasm

Resistance Gene	Frequency in GSR Lines (%)	Nature	Type of Resistance
xa5	91.8	Recessive	Broad-spectrum (Naheed et al., 2024)
Xa21	90.9	Dominant	Broad-spectrum

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Xa4	78.2	Dominant	Broad-spectrum
Xa10	76.4	Dominant	Race-specific
xa13	44.5	Recessive	Durable

4.2 Genetic Basis of False Smut Resistance

Resistance to false smut is genetically more complex, typically governed by quantitative trait loci (QTLs) rather than single major genes. More than 40 QTLs have been identified globally, including *qRFS3.01* and *qRFS12.01-1*, which have shown consistency across mapping populations (Zhou et al., 2024). In Pakistan, the identification of resistant sources is an ongoing priority, as current varieties lack the high-level immunity required to withstand epidemic outbreaks. Screening of International Rice Research Institute (IRRI) lines has identified IR77298-14-1-2: IRGC117374-1 as a potential donor of RFS resistance (Neelam et al., 2022).

5. Breeding for Multi-Disease Resistance in Pakistan

The integration of multiple resistance genes into a single cultivar a process known as gene pyramiding is the primary objective of Pakistan's rice improvement programs (Ullah et al., 2023).

5.1 Marker-Assisted Backcross Breeding (MABB)

MABB has revolutionized the breeding of aromatic Basmati rice. By using molecular markers, breeders can track the introgression of target R genes (foreground selection) while ensuring maximum recovery of the high-quality Basmati genome (background selection) (Reddy et al., 2025). A significant achievement was the development of Basmati backcross inbred lines (BILs) derived from Super Basmati using IRBB57 as the donor parent for *Xa4*, *xa5*, and *Xa21* (Li et al., 2024). Foreground selection identified that the three-gene combination (*Xa4*+*xa5*+*Xa21*) was significantly more effective than single or double gene introgression (Naheed et al., 2022). Genome recovery analysis using the Cornell 7K Array SNP chip showed that most lines recovered >90% of the Super Basmati genome (Munir et al., 2021).

Table 4: Genomic Recovery and Disease Resistance of Basmati Backcross Inbred Lines (BILs)

Line Description	Gene Content	Recurrent Genome Recovery (%)	Disease Reaction (Xoo)
BIL 46	*Xa4* + *xa5* + *Xa21*	93.8	Highly Resistant (Naheed et al., 2022)
BIL 25	*Xa4* + *xa5* + *Xa21*	ND	Min Lesion (Naheed et al., 2022)
BIL 13	*Xa4* + *xa5* + *Xa21*	73.5	Resistant (Naheed et al., 2022)
Super Basmati	Nil	100.0	Susceptible (Naheed et al., 2022)

5.2 Pyramiding in Basmati-385

Similar successes have been recorded in the improvement of Basmati-385. Through crosses with IRBB59, researchers developed lines carrying the quadruple pyramid

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(*Xa4+xa5+xa13+Xa21*), which exhibited superior field performance. The study identified Line 23 as a potentially homozygous recombinant inbred line (RIL) carrying all four genes (Ullah et al., 2023).

5.3 The Role of Advanced Biotechnological Tools

Beyond traditional MAS, precision genome editing tools such as CRISPR/Cas9 are being explored. By editing the promoter regions of susceptibility (S) genes such as the SWEET genes researchers can create varieties that are fundamentally immune. These technologies provide a faster alternative to backcrossing (Eom et al., 2019;).

6. Institutional Milestones and Released Varieties

The Rice Research Institute (RRI) at Kala Shah Kaku and the National Agricultural Research Centre (NARC) have been at the forefront of developing varieties that meet productivity and disease resistance demands (Rice Research Institute, 2025).

6.1 RRI Kala Shah Kaku: A Legacy of Innovation

RRI Kala Shah Kaku has released 33 rice varieties since its inception (Rice Research Institute, 2025). The release of Super Gold in 2019 marked a significant step in providing farmers with a Basmati variety specifically bred for BLB resistance (Whitney & Cartwright, 2018). Furthermore, short-duration varieties like Kissan Basmati (94 days) have allowed for more flexible rotations, reducing the window for pathogen buildup (He et al., 2024).

Table 5: Historical and Recent Rice Variety Releases from RRI Kala Shah Kaku

Variety Name	Year of Release	Type	Key Trait	Resistance Status
Basmati 370	1933	Fine	Aromatic	Susceptible
IR 6	1971	Coarse	High Yield	Susceptible
Super Basmati	1996	Fine	Aroma	Susceptible
Basmati 515	2011	Fine	High Yield	Susceptible
Super Gold	2019	Fine	BLB Resistance	Resistant
KSK 111H	2021	Hybrid	Basmati Hybrid	High Yield
Sona Super Basmati	2023	Fine	9.5mm Grain	Improved Yield
Vital Super Basmati	2023	Fine	Biofortified	Fe & Zn rich
Sultan Super Basmati	2025	Fine	Yield (77 m/a)	High Potential

6.2 NARC and the Green Super Rice Initiative

NARC has focused heavily on the characterization and deployment of Green Super Rice (GSR) germplasm, which is designed to produce high yields under minimal input conditions. Screening of GSR lines has identified several accessions that combine superior resistance to BLB with high paddy yield potential (Biswas, 2003). Specifically, Cluster 1 of the GSR germplasm (representing 36.4% of the lines) combines an average yield of 2520 kg/ha with robust resistance to local Xoo strains (Naheed et al., 2024).

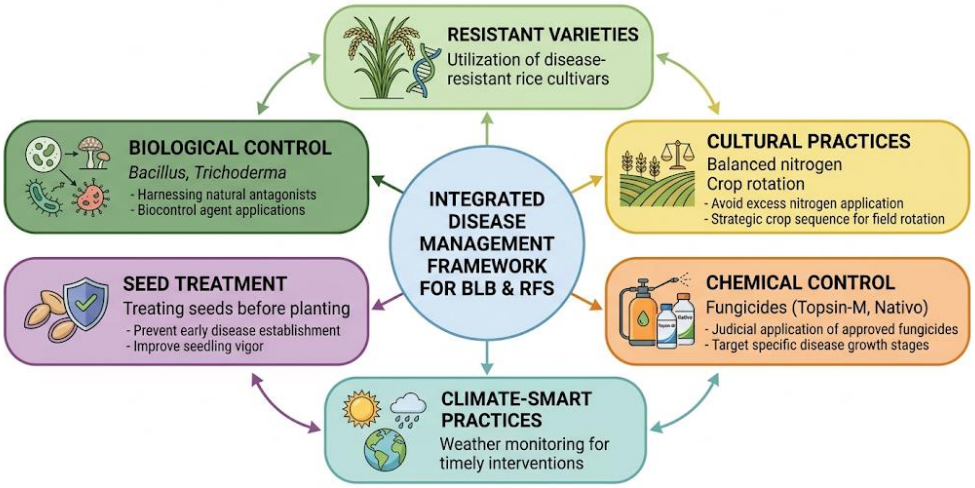
7. Management and Integrated Control Strategies

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
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Online ISSN 3007-3197
Print ISSN 3007-3189
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An integrated disease management framework combining genetic and agronomic strategies is presented in Figure 2. Such multi-layered approaches are essential for sustainable disease control. Resistant varieties must be integrated with robust management practices (Zhou et al., 2024).

Figure 2. Integrated Disease Management Framework for BLB and RFS



7.1 Chemical and Biological Interventions

Field studies in Sindh have shown that fungicides such as Topsin-M and Nativo provide 100% inhibition of *U. virens* at high concentrations. Biological control agents, including *Bacillus* and *Trichoderma*, have also shown promise, inhibiting fungal growth by over 70% (Mubeen et al., 2025).

Table 6: Effectiveness of Various Management Strategies for Rice False Smut

Treatment	Type	Application	Efficacy (%)
Topsin-M	Chemical	1500 ppm	100 (Mubeen et al., 2025)
Nativo	Chemical	1500 ppm	100 (Mubeen et al., 2025)
Pyraclostrobin	Chemical	Booting stage	Up to 95 (Zhou et al., 2024)
Bacillus subtilis	Biological	Seed/Root treatment	44-93 (Zhou et al., 2024)
Garlic Extract	Botanical	Foliar spray	Antifungal (Zhou et al., 2024)

7.2 Cultural Practices and Inoculum Management

Effective management requires adherence to improved cultural practices. Avoiding excessive nitrogen is critical, as high levels promote luxuriant growth that pathogens exploit. Treating seeds with hot water (52 degrees C to 54 degrees C for 20-30 minutes) eliminates primary inoculum (National Institute for Agriculture and Biology, 2025).

8. Future Perspectives and Research Priorities

Future breeding must address pathotype variation in Xoo, which remains high. Breeders must prioritize novel R genes and QTLs, including temperature-stable genes like *Xa7* (Hsu et al., 2020). Advancements in pathogen genomics are providing new insights through effector protein identification, allowing for the development of "smart" rice varieties (Shakouka et al., 2026).

9. Conclusions

Bacterial leaf blight and rice false smut pose severe threats to rice productivity and quality in Pakistan, causing substantial yield losses and, in the case of false smut,

Online ISSN 3007-3197
Print ISSN 3007-3189

introducing mycotoxin risks that compromise food safety. Effective management requires an integrated approach centered on resistant varieties developed through molecular breeding and gene pyramiding. Successful introgression of major BLB resistance genes (Xa4, xa5, xa13, Xa21) into elite Basmati backgrounds via marker-assisted backcrossing has produced high-performing lines with durable, broad-spectrum resistance while preserving grain quality and aroma. For false smut, ongoing identification of QTLs and resistant donors, combined with cultural and chemical interventions, offers promising control options. Institutional efforts at RRI Kala Shah Kaku and NARC, including the release of varieties like Super Gold and advancement of Green Super Rice germplasm, demonstrate the practical impact of these strategies. However, challenges such as evolving pathogen pathotypes, climate-driven disease pressure, and the need for multi-stress tolerance necessitate continued research into novel resistance sources, CRISPR-based editing of susceptibility genes, precise pathotype monitoring, and climate-smart agronomic practices. Strengthening seed systems, farmer awareness, and policy support for resistant varieties will be essential to minimize losses, enhance export competitiveness, and ensure long-term food security in Pakistan's rice sector. Ultimately, a robust, genomics-assisted breeding pipeline combined with integrated disease management will be key to building resilient rice production systems capable of withstanding future biotic and abiotic challenges.

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