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Phenotypic Evaluation and Genetic Assessment of Advanced Bacterial Leaf Blight (BLB)-Resistant *Gal-ong* Rice Mutants Using the BLB Differential System

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Abstract

Bacterial leaf blight (BLB) is a major rice disease in the Philippines, making the identification of BLB-resistant varieties and their underlying mechanisms critical for breeding. This study evaluated twenty gamma-ray-induced advanced (M_{12}) mutant rice lines derived from the traditional variety *Gal-ong*, alongside susceptible checks and IRBB differential lines that possess known *Xa* genes and with defined reactions to different *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) races. All the materials were screened against ten *Xoo* strains representing nine major Philippine races prevalent in local rice-growing regions across the country. This revealed that some of the mutants (GXB-229, GXB-SB-9, GXB-51, GXB-61, GXB-225-1 and GXB-1) had a wide spectrum of resistance against the different *Xoo* races, with broad-spectrum resistance indices (BSRI ≥ 0.8) comparable to those of the broad-spectrum resistant checks. In trying to estimate the causal genes of the mutants, a discrepancy was observed between the reaction profiles of the mutants with those of the resistant IRBB checks, indicating possible gain-of-function or novel genetic mechanisms in the *Gal-ong* mutants. This is supported by the sequence analysis (BLASTN) of 32 *Xa* genes in *Gal-ong* and GXB207 genomes, which showed that 15 truncated *Xa* genes in *Gal-ong* were partially to fully restored in the mutant in terms of homology length to the full-length query sequence as a random effect of gamma irradiation. Overall, this study identified new BLB-resistant materials that, when further subjected to genetic analysis, could become invaluable sources of novel BLB resistance genes for rice breeding.

Introduction

The sustainability and management of effective and durable resistance gene(s) to biotic stresses remain a continuous challenge worldwide (Mundt, 2014). Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases that result in substantial yield losses of 20–30% in moderately affected fields and up to 50% in severely affected fields (Jiang *et al*., 2020). Although extensive breeding efforts have produced improved rice varieties with better BLB resistance, yield, and tolerance to environmental stresses (Raza *et al*., 2019), the effectiveness of BLB resistance genes often declines over time due to the rapid evolution of pathogen populations. This is especially true for varieties that are largely governed by single dominant (*Xa*) or recessive (*xa*) genes that are often overcome by multiple *Xoo* races (Mew *et al*., 1992; Dossa *et al*., 2015). It is therefore ideal to stack

different BLB resistance genes together for broad-spectrum resistance. Such lines are already available, particularly the IRBB differential lines, which are near-isogenic lines that carry different combinations of *Xa* genes (*Xa4*, *xa5*, *Xa7*, *Xa10*, *xa13*, *Xa14*, and *Xa21*) in IR24 background and have well-defined reactions to multiple *Xoo* races, including the Philippine PXO series. These lines, therefore, serve as references to interpret race-specific responses and *Xa* gene composition of candidate breeding lines, guiding the selection of those with broad-spectrum or durable resistance.

Broad-spectrum resistance refers to the ability to resist the majority of races of a pathogen species (Kou & Wang, 2010), making it a critical objective in breeding programs (Cheng *et al*., 2024). Recent studies indicate that broad-spectrum resistance can arise not only from pyramided R genes but also from modifications in susceptibility-related genes, such as members of the *OsSWEET* family, or through regulatory changes that enhance basal defense responses (Oliva *et al*., 2019). Induced mutagenesis offers a promising avenue to expand the genetic diversity of rice and generate novel resistance alleles. Gamma irradiation, in particular, has been widely used to induce a high frequency and spectrum of mutations, producing mutants with improved traits such as pest and disease resistance, enhanced plant type, higher yield, and tolerance to abiotic stresses (Ahloowalia & Maluszynski, 2001; Baloch *et al*., 2003). Notably, gamma irradiation is capable of producing gain-of-function mutations, which can result in novel phenotypes, including enhanced disease resistance, in addition to loss-of-function mutations (Lee *et al*., 2003; Alfonso *et al.*, 2015). To date, induced mutation has already been demonstrated to generate mutants with broad-spectrum and durable resistance to both rice blast and BLB (e.g., sodium azide-induced SA0169) (Lo *et al.*, 2022).

In this study, we analyzed gamma–ray–induced rice mutants that exhibited resistance to BLB. These mutants were derived from *Gal-ong*, a traditional Benguet variety valued for its eating quality, red pericarp, and strong aroma, but is susceptible to BLB and other diseases. The study aimed to characterize the resistance spectrum of twenty advanced (M_{12}) *Gal-ong* mutants against a total of 10 strains of *Xoo*, representing nine Philippine *Xoo* races, and inferred candidate resistance loci by comparing their reaction patterns with those of the BLB differential lines. Additionally, sequence homology comparisons by BLASTN between *Gal-ong* and the standout mutant GXB-207 were conducted at selected *Xa* loci to identify genetic clues for putative gain-of-function mutations underlying the improved resistance phenotype. The findings provide insight into the potential of induced mutagenesis for generating broad-spectrum and durable BLB resistance, providing valuable genetic resources for future breeding and possibly for the discovery of novel resistance loci.

Materials and Methods

Bacterial Leaf Blight Resistance Screening/ Phenotypic Evaluation

Twenty gamma-ray-induced *Gal-ong* advanced (M_{12}) mutant lines were evaluated against ten local *Xoo* isolates representing nine major Philippine races (Table 1). For the screening, each line was established in triplicate (3 seedlings per pot) and arranged in a Completely Randomized Design (CRD), with susceptible checks and eight IRBB differential lines (Table 2) serving as references. Screening for bacterial leaf blight (BLB) followed the standard clip-inoculation technique of Kauffman *et al.* (1973), with refinements based on the IRRI Standard Evaluation System (IRRI, 2013) and PhilRice guidelines (PhilRice, 2020), guidelines to align with current diagnostic practices. The *Xoo* isolates PXO61 (Race 1), PXO86 (Race 2), PXO79 (Race 3b), PXO340 (Race 3c), PXO71 (Race 4), PXO112 (Race 5), PXO99 (Race 6), PXO145 (Race 7), PXO280 (Race 8), PXO339 (Race 9a) and PXO341 (Race 10) were sourced through the IRRI–PhilRice collaboration (2018 MTA) and maintained at the Crop Protection Division. All experimental procedures adhered to PhilRice and the National Committee on Biosafety of the Philippines (NCBP) regulations. Twenty-one-day-old seedlings of each entry were transplanted into triplicate pots (three seedlings per pot) and arranged in a Completely Randomized Design (CRD) under screenhouse conditions during the 2024 dry season. Bacterial inoculum was prepared from three-day-old cultures grown on modified Wakimoto medium and suspended in sterile distilled water to approximately 10^9 CFU mL^{-1}. Plants were inoculated at 45–55 days after transplanting (DAT) at maximum tillering by clipping 2–3 cm of the leaf tip using sterile scissors dipped in the bacterial suspension (Figure 1A). For each entry, 5–10 leaves across nine plants were inoculated, with each race labeled using color-coded tags. Lesion lengths were recorded 21 days after inoculation, following the IRRI Standard Evaluation System (IRRI, 2013).

Disease Evaluation and BSRI Computation

Disease reactions were assessed 21 days post-inoculation (DPI) following IRRI SES (IRRI, 2013) and PhilRice (2020) protocols. Lesion length (LL) was measured from the clipped tip to the lesion end on five leaves per plant across nine plants per entry. Mean LL values were used to classify reactions as Resistant (R, 0–5 cm), Moderately Resistant (MR, >5–10 cm), Moderately Susceptible (MS, >10–15 cm), or Susceptible (S, >15 cm)

(Figure 1B). Lesion length data were subjected to one-way ANOVA, and mean separation was conducted using Tukey's HSD test ($\alpha = 0.05$) in STAR software (IRRI, 2014). To quantify multi-race resistance, a Broad-Spectrum Resistance Index (BSRI) was calculated as *BSRI = (N(R) / N(T))*, where *N(R)* is the number of races eliciting R or MR reactions and *N(T)* is the total number tested (Liu *et al*., 2021). Disease reaction profiles of the mutants were benchmarked against those of IRBB differential lines/NILs to infer possible resistance loci in the mutant lines.

Reaction Profile Comparison and Cluster Analysis

To visually assess the degree of dissimilarity and underlying genetic relationships among the *Gal-ong* mutants and the IRBB differential lines, a cluster analysis was performed using the reaction profiles (strings of 'A', 'C', and '-') as input data. The dissimilarity between all pairs of materials was quantified using a custom distance metric based on the proportion of mismatches, which was implemented using a custom script in the R programming environment. Prior to calculation, all reaction profiles were standardized to a uniform length (10 positions) through padding with the missing data symbol ('-'). The custom metric calculated the distance (D) between two profiles (S_i and S_j) by taking the ratio of the number of mismatched positions to the number of comparable positions. Positions were deemed comparable only if neither profile contained the missing data symbol ('-'). The distance function is defined as:

$$D(S_i, S_j) = \frac{\text{Number of Mismatches}}{\text{Number of Comparable Positions}}$$

This approach ensured that positions with undetermined resistance (gaps) did not contribute to or skew the calculated dissimilarity between profiles. These pairwise distance values were compiled into a condensed distance matrix.

Hierarchical agglomerative clustering was applied to the distance matrix using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), also known as average linkage, which was executed using the hclust function within the R stats package. This method sequentially merged the closest (most similar) clusters, where the distance between two clusters was defined by the average distance between all profiles in the first cluster and all profiles in the second cluster. The resulting linkage data were then used to construct a dendrogram using the dendextend package in R, which provided a visual representation of the grouping patterns. The height of each branch on the dendrogram corresponds to the distance value at which the two sub-clusters were joined, allowing for clear interpretation of the relative genetic similarity among the tested rice materials.

Genome Sequencing and Assembly

High-molecular-weight genomic DNA was extracted from young leaf tissue of wild-type *Gal-ong* and its mutant line GXB207, and sequencing libraries were prepared following standard Illumina protocols. Libraries were sequenced on an Illumina NovaSeq platform to generate 2 × 150 bp paired-end reads at ~60X coverage. Raw reads were quality-trimmed using Trimmomatic, and de novo genome assembly was performed using SPAdes. Assembly quality was assessed via N50 and BUSCO metrics.

Localized BLAST Analysis of *Xa* Genes

Full-length nucleotide sequences of known *Xa* resistance genes were compiled into a single FASTA query file. De novo assemblies of *Gal-ong* and GXB207 were converted into local BLAST databases using makeblastdb (NCBI BLAST+; -dbtype nucl). BLASTN searches were conducted using the *Xa* query file against each database independently, with an E-value threshold of 1e−5 and tabular output (-outfmt 6). BLAST hits were filtered for high identity and coverage to identify *Xa* homologous regions. Comparative analysis of alignment metrics between *Gal-ong* and GXB207 enabled the detection of differential events presence/absence variation, insertions/deletions, or SNPs within *Xa* loci, providing genomic context for phenotypic resistance differences.

Results and Discussion

Phenotypic Resistance Profiling

Exactly 21 days after inoculating all the plant materials with 10 strains (9 races) of *Xoo*, the 20 advanced (M_{12}) *Gal-ong*-derived mutant rice lines exhibited resistant phenotypes (Figure 2A-2C). Based on lesion lengths, some materials were resistant to most of the strains, while some were only resistant to fewer or specific strains, with the former indicating a higher broad-spectrum resistance index (BSRI) (Figure 2D). In terms of BSRI, the materials were grouped into four (Table 3). Group I, with BSRI of 0.8 to 1.0, included the broadest-spectrum mutants (*Xa21*-like resistance), namely, GXB-229, GXB-SB-9, GXB-1, GXB-225-1, GXB-51, and GXB-61, along with the resistant checks IRBB21, IRBB57, and IRBB5 (Song *et al.*, 1995; Jiang *et al*., 2020). These lines remained resistant to highly virulent races such as PXO61, PXO79, PXO340, and PXO145 (Figure 2). Group II, with BSRI of 0.5 to 0.7, included materials with partial or race-specific resistance and includes the rest of the 14 *Gal-ong* mutants along with IRBB7. The other two groups are Group III, which includes 4 IRBB differential lines with narrower resistance, and the susceptible Group IV, which includes *Gal-ong*, IR24, and

TN1. Interestingly, none of the mutants were classified as Group III or Group IV, indicating the value of these mutants for resistance breeding. It is also important to note that the IRBB differential lines and susceptible checks displayed the expected reaction patterns in Table 1, validating the bioassay.

Causal Gene Estimation

The similar BSRI values observed between the test materials and the IRBB checks in the same group (Group I or II, Table 3) initially suggested a shared causal gene for Bacterial Leaf Blight (BLB) resistance. To validate this suggestion, the potential causal gene(s) responsible for resistance in the mutants were first estimated by directly comparing their disease reaction patterns to those of the reference IRBB differential lines (Ashwini *et al.*, 2024). A definitive causal gene match was established only when the mutant and the corresponding IRBB check shared 100% identical disease reactions (susceptible 'S' or resistant 'R') across all 10 test points. Conversely, any phenotypic response mismatch to the expected reaction profile indicated that the resistance observed in the mutant could not be solely attributed to the known *Xa* resistance gene in the IRBB check. Consequently, the analysis revealed that the majority of mutant phenotypes did not exhibit 100% similarity to that of any IRBB line and, therefore, could not be explained by the genes within the established BLB differential system. The mutant line GXB-229 is a prime example. Its resistance against Races 1, 5, 6, 7, and 8 could plausibly be conferred by a combination of genes such as *Xa4*, *Xa14*, *Xa8*, and *xa13*. However, its corresponding resistance against Races 3b, 3c, 4, and 9a remains unexplained by the other genes in the differential panel. Another example is the similar reaction pattern exhibited by GXB-SB-9 and GXB-1, which were only susceptible to Race 7 (PXO145). While their resistance against Races 5, 6, and 8 can be explained by *Xa14*, *Xa8*, and *xa13*, their resistance to the rest of the isolates in the system cannot be accounted for. These results negate the hypothesis that the same BSRI grouping in Table 3 translates directly to a shared causal gene. More importantly, the discrepancies provide strong evidence that the observed resistance is conferred by genes outside the standard BLB differential system, potentially by entirely novel resistance alleles generated by gamma ray irradiation.

Cluster Analysis

To better visualize the discrepancy between the disease reaction profiles of the IRBB lines and the mutants when estimating the causal genes, a dendrogram was constructed (Figure 3). The resulting dendrogram effectively grouped entries with similar reaction profiles from Table 3, such as GXB-1 and GXB-SB-9, IRBB57 and IRBB21, and the susceptible checks *Gal-ong*, TN1, and IR24, which clustered onto the same terminal nodes. Crucially, the IRBB lines did not share terminal nodes with any of the mutants. This graphical evidence strongly supports the initial observation: the disease reaction profiles of the IRBB differential lines and the mutants do not match, suggesting that alternative genetic mechanisms (e.g., alternative gain-of-function alleles or novel genes) may underlie the broad-spectrum resistance observed in the mutants.

Homology Profile of *Xa* genes in *Gal-ong* and GXB207 mutant

Since the possibility of gain-of-function or novel mutations in the *Gal-ong*-derived mutants has become apparent, the genomes of *Gal-ong* and one of the mutants with desirable traits (GXB207; Table 1) were compared to have a general insight into how genes are altered by gamma ray irradiation. In particular, BLASTN was used to query their respective genome assemblies with full-length sequences of 32 published *Xa* genes. The results showed that the homology length to the query sequence of 15 (46.9%) truncated *Xa* genes in GXB207 increased compared to that in *Gal-ong* (Figure 4), resulting in partial (≥3.35%) (e.g., *Xa25*, *etc.*) to full (100%) (e.g., *xa13*) gene sequence restoration (28.03% average increase). In contrast, *Xa3*/*Xa26*, *Xa23* and *Xa40* exhibited reduced homology length. The observed sequence length changes offer a genetic indicator of a high probability of gain-of-function or novel mutations occurring throughout the genome as a random effect of gamma ray irradiation. This random mutagenesis may have also failed to affect certain genes, a point supported by the 13 *Xa* genes that showed no alteration. Notably, *Xa7* was absent in both genomes examined.

As mentioned, *Xa13* was fully restored in GXB207. However, this is unlikely to be the causal gene because *Xa13* is a susceptibility gene and only the recessive *xa13* allele confers BLB resistance in previous mutants (Yang *et al.*, 2006) by preventing pathogen induction of the gene. Normally, the pathogen's TAL effector (PthXo1) targets this gene to acquire sucrose. The phenotypic data of GXB207 strongly support this narrative, showing that the restoration of *xa13* in GXB207 correlates perfectly with this mutant's susceptibility (classified as "MS" or Moderately Susceptible) to the target pathogen, Race 6 (PXO99). In contrast, the true resistant check IRBB13 (carrying the recessive *xa13* allele) was correctly observed to be "R" (Resistant) to PXO99 in the same table. Another gene in question is *Xa25 (d)*, which was almost completely restored (97.38%) in GXB207. Unlike the recessive *xa25* (an allele of *OsSWEET13*), *Xa25(d)* is a dominant resistance gene originally identified from the somatic mutant HX-3 (Chen,

2002). Because *Xa25(d)* functions as a dominant R-gene, its restoration by gamma irradiation would result in a gain-of-function phenotype. However, it cannot explain the broad-spectrum resistance in the mutants because while *Xa25(d)* confers resistance against races 1, 3, and 4, the mutant GXB207 displayed resistance to a wider range of strains (Table 3). This indicates that *Xa25(d)* alone cannot explain the resistance in broad-spectrum resistance in GXB207, and there are other unknown genetic mechanisms at play. Another gene, *Xa21*, was 83.19% restored in GXB207. This gene is known for the broad-spectrum resistance of IRBB21, but as discussed earlier, its disease reaction profile does not completely match the profile of the Gal-ong mutants, and so, it cannot explain the resistance in the mutants. Specifically, while *Xa21* (in IRBB21) confers moderate resistance against the highly virulent Race 6 (PXO99), the mutant GXB207 was found to be moderately susceptible to this race. This phenotypic discrepancy indicates that the resistance in GXB207 cannot be attributed to the partial restoration of the *Xa21* locus. Finally, susceptibility genes aside from *Xa13* (*Xa25* and *Xa41* (t)) were also examined in both *Gal-ong* and the GXB207 mutant, but yielded confusing results for *Gal-ong*. Therefore, at this point, no known *Xa* gene so far has been able to explain the resistance in the GXB207 and other *Gal-ong* mutants. Nevertheless, it is expected that further bioinformatics analysis, comparative genomics, and functional analyses will reveal the causal gene(s) of the mutants in the future.

Integrated Genomic–Phenotypic Interpretation

By integrating BSRI evaluation, phenotypic classification, dendrogram clustering, and BLASTN homology profiles, a cohesive mechanism for the BLB resistance observed in gamma-induced *Gal-ong* mutants emerges. The phenotypic analysis revealed that high-value mutants, such as GXB-229, GXB-SB-9, GXB-1, GXB-225-1, GXB-51, and GXB-61, possessed broad-spectrum resistance comparable to the most effective IRBB differential lines (Group I in Table 3). However, while these mutants fell into the same broad-spectrum category, the cluster analysis demonstrated that they formed distinct branches separate from the established checks (e.g., IRBB21 and IRBB57), rather than sharing terminal nodes. This clear separation, coupled with the lack of identical reaction profiles, strongly suggests that gamma irradiation generated mutants containing putatively novel genetic mechanisms or modified alleles that cannot be fully explained by existing *Xa* genes alone (Ahloowalia & Maluszynski, 2001; Zhong *et al*., 2024). This hypothesis is supported by the genomic analysis of GXB207. The observed restoration and enhanced homology of several *Xa* loci in this mutant provide physical evidence that radiation-induced random structural changes underlie the phenotypic improvements. Consequently, the resistance observed, based on genetic examination above, is likely a result of a dual mechanism: the restoration of dominant R-gene functionality and the potential generation of novel alleles, which need to be further validated. Collectively, these findings demonstrate that gamma-ray mutagenesis is an effective approach for expanding the genetic diversity of rice, providing valuable resources for gene pyramiding and the development of durable, multi-race BLB-resistant cultivars (Ahloowalia & Maluszynski, 2001; Lu *et al*., 2021; Vera Cruz *et al*., 2000; Dossa *et al.*, 2015).

Table 1. The bacterial leaf blight differential system comprising IRBB near-isogenic lines, their associated resistance genes, and representative strains of nine Philippine *Xanthomonas oryzae pv. oryzae* (*Xoo*) races.

		Reactions to Xoo Races									
Near-isogenic lines (NILs)	***Xa* gene(s)**	**PXO 61/ Race 1**	**PXO86/ Race 2**	**PXO79/ Race 3b**	**PXO340/ Race 3c**	**PXO71/ Race 4**	**PXO112 / Race 5**	**PXO99/ Race 6**	**PXO145 / Race 7**	**PXO280/ Race 8**	**PXO339/ Race 9a**
IRBB-4	*Xa4*	R	S	S	S	MR-MS	R	S	R	R	S
IRBB-5	*xa5*	R	R	R	R	S	R	S	R	R	R
IRBB-7	*Xa7*	MS	R	R	R	S	R	S	R	R	S
IRBB-10	*Xa10*	S	R	S	S	S	R	S	R	S	S
IRBB-13	*xa13*	S	S	S	S	S	S	R	S	S	S
IRBB-14	*Xa14*	S	S	S	S	S	R	S	S	R	S
IRBB-21	*Xa21*	R	R	R	MR	R	R	MR	MR	MR	MR
IRBB-57	*Xa4+ xa5 +Xa21*	R	R	R	R	R	R	R	R	R	R
TN1	*None*	S	S	S	S	S	S	S	S	S	S
[a]IR24	*None*	S	S	S	S	S	S	S	S	S	S

[a]IR24 is the recurrent parent of the IRBBs, which does not harbor known Xa genes. Reaction patterns lifted from the IRRI knowledgebank (https://www.knowledgebank.irri.org/ricebreedingcourse/Breeding_for_disease_resistance_Blight.htm).

Disease reaction based on IRRI SES (IRRI, 2013): *0–5 cm, Resistant (R); >5–10 cm, Moderately Resistant (MR); >10–15 cm, Moderately Susceptible (MS); and >15 cm, Susceptible (S).*

Table 2. Advanced (M_{12}) *Gal-ong*–derived mutant rice lines and reference checks used for bacterial leaf blight (BLB) resistance screening, along with their special traits.

Code	Line Designation	Special Trait
1	GXB-54	good eating quality, improved plant type
2	GXB-229	good eating quality, improved plant type
3	GXB-319	premium eating quality
4	GXB-SB-1	SB resistant, good eating quality,
5	GXB-SB-3	SB resistant, good eating quality,
6	GXB-SB-5-1	SB resistant, good eating quality,
7	GXB-SB-9	SB resistant, good eating quality,
8	GXB-31	good eating quality, improved plant type
9	GXB-36	good eating quality, improved plant type
10	GXB-51	good eating quality, improved plant type
11	GXB-61	good eating quality, improved plant type
12	GXB-207	premium eating quality, high-yielding
13	GXB-322	aromatic, high-yielding
14	GXB-210-1	premium eating quality, high-yielding
15	GXB-225-1	premium eating quality, high-yielding
16	GXB-209	aromatic, high-yielding
17	GXB-309	aromatic, high-yielding
18	GXB-317	aromatic, high-yielding
19	GXB-310-2	aromatic, high-yielding
20	GXB-1	high-yielding, good eating quality
21	*Gal-ong* (WT)	Wildtype (WT)
22	IR24	Susceptible control
23	TN1	Susceptible control
24	IRBB 4	*Xa4*
25	IRBB 5	*xa5*
26	IRBB 7	*Xa7*
27	IRBB 10	*Xa10*
28	IRBB 13	*xa13*
29	IRBB 14	*Xa14*
30	IRBB 21	*Xa21*
31	IRBB 57	*Xa4, xa5, Xa21*

Notes: IRBB refers to the IRBB differential lines (NILs) carrying 1-3 known Xa gene(s) in the IR24 background; GXB refers to the advanced (M12) mutant lines selected based on agronomic performance and grain quality traits.

Table 3. Phenotypic reactions of 20 *Gal-ong*-derived mutant rice lines, IRBB differential lines, and susceptible checks evaluated against nine *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) races (10 strains) under screenhouse conditions.

		Reactions/Numerical (cm) to *Xoo* races*										
Group	Designation	PXO 61 Race 1	PXO86 Race 2	PXO79 Race 3b	PXO 340 Race 3c	PXO71 Race 4	PXO112 Race 5	PXO99 Race 6	PXO145 Race 7	PXO280 Race 8	PXO339 Race 9a	BSRI**
	IRBB 21	R (2.7)	R (3.3)	R (2.0)	MR (5.8)	R (4.6)	R (4.6)	MR (5.6)	MR (6.9)	MR (7.3)	MR (4.4)	1.0
	IRBB 57	R (3.1)	R (3.60)	R (1.9)	R (2.8)	R (2.2)	R (3.5)	R (3.5)	R (4.7)	R (2.8)	R (3.5)	1.0
	GXB-229	R (4.4)	S (18.3)	MR (8.0)	R (3.6)	MR (9.0)	MR (6.0)	MR (6.4)	R (3.7)	MR (5.6)	MR (5.7)	0.9
	GXB-SB-9	MR (6.7)	MR (8.4)	R (2.3)	R (2.5)	MR (7.6)	R (3.2)	MR (5.1)	S (15.1)	MR (6.7)	R (3.2)	0.9
I	GXB-1	R (4.4)	MR (6.4)	R (2.6)	R (3.2)	MR (8.6)	MR (9.3)	MR (9.1)	S (16.5)	MR (6.2)	R (4.9)	0.9
	IRBB 5	R (2.9)	R (4.3)	R (3.4)	R (4.0)	S (17.5)	R (2.5)	S (22.5)	R (3.1)	R (4.0)	R (4.6)	0.8
	GXB-225-1	R (4.5)	MR (8.4)	MS (12.2)	R (2.3)	MR (9.3)	R (1.8)	MR (8.9)	S (17.3)	R (2.3)	R (5.0)	0.8
	GXB-51	MS (11.7)	MR (8.5)	MR (5.6)	R (4.6)	R (3.3)	MR (9.6)	MS (14.1)	MR (6.4)	R (4.6)	R (4.8)	0.8
	GXB-61	MR(8.0)	MR (8.6)	MR (6.9)	R (4.4)	MR (8.9)	S (17.5)	S (16.5)	MR (8.2)	MR (7.0)	R (4.8)	0.8
	GXB-319	R (2.8)	S (17.64)	R (4.4)	MR (6.2)	MR (9.5)	MR (8.6)	MR (8.6)	S (19.6)	MS (13.5)	MR (5.6)	0.7
	GXB-SB-1	MS (14.6)	MR (8.3)	R (3.9)	R (4.0)	MR (9.3)	R (2.3)	MR (6.7)	S (18.8)	S (15.6)	MR (5.9)	0.7
	GXB-SB5-1	MR (7.5)	MR (6.3)	MS (13.7)	R (4.6)	MS (10.2)	R (3.5)	R (5.0)	S (16.6)	MR (6.4)	R (2.8)	0.7
	GXB-207	MR (8.0)	MR (8.8)	MR (6.8)	R (4.6)	MR (6.5)	S (18.2)	MS (13.6)	MR (7.2)	MS (13.6)	R (4.8)	0.7
	GXB-309	S (22.8)	MR (8.2)	MR (5.8)	MR (5.3)	MR (7.0)	S (25.2)	MR (7.0)	MS (13.9)	R (5.0)	MR (7.2)	0.7
	GXB-31	MS (12.9)	MR (6.7)	R (3.8)	R (2.5)	S (23.2)	MR (10.0)	R (4.7)	S (15.6)	R (2.5)	MR (9.3)	0.7
II	GXB-322	MR (7.3)	MS (14.1)	MR (6.7)	R (4.6)	MR (9.0)	S (17.1)	MS (13.5)	MS (14.6)	MR (5.8)	MR (7.3)	0.6
	GXB-210-1	S (18.3)	MR (8.5)	MR (6.6)	R (4.6)	S (17.4)	S (27.9)	MS (13.4)	MR (7.8)	MR (7.8)	MR (5.7)	0.6
	GXB-209	MS (14.6)	R (3.3)	MR (6.8)	R (4.6)	MR (8.9)	S (16.6)	R (4.7)	MS (11.5)	S (17.6)	MR (6.6)	0.6
	GXB-310-2	S (16.2)	MR (6.2)	R (4.5)	R (3.5)	MR (8.1)	MR (8.8)	MS (10.7)	S (18.8)	S (19.3)	MR (6.5)	0.6
	GXB-SB-3	MR (9.1)	MR (7.9)	MS (14.3)	R (4.1)	MR (9.2)	MR (6.8)	MS (10.7)	S (15.9)	MR (6.1)	MS (10.9)	0.6
	GXB-54	S (16.2)	S (22.6)	MS (14.3)	R (3.5)	MR (9.5)	MS (11.2)	MS (11.0)	MR (8.5)	MR (6.9)	MR (5.7)	0.5

	GXB-36	S (18.7)	R (4.1)	R (4.2)	MS (11.6)	MR (9.6)	MS (14.5)	MR (7.8)	S (20.2)	MS (11.6)	MR (9.6)	0.5
	GXB-317	MR (9.0)	MR (7.8)	S (15.9)	R (3.1)	MR (7.4)	MS (13.0)	MS (10.7)	S (15.7)	S (18.2)	MR (5.9)	0.5
	IRBB 7	MS (13.4)	R (4.1)	R (1.9)	R (3.9)	MS (12.7)	R (2.5)	S (21.5)	R (4.0)	-	-	0.5
III	IRBB 4	R (3.6)	S (18.0)	S (17.8)	S (17.6)	-	R (2.1)	S (20.1)	MR (6.9)	R (5.0)	S (34.6)	0.4
	IRBB 10	S (23.5)	S (20.3)	S (17.9)	S (18.7)	S (28.9)	R (3.0)	S (23.0)	R (4.1)	S (18.7)	-	0.2
	IRBB 14	S (20.9)	S (25.5)	S (16.4)	S (19.0)	S (20.7)	R (3.1)	S (20.8)	S (19.0)	-	S (31.3)	0.1
	IRBB 13	S (22.8)	S (20.3)	S (16.7)	S (18.5)	S (20.8)	S (20.3)	R (3.1)	S (26.1)	S (18.5)	S (32.4)	0.1
IV	*Gal-ong* (WT)	S (20.5)	S (21.4)	S (16.2)	S (16.5)	S (17.3)	S (25.2)	S (35.6)	S (22.4)	S (16.5)	S (17.5)	0.0
	IR24	S (25.2)	S (20.7)	S (19.0)	S (19.8)	S (26.7)	S (21.7)	S (21.7)	S (25.6)	S (19.8)	S (33.0)	0.0
	TN1	S (25.1)	S (24.3)	S (17.0)	S (17.7)	S (27.6)	S (24.6)	S (24.6)	S (22.5)	S (17.7)	S (32.1)	0.0

Notes: IRBB and GXB refer to IRBB differential lines (NILS) carrying 1-3 known Xa gene(s) and Gal-ong mutant lines, respectively; Lesion lengths (cm) were measured 21 days post inoculation (DPI) and classified based on SES (IRRI, 2013): resistant (R, 0–5 cm), moderately resistant (MR, >5–10 cm), moderately susceptible (MS, >10–15 cm), and susceptible (S, >15 cm). Values represent mean lesion lengths from nine plants per entry. NILs with unmatched/invalid known reaction results are indicated with a dash (-); Broad-Spectrum Resistance Index (BSRI) was calculated as: BSRI = (N(R) / N(T)), where N(R) is the number of races eliciting R or MR reactions, and N(T) is the total number tested (Liu et al., 2021). The broad-spectrum resistance index (BSRI) indicates the proportion of races showing R or MR reactions. Data were subjected to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$)

Figure 1. Bacterial leaf blight (BLB) screening methodology and phenotypic classification. (A). Inoculation and evaluation steps (left to right): color-coded tagging of plants; clip-inoculation using nine *Xanthomonas oryzae pv. oryzae (Xoo)* races; active lesion development at 4 days post-inoculation (DPI); and lesion length measurement at 21 DPI. **(B)** Classification of *Gal-ong* mutant reactions at 21 DPI, showing (left to right) Resistant (R), Moderately Resistant (MR), and Susceptible (S) leaves.

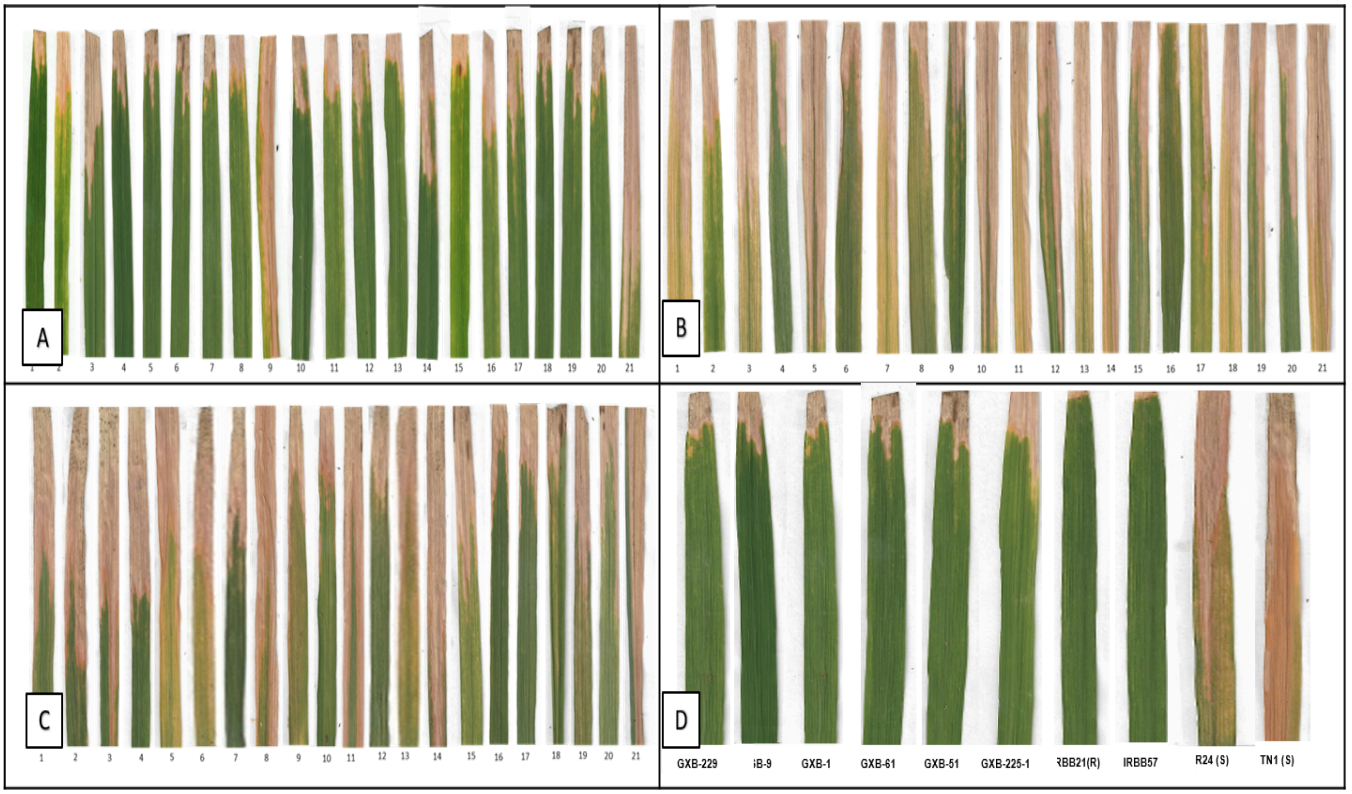

Figure 2. Phenotypic reaction profiles of Gal-ong mutant rice lines inoculated with representative *Xanthomonas oryzae pv. oryzae (Xoo)* races at 21 days post-inoculation (DPI). (A) Reactions of the 20 mutants and *Gal-ong* to PXO340 (Race 3c), showing a high frequency of resistance among the mutants; (B) Reactions of the 20 mutants and *Gal-ong* to the highly virulent strain PXO99 (Race 6); (C) Disease symptoms in the same set of materials observed against PXO71 (Race 4); (D) Leaf phenotypes of the top-performing mutants (GXB-229, GXB-SB-9, GXB-51, GXB-61, GXB-1) exhibiting broad-spectrum resistance with BSRI values of 0.8–0.9.

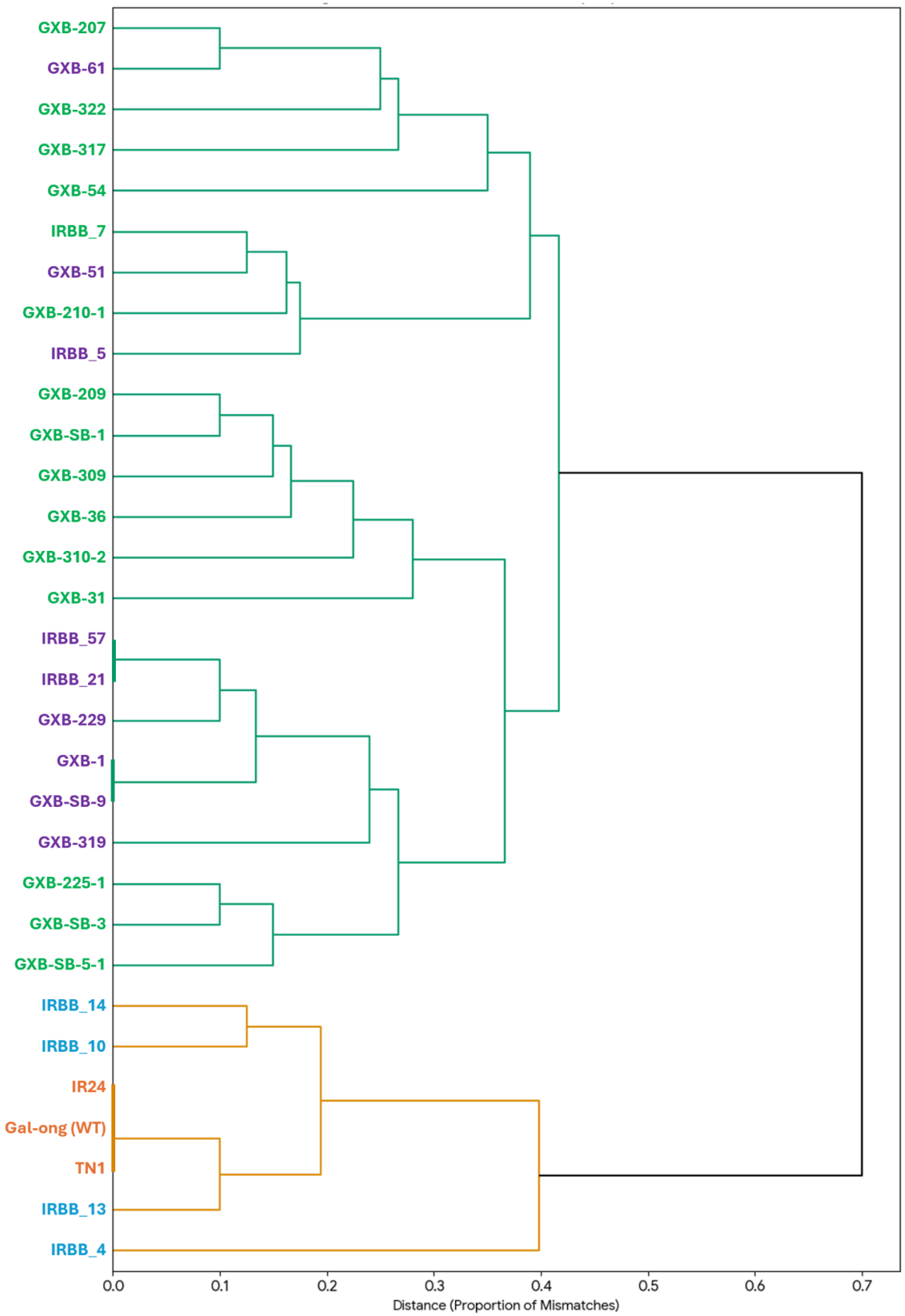

Figure 3. Dendrogram of phenotypic reaction-profile similarities among *Gal-ong* gamma ray-induced mutant lines, IRBB differential lines, and susceptible checks across nine Philippine *Xoo* races. Legend: Group I (in Table 3): purple boxes; Group II = green boxes; Group III = blue boxes; and Group IV = orange boxes.

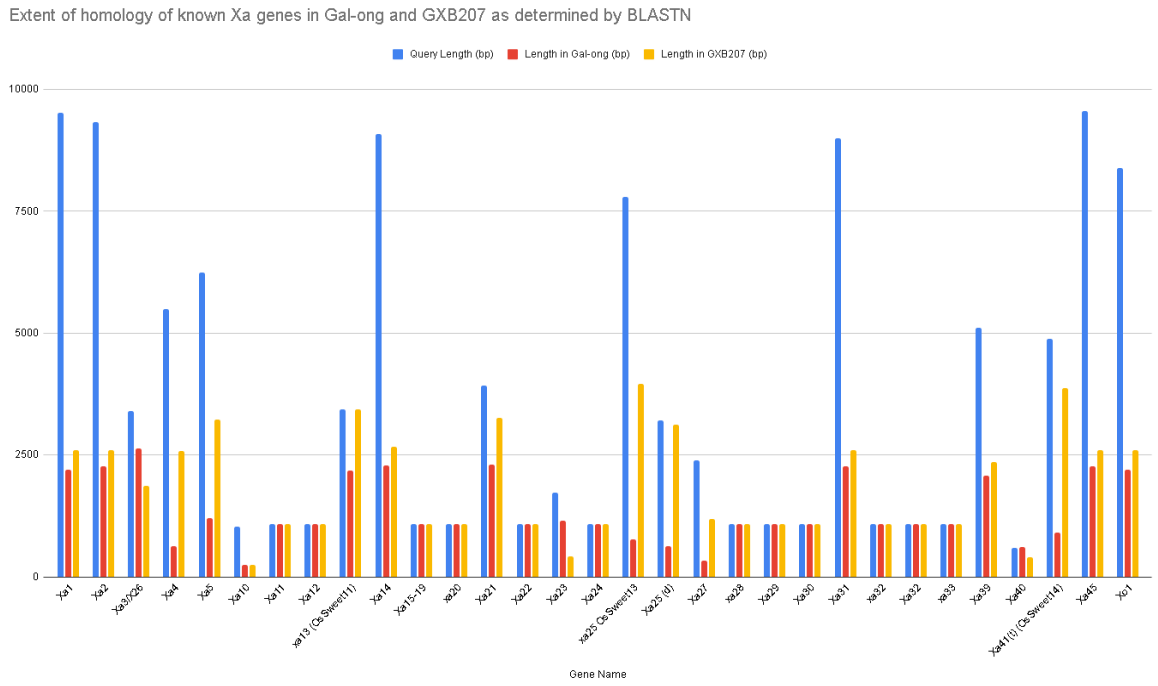

Figure 4. Comparative sequence homology of 31 known *Xa* resistance genes in wildtype *Gal-ong* and the mutant GXB207. Full-length sequences of 31 *Xa* genes were used as a query in BLASTN homology searches. These genes represent known bacterial leaf blight (BLB) resistance loci in rice and were used to identify homologous regions in the gamma-irradiated mutant lines. Note: *Xa7* homologues were not found in the mutants and so were not included in the graph.

Conclusion

This study successfully demonstrated that gamma-ray mutagenesis is a potent tool for improving the disease resistance of the traditional rice variety *Gal-ong*, generating advanced mutant lines with significant broad-spectrum resistance to Bacterial Leaf Blight (BLB). Phenotypic screening against nine major Philippine *Xoo* races identified superior mutants—specifically GXB-229, GXB-SB-9, GXB-51, GXB-61, GXB-225-1, and GXB-1—that exhibited high Broad-Spectrum Resistance Indices (BSRI ≥ 0.8), performing comparably to the resistant differential checks IRBB5, IRBB21, and IRBB57. Critically, none of these mutants were classified into the narrower resistance groups (Group III) or susceptible groups (Group IV), validating the efficacy of the induced mutations in enhancing the host defense response. However, the resistance mechanisms in these mutants appear distinct from those of the established *Xa* genes. Cluster analysis revealed that the mutants formed unique branches separate from the standard IRBB checks, and their reaction profiles did not perfectly match the known *Xa* gene differentials. This suggests the presence of gain-of-function mutations or novel resistance alleles, a hypothesis supported by genomic analysis of mutant GXB-207, which showed the partial-to-full sequence restoration of previously truncated *Xa* genes (such as *xa13, Xa25(d), Xa21, etc.*). While these molecular changes offer a plausible explanation for the enhanced BLB resistance, the specific causal mechanisms have yet to be pinpointed. Consequently, the identification of the precise gene(s) responsible is reserved for subsequent studies. It is anticipated that further bioinformatics analyses, comparative genomics, and functional analyses will ultimately unveil the causal gene(s) underlying these mutants in the future.

Ethical Statement

This study involved the evaluation of plant materials (gamma-ray-induced mutant rice lines) and laboratory-based screening against bacterial pathogens. The research did not involve human subjects, animal experiments, or the collection of sensitive personal data; therefore, formal ethical approval and informed consent were not required for the conduct of this study.

Conflict of Interest Statement

The authors declare no conflict of interest related to the conduct and publication of this research. All procedures followed were in accordance with institutional and ethical standards, and there were no financial or personal relationships that could have influenced the outcomes of this study.

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Declaration of Generative AI and AI-Assisted Technologies

During the preparation of this work, the author(s) utilized Google Gemini for grammatical assistance. Following the use of this tool/service, the author(s) conducted a review and made necessary modifications, assuming full responsibility for the content of the publication.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

ESA: Investigation, Writing – Original Draft; **RTT:** Methodology, Supervision, Writing – Review & Editing; **FMNG:** Investigation, Formal Analysis; **JOV:** Investigation; **MCG:** Data Curation, Investigation, Writing - Review and Editing; **ALGS:** Investigation, Writing - Review and Editing; **RLO:** Conceptualization, Supervision, Writing – Review & Editing.

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