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International Collaborative Research for the Implementation and Dissemination of a Differential System of Rice Blast

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Abstract

Rice blast, caused by *Magnaporthe oryzae* is one of the most devastating diseases affecting rice production in the Asia-Monsoon region, with estimated global yield losses ranging from 10% to 30%. Introducing resistant varieties is a promising strategy to simultaneously decrease environmental impact and improve sustainable productivity. However, the breakdown of resistance owing to pathogen evolution is a recurring issue. In the context of sustainable rice production in the Asia-Monsoon region, establishing an international collaboration system that enables the consistent evaluation of blast pathogen virulence across the region is essential, as it facilitates the identification of resistance traits in rice genetic resources, and tracks regional pathogen dynamics.

This report introduces the concept of differential system for blast resistance and international deployment of a blast differential system via collaborative research between JIRCAS and IRRI. Subsequently, this report presents case studies of applying the Differential System in Vietnam and Bangladesh under the Green Asia Project, aligned with Japan's "Strategy for Sustainable Food Systems, MIDORI." The case studies include the analysis of long-term monitoring data on the dynamics of rice blast pathogen virulence in Vietnam, field assessment of the resistance of introgression lines in Vietnam, and validation of pesticide reduction using introgression lines with partial resistance genes in field trials conducted in Bangladesh.

These results indicate that the effectiveness of resistance genes significantly differs by location and time, highlighting the criticality of continuous surveillance and strategic gene combinations, whereas introgression lines introducing resistance genes (*Pb1* and *pi21*) showed broader and more durable resistance compared to the original variety and the potential for pesticide reduction without considerable yield loss via resistant variety adoption.

These results support key policy goals such as decreasing environmental burdens, enhancing disease management efficiency, and optimizing pesticide use. Accelerating technology dissemination through farmer demonstrations and strengthening breeding strategies and pathogen monitoring systems are necessary

for the development of sustainable blast management.

This report provides practical insights into rice blast management and contributes to the realization of sustainable rice production in the Asia-Monsoon region.

1: Introduction

Rice blast caused by *Magnaporthe oryzae* is one of the most devastating diseases affecting rice production in the Asia-Monsoon region, with global yield losses estimated at 10%–30% (Skamnioti and Gurr, 2009). Hot and humid climates promote the pathogen proliferation. Moreover, outbreaks have even been observed during the dry season in recent years. The most economical and sustainable technique of controlling rice blast is using resistant rice varieties (Ashikani et al., 2015). Farmers commonly rely on resistant varieties and fungicides; however, the rapid evolution of pathogen often renders these measures ineffective (Phi, 2023).

Historically, the main strategy for blast control has been introducing resistant rice varieties. However, resistance breakdown occurs repeatedly owing to pathogen evolution. Against this backdrop, the accurate identification of pathogen virulence and rice resistance has become crucial for breeding and deployment decisions, as well as for establishing pathogen monitoring systems. Thus, international collaborative research has advanced the development of differential systems to achieve broader and more durable resistance.

This report first discusses the criticality of establishing a differential system for blast resistance in the context of sustainable rice production in the Asia-Monsoon region. Furthermore, it outlines the development and expansion of international cooperation frameworks that support this effort. Subsequently, it introduces case studies from Vietnam, where the system was utilized to examine the dynamics of blast pathogen virulence and assess improved rice lines. In addition, preliminary findings from pesticide reduction trials performed in Bangladesh using blast-resistant lines are presented.

Based on these findings, the report concludes with a discussion of the future difficulties and perspectives for technology dissemination and social implementation.

2: Overview of the Differential System for Blast Pathogen Virulence and Resistance

2.1: Discovery of Blast Pathogen Virulence and Resistance Breakdown

Rice blast pathogens comprise races with varying virulence profiles, depending on the rice variety. Sasaki's earliest report in Japan dates back to 1922 (Nakajima, 2002). Resistance is classified into "complete resistance" and "partial resistance." Complete resistance is race-specific, controlled by a single gene (Flor, 1971) and has long been used in Japanese breeding programs (Ezuka, 1972). However, the continued use of the same resistance genes results in the emergence of corresponding virulent races, leading to resistance breakdown. This became a problem in Japan during the 1960s (Ezuka et al., 1969). Similar breakdowns have been reported internationally, often occurring within a few years of the deployment of resistant varieties.

2.2: Disease Control Technologies Utilizing Resistance Genes

Various strategies have been developed to control rice blast with a focus on resistance gene use. These technologies directly contribute to policy goals such as sustainable disease management, decreased environmental impact, and optimized pesticide use. Key approaches include:

1. Partial resistance genes: Quantitative resistance controlled by multiple genes, with low race specificity and high durability (Ezuka, 1972; Fukuoka & Okuno, 2019).
2. Gene pyramiding: combining multiple resistance genes to achieve broad and durable resistance (Fukuoka et al., 2015).
3. Complete resistance genes (NLR-type): These provide strong protection against specific races but are prone to breakdown owing to pathogen evolution (Flor, 1971; Koizumi, 1994).
4. Multiline varieties: mixtures of near-isogenic lines with various resistance genes that suppress pathogen spread (Koizumi, 2010).
5. Induced resistance: Pre-inoculation with non-pathogenic strains activates plant defense responses (Ashizawa, 2007).
6. RNA interference (RNAi) suppresses pathogen gene expression to decrease infectivity (Saitoh et al., 2016).

Each method has advantages and limitations, and the selection must be tailored to the local pathogen dynamics and cropping systems. The Asia-Monsoon region has high pathogen diversity; therefore, breeding strategies should not solely rely on complete resistance.

2.3: Utilization of Partial Resistance Genes

Partial resistance has gained traction as a core technology for sustainable disease management, considering the repeated breakdown of complete resistance. However, the evaluation of partial resistance is complicated by the presence of complete resistance genes, which can mask the effects of partial resistance. Thus, understanding the complete resistance profile of a variety is necessary for evaluating partial resistance. A differential system serves as the foundation for this purpose.

2.4: Need for an International Differential System

Rice blast pathogens can spread across borders, and regional differences in virulence can substantially influence varietal resistance. Thus, a standardized evaluation system using standard differential varieties and pathogen isolates is necessary for consistent comparison. Additionally, such standardization facilitates integration with national pest surveillance systems.

Different varieties with known resistance genes are used to assess the pathogen virulence. Determining whether a pathogen causes a disease in these standard varieties reveal its virulence profile. Conversely, standard pathogen isolates with known virulence profiles are necessary to assess genetic resistance in rice varieties.

In Japan, Yamada et al. (1976) and Kiyosawa (1984) proposed differential varieties carrying genes such as *Pik-s*, *Pia*, and *Pii*. However, these varieties often carry additional resistance genes, limiting their use for cross-regional comparisons. For instance, *Pish*, which is common in Japanese varieties, is susceptible to Japanese races but resistant to several foreign races, making it challenging to isolate the effects of the target genes.

At the IRRI, near-isogenic lines of the susceptible variety CO39 carrying *Pi-1*, *Pi-2*, *Pi-3*, and *Pi-4* have been developed (Mackill & Bonman, 1992); however, the limited gene number restricts their use for assessing diverse pathogen populations.

Over 100 blast resistance genes have been reported globally (Koide et al., 2009). However, many several have been identified using local pathogen isolates, making cross-study comparisons challenging.

2.5: Establishment of an International Differential System

To solve the problems described in the previous section, the IRRI-Japan collaborative research Project supported by the Japanese government aimed to establish a global evaluation platform for blast resistance. It developed a set of differential lines by individually introducing 23 complete resistance genes into the susceptible Chinese variety Lijiangxintuanheigu (LTH) (Tsunematsu et al., 2000), which is now utilized as the international standard differential variety.

Furthermore, the corresponding standard pathogen isolates were selected. Twenty standard differential blast isolates (SDBIs) were identified from the Philippine isolates, enabling the international assessment of resistance and virulence.

The Differential System has two primary applications as follows (Figure 2.1):

- Virulence profiling: Using standard differential varieties to identify pathogen virulence.
- Resistance profiling: Using standard pathogen isolates to identify the composition of resistance genes in the rice varieties.

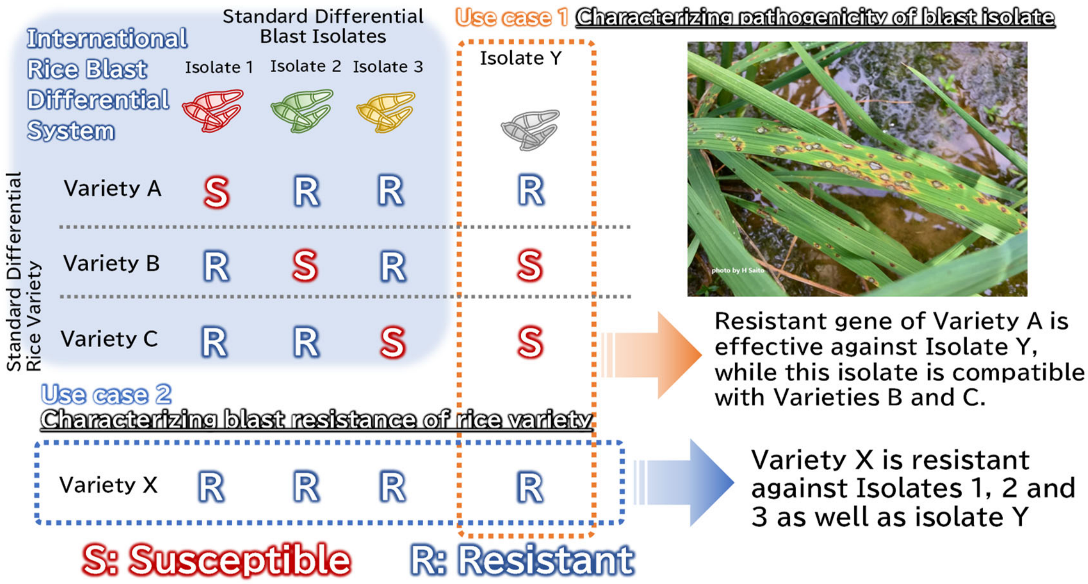

Figure 2.1. Overview of the Differential System for rice blast resistance.

2.6: Application of the International Differential System

Standard differential varieties have been distributed to over 50 research institutions globally and are utilized for virulence evaluation in Asia and Africa. Owing to strict phytosanitary regulations, pathogen isolates cannot be internationally distributed. Thus, each country should establish its own set of standard isolates.

JIRCAS and IRRI built an international network for blast research, collaborating with institutions in Japan, Korea, China, Vietnam, Laos, Cambodia, Thailand, the Philippines, Indonesia, Bangladesh, and West Africa (AfricaRice). Pathogenic isolates have been collected and assessed using standard differential varieties to reveal geographic variations in virulence (Fukuta et al., 2014, 2019, 2020; Kadeawi et al., 2021; Kawasaki-Tanaka et al., 2016; Khan et al., 2016; Li et al., 2016; Nguyen et al., 2020; Odjo et al., 2014; Xangsayasane et al., 2020). Standard isolates have been selected for each country, such as Bangladesh, Vietnam, Indonesia and Laos, based on the findings of pathogen isolates profiling supported by JIRCAS.

This international framework enhances the comparability of breeding research and supports region-specific varietal deployment. Standardized evaluation systems are necessary for institutional disease control strategies, given the transboundary nature of blast pathogens.

The differential system provides scientific evidence for variety registration and supports policy decision making. Additionally, it is indispensable for technologies, such as partial resistance utilization, gene pyramiding, and varietal rotation.

Monitoring of pathogens provides crucial information for regional disease control and variety selection. This system can be integrated with pest surveillance and warning systems to track regional virulence dynamics. Therefore, it serves as a technical foundation for policymaking associated with the deployment of resistant varieties and pesticide optimization.

3: Case Study 1 – Dynamics of Rice Blast Pathogen Virulence in Vietnam

3.1: Background

In Vietnam, crop losses owing to diseases, such as rice blast, have a serious effect on agricultural productivity. Establishing systems to monitor pathogen dynamics is a national policy priority. The "Law on Plant Protection and Quarantine" (No. 41/2013/QH13) enacted by the National Assembly of Vietnam stipulates that the government should support system development for pest information collection, monitoring, and early warning (National Assembly of Vietnam, 2013).

This section introduces a case study in Vietnam that utilizes the blast resistance differential system to examine the dynamics of pathogen virulence of rice blast.

Noda et al. (1999) used 12 Japanese differential varieties to assess the virulence of 129 isolates collected from Vietnam between 1995 and 1996. Their study showed that resistance genes, such as *Pia*, *Pit*, and *Pik-s* had already lost their effectiveness, whereas *Pish*, *Pik*, *Piz*, *Pita-2*, *Piz-t*, and *Pik-p* remained effective.

Fukuta et al. (2020) analyzed isolates collected in 2007 from provinces around the Mekong Delta. In addition to *Pit*, *Pia* and *Pik-s*, *Pib* and *Pi12* had lost resistance, whereas *Pish*, *Pii*, *Pi3*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*, *Pi9*, *Piz*, *Piz-5*, and *Pita* (IRBLta-K1) remained effective.

Nguyen et al. (2020) examined the isolates collected from northern and central Vietnam between 2012 and 2016. In addition to *Pit*, *Pia* and *Pik-s*, increased virulence was noted for *Pib*, *Pii*, *Pi3*, *Pi5*, *Piz*, *Piz-t*, *Pi12*, *Pi19*, and *Pi20*, whereas *Pish*, *Pik-m*, *Pik-h*, *Pi1*, *Pi9*, *Pita2*, *Pik*, *Pik-p*, *Pi7*, *Piz-5*, and *Pita* remained effective. *Pik*, *Pik-p*, *Pi7*, *Piz-5*, *Pita-2* (Re), and *Pita* (two differential varieties) indicated that the number of isolates showing virulence was increasing, although only a few virulent isolates were present.

However, the alterations in race composition following 2016 remains elusive. This study aimed to clarify the virulence of blast isolates collected across Vietnam since

2016 using a differential system and to investigate the implications for resistance breeding strategies.

3.2: Methods

1) Isolation and Inoculation of Blast Pathogens

Blast isolates were collected between 2007 and 2024 from infected rice plants across Vietnam. Of the 807 isolates, 94 from 2007 and 2013 (Mekong Delta, Central Highland, and Southeast) were previously reported by Fukuta et al. (2020) and 239 from 2012 to 2015 were reported by Nguyen et al. (2020). The remaining 474 isolates were assessed using the differential system. Following Hayashi et al. (2009), the infected leaves or panicles were placed on moist filter paper for 24 h at room temperature to induce sporulation. Spores were isolated and stored in filter paper medium. Inoculation tests were conducted according to protocols described by Hayashi and Fukuta (2009).

Table 3.1. Collection periods and regions of rice blast isolates used for virulence evaluation

Ecotypic Area	Year														
	2007	2012	2013	2014	2015	2017	2018	2019	2020	2021	2022	2023	2024	Unknown	Total
Red Rive Delta (RRD)	0	65	25	4	2	0	2	7	0	0	24	42	26	0	197
North Mountain (NM)	0	10	41	28	5	0	7	9	1	0	64	8	57	0	230
North Central (NC)	0	13	1	32	21	10	13	13	1	1	0	0	61	0	166
South Central (SC)	0	0	0	0	36	0	2	0	0	0	0	0	0	0	38
Central Highlands (CH)	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2
South East (SE)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Mekong river delta (MD)	63	0	17	0	0	0	0	0	0	79	0	0	1	0	160
Unknown	1	0	0	0	0	0	0	0	0	0	0	0	0	12	13
Total	64	88	85	64	66	10	24	29	2	80	88	50	145	12	807

2) Virulence Evaluation Using the Differential System

Virulence was scored on a scale of 0–5 based on the lesion size. Scores of 0–2 were classified as resistant (R) and 3–5 as susceptible (S). Exceptions were made for IRBLsh-B, IRBLta2-Pi, and IRBL5-M, based on the lesion progression. Race codes were assigned using the findings from 25 different varieties and the susceptible variety LTH. Diversity indices (Simpson, 1949) and evenness (Arnaud-Haond et al., 2005) were evaluated for the isolates from the four regions (NW, RRD, NC, and MD).

3) Hierarchical Clustering

Hierarchical clustering was conducted using the pheatmap function in R with Euclidean distance and ward. D2 method (Kolde, 2025).

4) Geospatial Data Analysis

The average infection scores were calculated for each province and each differential variety before and after 2016, and visualized using the sf package in R (Nguyen et al., unpublished date) to observe the regional changes in virulence.

3.3: Results and Discussion

1) Temporal and Regional Distribution Analysis

A comparison of the isolates collected before and after 2016 showed four major categories of resistance genes (Figure 3.1).

Type 1: Genes with nationwide resistance breakdown and unsuitability for breeding (*Pia*, *Pib*, *Pik-s*, *Pi3, Pi5, Pit* and *Pish*). Notably, *Pish* rapidly lost resistance following 2017.

Type 2: Genes with low virulence nationwide and suitable for breeding (*Pi9*, *Pik*, *Pik-h*, *Pik-m*, *Piz-5,* and *Pi1*).

Type 3: Genes with regional effectiveness; for instance, effective in the south but broken in the north (*Pita*, *Pi7* and *Pik-p*,). Human activity and seed movement may spread to virulent races.

Type 4: Genes with no clear spatial or temporal trends (*Pita-2* and *Piz-t*); however, virulent races already exist in Vietnam, posing risks for continued cultivation.

These results underscore the dynamic nature of the effectiveness of resistance genes and the criticality of the region-specific gene deployment and continuous pathogen monitoring.

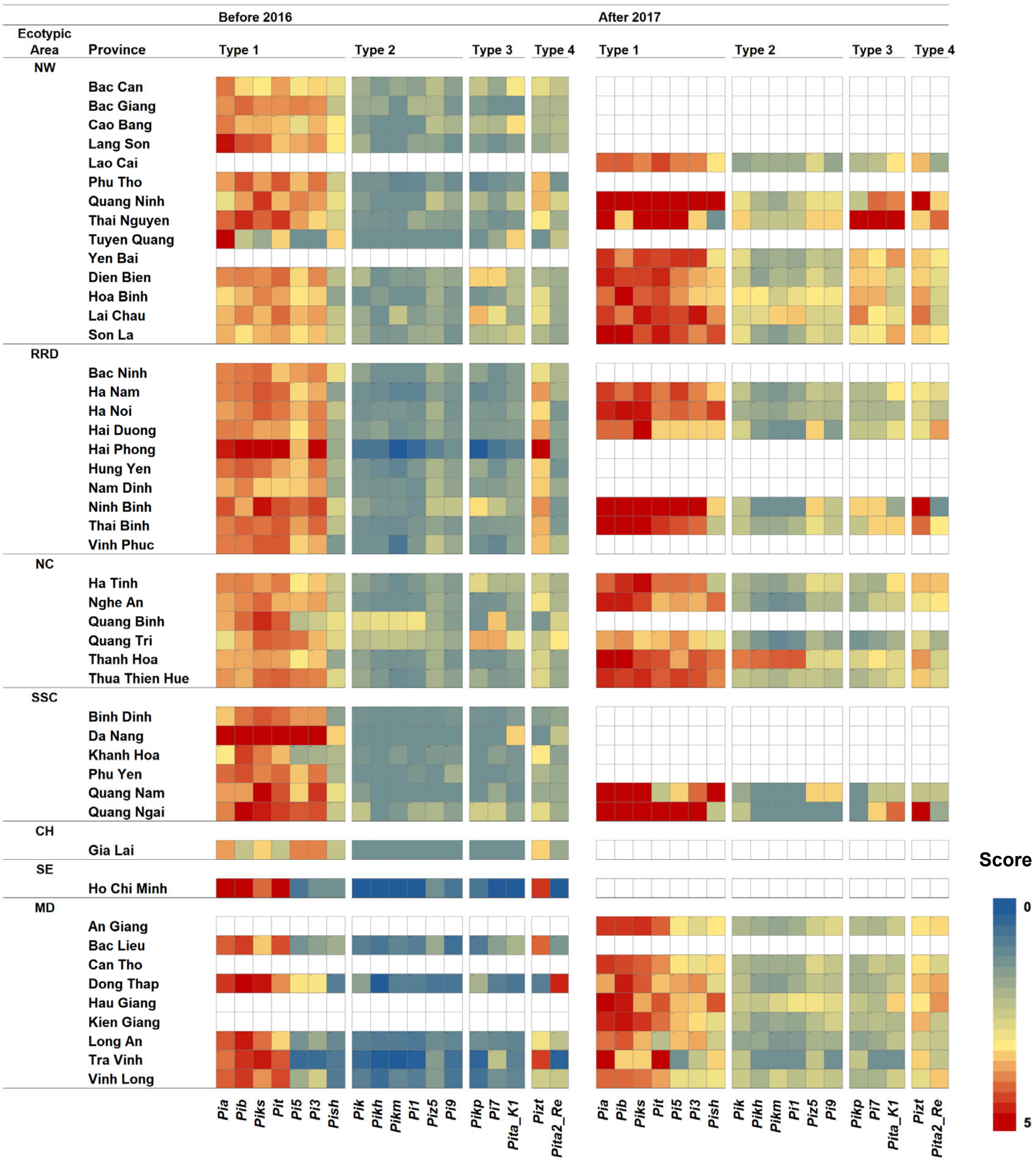

Figure 3.1. Heatmaps of the virulence scores against the differential varieties in the Vietnamese provinces.

The pathogenicity scores of the blast isolates collected from different provinces in Vietnam before 2016 and after 2017 were based on the inoculation tests using different varieties. Each panel represents the average pathogenicity scores for each province during the respective time periods. The color gradient indicates the infection severity, ranging from dark blue (low pathogenicity, score = 0) to deep red (high pathogenicity, score = 5).

2) Identification of Resistance Breakdown Factor in Thien Uu 8

In 2017, the popular variety Thien Uu 8 (TU8) experienced a sudden breakdown in resistance in Ha Tinh Province. Comparative analysis revealed that the isolates from TU8 in 2017 demonstrated high virulence against *Pita-2* (Figure 3.2). Genotyping showed that TU8 harbored *Pita-2*, *Pia*, *Pita*, *Pib*, and *Pi3*/*Pi5* (Table 4.1).

The spread of infection to TU8 in 2017 was considered to be owing to the pathogenicity of the infecting blast isolates against at least the *Pita-2* gene carried by TU8. Le et al. (2023) observed that central Vietnam has a low varietal diversity, with farmers often cultivating the varieties recommended by the government. The widespread monoculture of TU8 likely facilitated the emergence of virulent races capable of overcoming *Pita-2*.

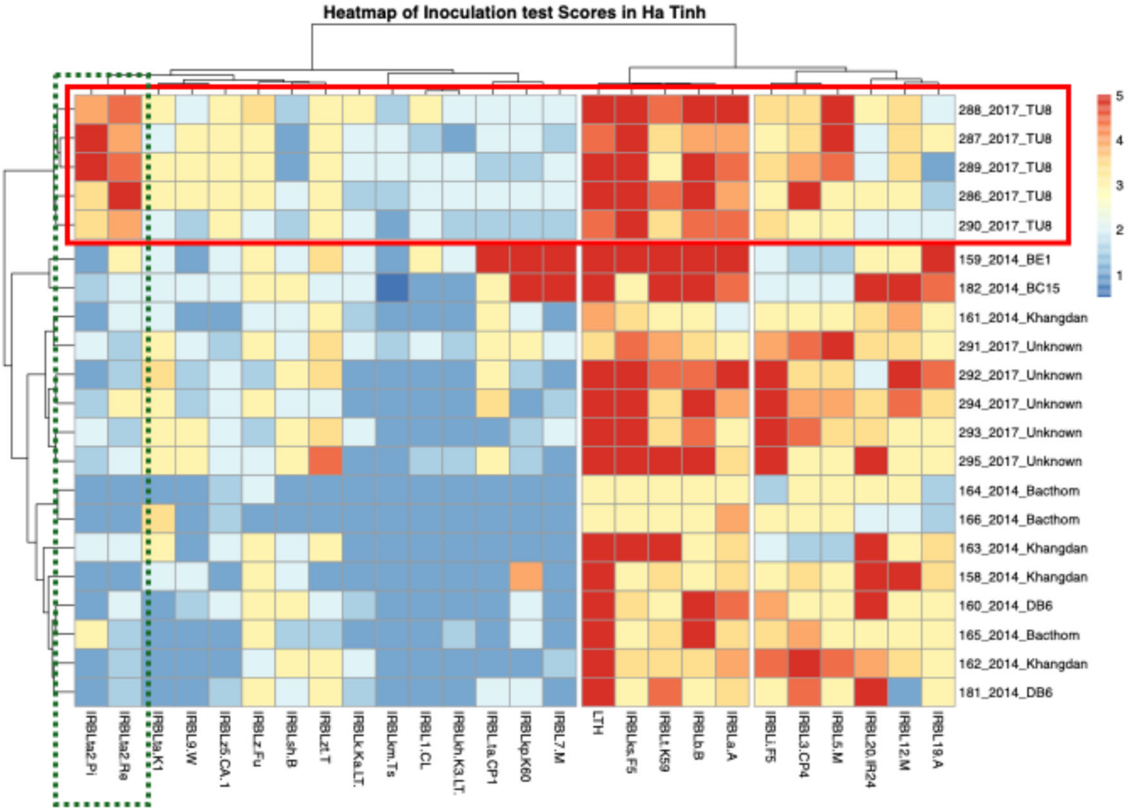

Figure 3.2. Heatmap of the virulence profiles of the blast isolates collected in Ha Tinh Province.

Rows represent the individual isolates labeled by sample ID, year, and host variety. Red boxes indicate isolates from the infected TU8 in 2017. Columns represent differential varieties and susceptible variety, LTH. Green dashed boxes highlight the varieties carrying the *Pita-2* gene. Virulence scores range from 1 (low, blue) to 5 (high, red).

4: Case Study 2 – Resistance Characteristics of Introgression lines in Vietnam

4.1: Background

In Vietnam, rice blast poses a substantial threat to food security and export income, affecting over 40% of the country's cultivated land (personal communication from Vietnamese collaborators). Thien Uu 8 (TU8), a high-yield and widely cultivated variety, has recently suffered from blast outbreaks, as discussed in Chapter 3.

Breeding for durable and broad-spectrum resistance requires the use of partial resistant genes (Fukuoka & Okuno, 2019). as discussed in Chapter 2. Advances in molecular genetics have resulted in the identification of several such genes, including *pi21* on chromosome 4 (Fukuoka & Okuno, 2001) and *Pb1* (Fujii et al., 2000).

JIRCAS and the Agricultural Genetics Institute (AGI) of Vietnam introduced *Pb1* and *pi21* into TU8 using marker-assisted selection to stabilize TU8 production. Additionally, lines combining both genes have also been developed (Appendix 4.1).

Given the resistance breakdown observed in TU8 owing to specific pathogen races, maintaining its agronomic traits while enhancing resistance is essential. In this study, we assessed the agronomic performance and resistance characteristics of TU8-derived lines carrying *Pb1* and/or *pi21*.

4.2: Complete Resistance Genes in Introgression lines Carrying *Pb1* and/or *pi21*

TU8 carries the *Pita-2* gene, which has recently been shown to be ineffective. The complete resistance gene profiles of the three introgression lines were determined to assess the effects of *Pb1* and *pi21*.

The three introgression lines were TU8 with *pi21* (J1), *Pb1* (J2), and both *pi21* and *Pb1* (No. 69). Using DNA markers for nine resistance loci (Kitazawa et al., 2019), we found that all lines carried *Pita-2*, like TU8. J1 retained the same alleles as TU8, whereas J2 lacked *Pia* and No. 69 lacked *Pib*, possibly because of the donor parent segments (Table 4.1).

Table 4.1. Estimated genotypes of the complete resistance loci in the TU8 and introgression lines with partial resistance genes

Line/Variety name	Introgressed gene(s)	Estimated allele[a]
TU8		*Pib, Pi5/Pi3, Pia, Pita, Pita-2*
J1	*pi21*	*Pib, Pi5/Pi3, Pia, Pita, Pita-2*
J2	*Pb1*	*Pi5/Pi3, Pita, Pita-2*
No. 69	*Pb1, pi21*	*Pi5/Pi3, Pia, Pita, Pita-2*

[a] Analysis of the *Pik* allele could not be conducted because fragments indicated by Kitazawa et al 2019 were not observed in Pik-ID007 and Pik-ID011, and fragment other than the sizes indicated by Kitazawa et al 2019 were detected in them. （Nguyen et al. unpublished data)

4.3: Agronomic Traits of Introgression Lines Carrying *Pb1* and/or *pi21*

For resistant varieties to be adopted, they must match the agronomic performance of the existing varieties. Farmers and markets widely accept TU8 and maintaining its traits is crucial.

In the spring season of 2023, TU8 and the three introgression lines were cultivated in Yen Bai District, Ha Nam Province, and Hanoi (Appendix 4.2). Agronomic traits were similar between TU8 and the introgression lines. Yields were comparable across sites, with minor variations in the 1000-grain weight (Appendix 4.3).

J2 had a higher 1000-grain weight than TU8, possibly because of the donor segments outside the target region of the partial resistance gene. Overall, the introgression lines retained TU8's agronomic traits and yield potential, making them suitable for dissemination.

4.4: Response to Vietnamese Standard Differential Blast Isolates (SDBIs)

Scientific assessment of resistance is necessary for making policy decisions regarding variety deployment. Partial resistance is durable but depends on the local pathogen dynamics, requiring inoculation tests with regional isolates.

Resistance was evaluated according to Hayashi and Fukuta (2009), using 25 Vietnamese SDBIs (Nguyen et al., 2020). Virulence was scored on a scale of 0 to 5. Scores ≥3 were considered susceptible, whereas scores <3 were considered resistant. While TU8 was resistant to 22 isolates, J1, J2, and No. 69 were resistant to 25, 24, and 25 isolates, respectively.

Despite the lack of *Pib* and *Pia*, J2 and No. 69 exhibited improved resistance, indicating the effectiveness of *Pb1* and *pi21* (Table 4.2). Disease scores were lower in all introgression lines, which is consistent with previous findings (Saito et al., 2022).

Table 4.2. Responses of TU8-derived introgression lines to the 25 Vietnamese standard differential blast isolates

Line/Variety name	Introgressed gene(s)	No. of isolates		Score difference with original variety [a]
		Resistant reaction	Susceptible reaction	
TU8		22	3	-
J1	*pi21*	25	0	-0.42 ± 0.55
J2	*Pb1*	24	1	-0.42 ± 0.61
No. 69	*Pb1, pi21*	25	0	-0.62 ± 0.73
LTH	S-check	0	25	

[a] The values for the 25 isolates were calculated by subtracting the scores of the introgression lines from those of the original variety. The mean and standard deviation of these differences are presented. Negative values indicate a shift toward increased resistance in the introgression lines compared to the original varieties. (Nguyen et al. unpublished data)

4.5: Response of the Introgression Lines to Vietnamese Isolates Infecting TU8

TU8 exhibits resistance to many SDBIs in Vietnam. However, assessing their response to blast isolates that have caused disease allows us to specifically determine the effectiveness of improved lines against local disease risks. As TU8 exhibits susceptibility to only a limited number of strains in SDBIs, inoculation tests using strains that have infected fields have become a critical verification method for showing the effectiveness of the lines.

This section presents the findings of assessing the response of the three lines into which *Pb1* and *pi21* were introduced against the strains to which the original TU8 variety is susceptible.

J1 and J2 revealed lower disease scores than TU8 against the 16 virulent isolates (Figure 4.1). While average score of TU8 was 3.09, those of J1 and J2 were 2.69 (resistant to 8 isolates) and 2.78 (resistant to 6 isolates), respectively. Despite the lack of *Pib* and *Pia*, both lines revealed broader resistance than TU8, suggesting the effectiveness of partial resistance genes. No. 69 (pyramided line) had the lowest score (2.22) and was resistant to 14 out of the 16 isolates. These findings support the strategy of gene pyramiding to enhance resistance (Fukuoka et al., 2015), and No. 69 is a promising candidate for durable resistance breeding.

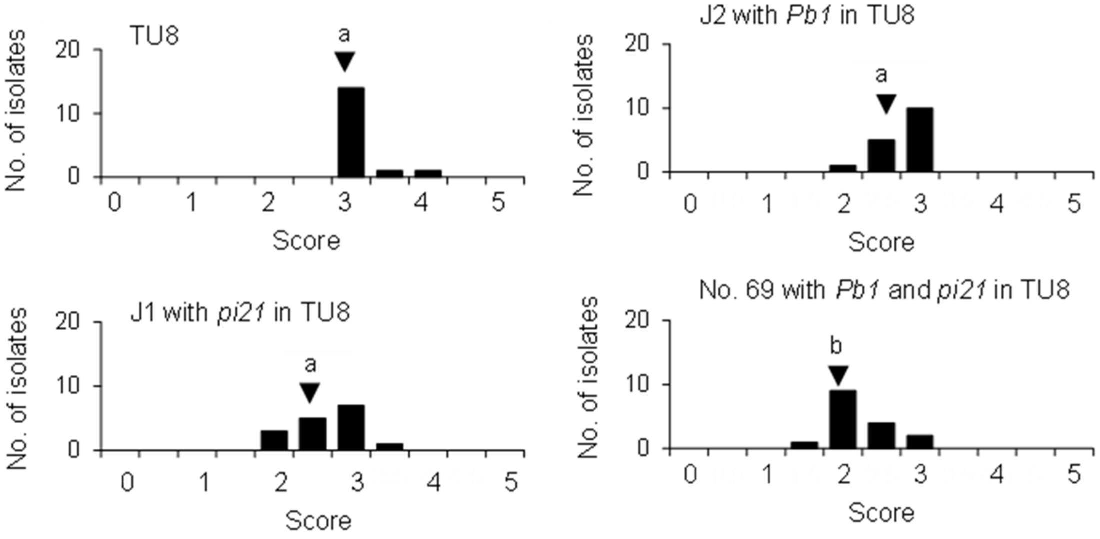

Figure 4.3. Frequency distribution of the disease scores against blast isolates virulent to TU8.

Inoculation tests were conducted in the introgression lines and original varieties using 16 blast isolates virulent to TU8. Blast disease score: 0 (no symptom) - 5 (severe symptom). Statistical comparisons among the four lines were conducted using Tukey’s test at a significance level of probability < 0.01. (Nguyen et al. unpublished data)

4.6: Pesticide Reduction Trials Using the introgression Lines

Field trials were conducted in Hue and Yen Bai, using three pesticide treatments: no application, 50% application, and conventional application. However, TU8 showed little infection, making evaluation difficult.

Vietnamese researchers noted that blast outbreaks were less frequent during the Green Asia Project period (2023–2025). The susceptible variety US2 showed infection at the same location during this period, indicating a latent risk. Artificial inoculation of infected leaves may be required to ensure consistent infection rates in future studies.

Despite limited infection, introgression lines retained TU8’s agronomic traits and revealed broad resistance, making them strong candidates for policy-based variety selection and farmer adoption.

5: Case Study 3 – Pesticide Reduction Trials in Bangladesh

5.1: Background

In Bangladesh, rice blast causes substantial yield losses and threaten farmers' livelihoods and national food security. Strong demand for technologies that decrease pesticide use while maintaining yield.

Rice cultivation in Bangladesh is divided into three cropping seasons: Boro (dry-season irrigated rice), Aman (late wet season rainfed rice), and Aus (early wet season upland rice). Among these, Boro is the most productive and is critical to national food security. However, chemical pesticide use is not always widespread in Boro, and the lack of proper control measures often results in severe blast outbreaks, particularly panicle blast.

Recently, blast outbreaks were reported during the Aman and Aus seasons, underscoring the criticality of disease management. While excessive pesticide use raises environmental concerns, the goal is not pesticide-free farming but rather sustainable production via optimized pesticide use and resistant varieties.

The National Seed Board of Bangladesh declared a policy to promote the introduction and dissemination of resistant varieties to enhance productivity and decrease disease risk (Bangladesh National Seed Board, 2023). Furthermore, JICA (2023) emphasizes the requirement for sustainable rice systems via appropriate pesticide use and the deployment of resistant varieties.

This report presents the findings of field trials using introgression lines with partial resistance genes under 50% reduced-pesticide conditions. Appendix 5.1 provides the details of the introgression lines and trial design.

5.2: Partial Resistance of Introgression lines

For each pesticide treatment (50% application and conventional application), the panicle blast incidence and grain yield were measured. The increase in the blast incidence under decreased pesticide conditions was calculated by comparing the

50% reduction treatment with the conventional treatment.

Consequently, under 50% pesticide reduction, the panicle blast incidence increased 125-fold in the original varieties (BRRI dhan 63 and BRRI dhan 64). Contrastingly, the three introgression lines revealed only a 37.1-fold increase on average. This represented a 70.3% reduction in the blast incidence rate (Figure 5.1).

These findings show that introgression lines can markedly suppress panicle blasts even under reduced-pesticide conditions.

Figure 5.1. Comparison of the panicle blast incidence under reduced pesticide conditions
Blast incidence rate is indicated as a fold increase in the 50% pesticide reduction treatment compared to the conventional treatment. Black bars indicate the original varieties; white bars represent the average of the three breeding lines.

5.3: Reduced chemical pesticide use in fields with the introgression lines

Pesticide reduction was calculated using the indicator: “amount of pesticide used per unit yield.” This was evaluated by comparing the yield under reduced-pesticide conditions with that under the conventional treatment.

The introgression lines achieved a 45.8% reduction in pesticide use per unit yield. This suggests that introgression lines can maintain yield while decreasing pesticide input.

Although the trial sites had similar climatic conditions to the surrounding regions, allowing for broader dissemination, the findings were based on limited field sites and may not be directly applicable nationwide. Differences in climate, soil, and farming practices may influence the effectiveness of the resistant varieties and pesticide reduction.

Randomized trials involving farmers are recommended to objectively assess pesticide reduction. For instance, providing varieties without disclosing names and monitoring pesticide use, timing, and yield under normal management can eliminate bias and allow the quantitative assessment of pesticide reduction per unit yield.

Furthermore, setting multiple pesticide levels (e.g., a 40% reduction) can help determine the tolerance of the resistant varieties to reduced pesticide inputs. Such trial designs can optimize pesticide use and provide practical guidance for farmers.

Future studies should include multi-location and multi-year trials, as well as randomized trials, to further validate the applicability of resistant varieties and pesticide reduction strategies in sustainable rice systems.

6: Future Directions and Methodologies for Social Implementation

6.1: Accelerating Technology Dissemination through Farmer Demonstrations

Farmer demonstrations are highly effective at accelerating the dissemination of new technologies. When farmers experience technology first-hand and observe its benefits, their understanding and acceptance increase, helping in broader adoption within the community. Demonstrations in actual fields help visualize outcomes such as yield, disease resistance, and input use, thus encouraging behavioral change.

In the spring of 2025, a preliminary demonstration featuring blast-resistant rice lines was conducted in Nghe An Province, central Vietnam. The participants included local researchers, farmer groups, and staff from plant protection centers. Introgression lines were presented in the field, and participants received explanations regarding their traits and management practices.

However, natural blast infection was limited in this region and even the parent lines revealed little disease. Thus, resistance assessment was explained using the findings from separate inoculation trials, which confirmed that introgression lines exhibited stronger resistance than the parent variety. Future studies should include additional validation under natural infection conditions.

This preliminary demonstration was valuable for evaluating the local adaptability and farmer acceptance and provided useful information for the design of formal demonstrations. Dialogues with farmers help to identify technical concerns and expectations, contributing to the development of support systems by extension agencies.

Public institutions such as agricultural extension centers and plant protection offices support extension efforts. These organizations play a key role in connecting farmers and promoting technology adoption. Activities such as providing information and field guidance contributes to productivity and sustainability. Collaboration with these institutions enables broader regional dissemination.

Farmer demonstrations linked with public institutions offer technical promotion as well as policy support and institutional backing, ensuring sustainable implementation. These institutions have established trust and networks with farmers, making them essential partners in increasing the acceptance of technology. Strengthening these partnerships is a key consideration in future policy design.

6.2: Breeding Strategies and Pathogen Monitoring for Sustainable Blast Resistance

Using blast-resistant varieties is a highly effective strategy for disease control. However, resistance based on complete resistance genes is often broken down within a few years owing to the spread of virulent races, particularly when the same variety is continuously cultivated. To develop sustainable resistance, identifying the composition of resistance genes in various varieties, monitoring pathogen virulence, and detecting emerging races through continuous surveillance are essential.

Nationwide, systematic monitoring should be regularly conducted to determine which resistance genes are effective in each region and to understand the spatiotemporal dynamics. This approach should enable efficient breeding and sustainable disease control. Such monitoring systems can be institutionalized by linking them with existing networks such as plant protection centers.

A preliminary monitoring survey was conducted in the Mekong Delta region of Vietnam in the spring of 2025. This study assessed the race composition of local blast pathogens before introducing resistant varieties. Pathogens were collected from the two provinces and tested against lines carrying representative resistance genes.

This preliminary monitoring provides the necessary information for various selection and breeding strategies. Understanding regional virulence enables a more effective deployment of resistance. Future plans include using the data to identify suitable resistance gene combinations for Mekong Delta and developing breeding strategies for sustainable disease control.

In cases of endemic outbreaks, emergency responses, such as increased monitoring frequency and rapid evaluation of resistance effectiveness are needed. Chapter 3.3.2 demonstrated the value of such responses, which can inform the re-evaluation of resistant varieties and breeding strategies. These insights provide scientific evidence for future disease control policies.

6.3: Breeding for Blast Resistance and Multi-Disease Resistance

In the Asia-Monsoon region, rice production is affected by blast as well as by other pests and diseases such as bacterial leaf blight and brown planthoppers. Decreasing pesticide use and controlling burden are policy priorities. Thus, developing rice varieties that are resistant to multiple pests and diseases is a key strategy for sustainable rice systems.

As described above, the blast differential system enables the identification of effective resistance genes and supports the breeding of lines with durable partial resistance. Moreover, it has facilitated the discovery of new partial resistance genes. However, in the current identification system, the spore inoculation assay for rice blasts is highly labor-intensive, which represents a substantial limitation that hinders the practical implementation of the system under field conditions. To overcome this limitation, DNA marker-assisted selection offers a highly promising method for the development of blast-resistant cultivars, enabling breeding with greater efficiency and precision.

Given the prevalence of multiple pests and diseases, breeding multi-resistant varieties is a high-priority policy. These strategies contribute to decreased pesticide use and environmental impact and can be positioned as a part of sustainable agricultural policy.

Authors contribution statement

M.O. conceived and coordinated the overall research framework and, in particular, designed the use of pathogen isolates capable of infecting the parental variety to enable the differential system to detect the effects of partial resistance genes—a methodological contribution unique to this report. H.S. provided plant-pathology expertise and generated new insights by interpreting large-scale virulence data, identifying temporal and regional shifts in pathogen populations. N.K. designed the multi-location inoculation trials for evaluating introgression lines and coordinated data acquisition with partner institutions, contributing new evidence on resistance performance across diverse environments. All authors jointly reviewed the literature, integrated the data, and contributed to writing and revising the manuscript.

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Appendix 1.1

Table. International standard blast differential varieties developed under the IRRI-Japan collaborative research project (Hayashi et al. 2009)

		International standard rice blast differential variety　(refference)			
Target R-gene	R-gene source	Monogenic line (Tsunematsu et al. 2000)	LTH-NIL (Telebanco-Yanoria et al. 2010)	CO39-NIL (Telebanco-Yanoria et al. 2011)	US2-NIL (Fukuta et al. 2022)
Pish	BL1	IRBLsh-B		IRBLsh-B[CO]	
Pish	Shin 2	IRBLsh-S		IRBLsh-S[CO]	
Pish	Kusabue			IRBLsh-Ku[CO]	
Pish	Fukunishiki			IRBLsh-Fu[CO]	
Pib	BL1	IRBLb-B	IRBLb-B[LT]		
Pib	IRAT 13			IRBLb-IT13[CO]	
Pit	K59	IRBLt-K59 1)			
Pia	Aichiasahi	IRBLa-A			US2NILPia-A
Pia	CO 39	IRBLa-C			US2NILPia-C
Pia	Zenith		IRBLa-Ze[LT]		
Pii	Fujisaka 5	IRBLi-F5			US2NILPii-F5
Pi3	C104PKT	IRBL3-CP4	IRBL3-CP4[LT]		US2NILPi3-CP4
Pi5	RIL249 (Moroberekan)	IRBL5-M		IRBL5-M[CO]	US2NILPi5-M
Pik-s	Fujisaka 5	IRBLks-F5			US2NILPiks-F5
Pik-s	Shin 2	IRBLks-S	IRBLks-S[LT]		US2NILPiks-S
Pik-s	B40		IRBLKs-B40[LT]		
Pik-s	Zhaiyeqing 8		IRBLKs-Zh[LT]		
Pik-s	Caloro			IRBLks-CO[CO]	
Pik-m	Tsuyuake	IRBLkm-Ts		IRBLkm-Ts[CO]	US2NILPikm-Ts
Pi1	C101LAC	IRBL1-CL		IRBL1-LA[CO]	US2NILPi1-CL
Pik-h	K3	IRBLkh-K3	IRBLkh-K3[LT]	IRBLkh-K3[CO]	US2NILPikh-K3
Pik	K51	IRBLk-Ka	IRBLk-Ka[LT]	IRBLk-Ka[CO]	US2NILPik-Ka
Pik	Kusabue			IRBLk-Ku[CO]	
Pik-p	K60	IRBLkp-K60		IRBLkp-K60[CO]	US2NILPikp-K60
Pi7	RIL29 (Moroberekan)	IRBL7-M	IRBL7-M[LT]	IRBL7-M[CO]	US2NILPi7-M
Pi9	WHD-1S-75-1-127	IRBL9-W	IRBL9-W[LT]		US2NILPi9-W
Piz	Fukunishiki	IRBLz-Fu			US2NILPiz-Fu
Piz-5(Pi2(t))	C101A51	IRBLz5-CA	IRBLz5-CA[LT]		US2NILPiz5-CA
Piz-t	Toride 1	IRBLzt-T			US2NILPizt-T
Pita-2	Reiho	IRBLta2-Re		IRBLta2-Re[CO]	US2NILPita2-Re
Pita-2	Pi No.4	IRBLta2-Pi	IRBLta2-Pi[LT]	IRBLta2-Pi[CO]	
Pita-2	IR 64			IRBLta2-IR64[CO]	
Pi12	RIL10(Moroberekan)	IRBL12-M			
Pita (Pi4(t))	K1	IRBLta-K1	IRBLta-K1[LT]		US2NILPita-K1
Pita (Pi4(t))	C101PKT	IRBLta-CP			US2NILPita-CP1
Pita (Pi4(t))	C105TTP2L9	IRBLta-CT2	IRBLta-CT2[LT]		US2NILPita-CT2
Pita (Pi4(t))	Zhaiyeqing 8		IRBLta-Zh[LT]		
Pita (Pi4(t))	Yashiromochi			IRBLta-Ya[CO]	
Pita (Pi4(t))	Metica 1			IRBLta-Me[CO]	
Pi19	Aichiasahi	IRBL19-A			
Pi20	ARL24(IR 24)	IRBL20-IR24			US2NILPi20-IR24

Appendix 4.1. Development of Introgression lines with Partial Resistance Genes

The partial resistance genes *pi21* and *Pb1* were introduced into the Thien Uu 8 (TU8) genetic background before the launch of the Green Asia Project. Selection was advanced to the BC_1F_8 generation, and fixed lines were selected (Figure).

DNA marker-assisted selection was conducted using RM16867 and RM1359 for *pi21* (Fukuoka et al., 2009), and RM206 for *Pb1* (Ishihara et al., 2014). After obtaining fixed lines via DNA marker analysis, candidate lines with phenotypes similar to the original variety were selected and those with stable phenotypes were further refined. The following three lines were developed:
J1: *pi21* introgression line (Figure a)
J2: *Pb1* introgression line (Figure b)
No. 69: pyramided line with both *pi21* and *Pb1* (Figure c)

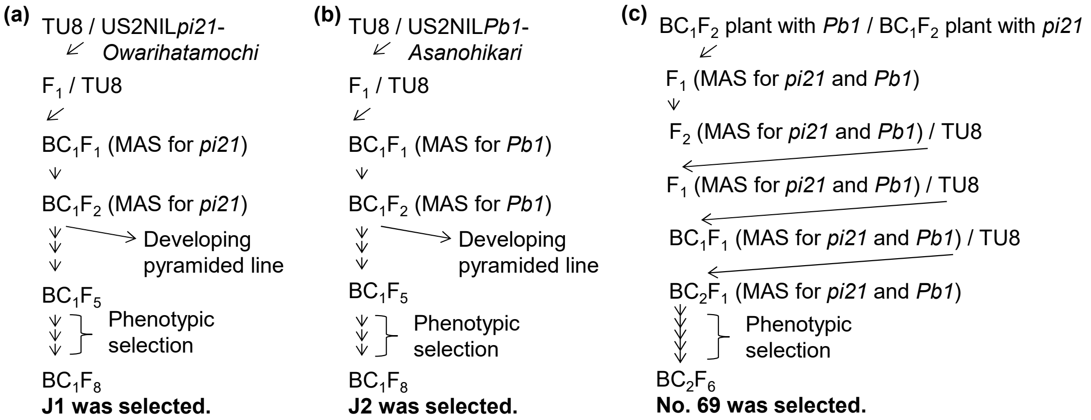

Figure. Development of introgression lines with partial resistance genes

Plants from the BC_1F_2 generation carrying *Pb1* were crossed with those carrying *pi21* (used as pollen donors) to develop the pyramided line. The resulting F_1 plants were backcrossed two times using TU8 as the pollen parent. Finally, plants that were morphologically similar to TU8 were selected. (Modified from Nguyen et al. unpublished data)

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Ishihara, T. et al. (2014) Quantitative trait locus analysis of resistance to panicle blast in the rice cultivar Miyazakimochi. Rice 7: 2.

Appendix 4.2. Cultivation and Agronomic Evaluation of the Introgression lines

In the spring season 2023, three introgression lines with partial resistance genes (*pi21*: J1, *Pb1*: J2, and pyramided line: No. 69) and Thien Uu 8 (TU8) were cultivated in paddy fields in Yen Bac, Duy Tien District, Ha Nam Province, and Hanoi City, Vietnam (Photo).

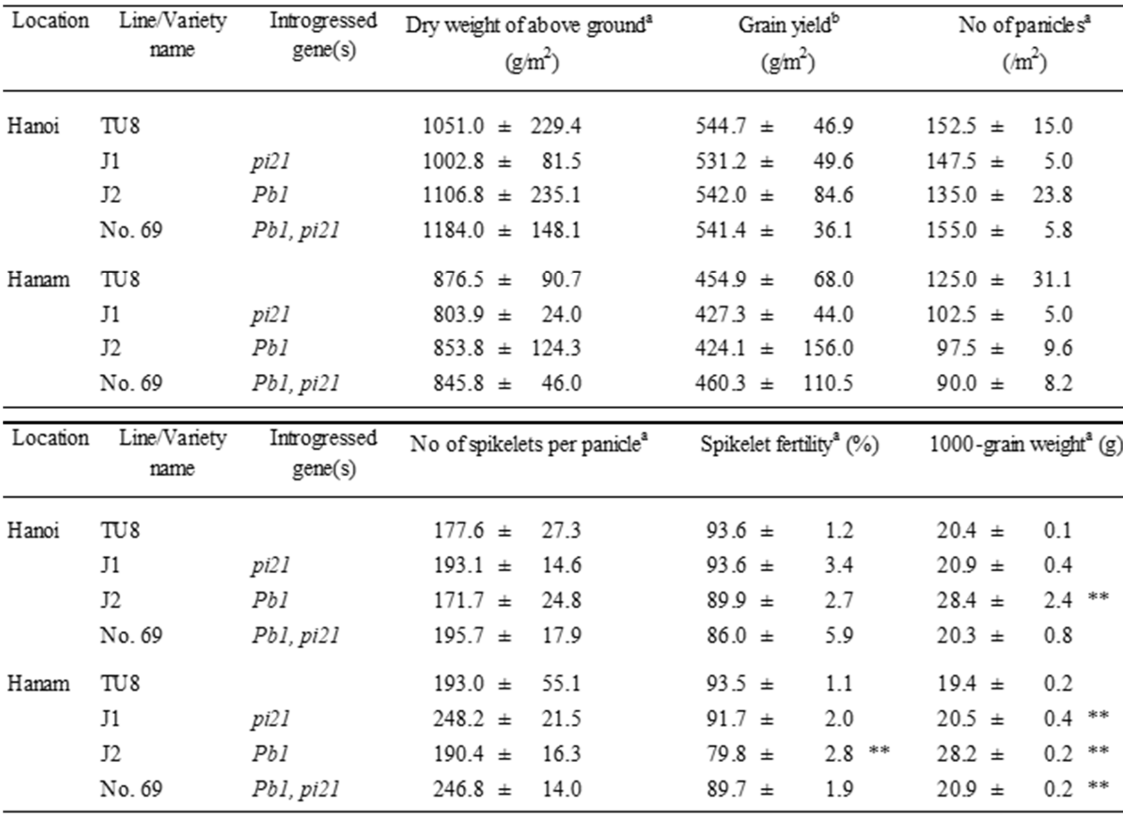
One-month-old seedlings were transplanted at a spacing of 25 cm between rows and 20 cm between plants, at a density of 20 plants per square meter. Each variety or line was planted in plots of 10 plants × 4 rows, with four replicates.

Ten plants were harvested from each plot to assess grain yield. Furthermore, two representative plants per plot were harvested to evaluate five yield-related traits: aboveground dry weight, number of panicles, number of grains per panicle, grain-filling rate, and 1000-grain weight.

Photo. Experimental field in Hanoi in the spring season 2023.

Appendix 4.3. Yield and Related Traits of Thien Uu 8 (TU8) and Introgression lines in Spring 2023 (Hanoi and Ha Nam)

Table. Yields and related traits of TU8 and introgression lines with blast field resistant gene(s) in Hanoi and Hanam in the 2023 spring season



Location	Line/Variety name	Introgressed gene(s)	Dry weight of above ground[a] (g/m^2)	Grain yield[b] (g/m^2)	No of panicles[a] (/m^2)
Hanoi	TU8		1051.0 ± 229.4	544.7 ± 46.9	152.5 ± 15.0
	J1	*pi21*	1002.8 ± 81.5	531.2 ± 49.6	147.5 ± 5.0
	J2	*Pb1*	1106.8 ± 235.1	542.0 ± 84.6	135.0 ± 23.8
	No. 69	*Pb1, pi21*	1184.0 ± 148.1	541.4 ± 36.1	155.0 ± 5.8
Hanam	TU8		876.5 ± 90.7	454.9 ± 68.0	125.0 ± 31.1
	J1	*pi21*	803.9 ± 24.0	427.3 ± 44.0	102.5 ± 5.0
	J2	*Pb1*	853.8 ± 124.3	424.1 ± 156.0	97.5 ± 9.6
	No. 69	*Pb1, pi21*	845.8 ± 46.0	460.3 ± 110.5	90.0 ± 8.2

Location	Line/Variety name	Introgressed gene(s)	No of spikelets per panicle[a]	Spikelet fertility[a] (%)	1000-grain weight[a] (g)
Hanoi	TU8		177.6 ± 27.3	93.6 ± 1.2	20.4 ± 0.1
	J1	*pi21*	193.1 ± 14.6	93.6 ± 3.4	20.9 ± 0.4
	J2	*Pb1*	171.7 ± 24.8	89.9 ± 2.7	28.4 ± 2.4 **
	No. 69	*Pb1, pi21*	195.7 ± 17.9	86.0 ± 5.9	20.3 ± 0.8
Hanam	TU8		193.0 ± 55.1	93.5 ± 1.1	19.4 ± 0.2
	J1	*pi21*	248.2 ± 21.5	91.7 ± 2.0	20.5 ± 0.4 **
	J2	*Pb1*	190.4 ± 16.3	79.8 ± 2.8 **	28.2 ± 0.2 **
	No. 69	*Pb1, pi21*	246.8 ± 14.0	89.7 ± 1.9	20.9 ± 0.2 **

[a] Mean values are presented as standard deviations (n=4). The values for each replicate were obtained from the average of two individuals. A significant difference between the parental variety and an introgression line grown in the same location was determined using Dunnett's test; ** *P*<0.01.

[b] Mean values are presented as standard deviations (n=4). Values for each replication were obtained using the average of ten individuals. A significant difference between the parental variety and an introgression line grown in the same location was determined using Dunnett's test. (Nguyen et al., unpublished data).

Appendix 5.1. Introgression lines with Blast Resistance and Pesticide Reduction Trial Design in Bangladesh

Herein, BRRI dhan 63 and BRRI dhan 64, which are widely cultivated in Bangladesh, were utilized as the background varieties. The partial resistance genes *Pb1* and *pi21* were introduced to develop introgression lines, using US2NILpi21-Owarihatamochi (carrying *pi21*) and US2NILPb1-Asanohikari (carrying *Pb1*) as the donor lines (Saito et al., 2022; Fukuta et al., 2022).

Lines adapted to the local cultivation environments were selected via two rounds of backcrossing and selfing. Finally, three introgression lines with partial resistance and high local adaptability were used.

2) Field Trials and Yield Evaluation

Field trials were conducted to assess the pesticide reduction and yield performance. The study design included the following:

Three pesticide treatments were used: no application, 50% application, conventional application, and six varieties: three introgression lines + three control varieties, with four replications per treatment.

Each plot measured at least 4 m^2. Two to three seedlings were transplanted per hill, and measures were taken to prevent missing plants. The grain yield was measured for 10 adjacent plants per plot.

3) Monitoring of Blast Infection

The incidence of panicle blasts was monitored in all the plots. The evaluation followed the IRRI's Standard Evaluation System (IRRI, 2013) and disease severity was scored. This allowed for an objective comparison between pesticide treatments and varietal resistance.

4) Data Analysis

Panicle blast incidence and grain yield were measured for each pesticide treatment (no application, 50% application, and conventional application). The increase in the blast incidence under 50% pesticide reduction was calculated by comparing the reduced treatment with the conventional treatment (double application).

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