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Characterizing the Genetic Resistance Mechanisms in Rice Against Rice Blast Disease: Exploring New Avenues for Disease Control

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Abstract

Rice blast (Magnaporthe oryzae) poses a major threat to global rice production, yet durable, broad-spectrum resistance remains elusive. Here, we combined high-throughput phenotyping of 300 diverse indica, japonica, and aus accessions with whole-genome resequencing (>10× coverage) and pangenome assembly to perform mixed-model GWAS and identify 45 SNPs ($p < 1\times10^{-5}$) across all 12 chromosomes. Meta-QTL integration with >400 published loci refined 23 candidate intervals and highlighted six robust regions enriched for NLR and receptor-like kinase genes. We prioritized 100 candidates by domain architecture and differential expression upon infection. Functional validation via CRISPR/Cas9 (knockout and allele-swap) in the susceptible Kitaake cultivar generated eight lines with on-target editing efficiencies of 71–99 %, no detectable off-target mutations, and lesion score reductions up to 80 %. The positive correlation between editing efficiency and resistance enhancement underscores the feasibility of precision allele pyramiding. Pangenome presence–absence analysis revealed 20 % novel resistance alleles absent from the Nipponbare reference, expanding the genetic toolkit for breeding. Growth-curve assays confirmed that genome editing did not impair early vegetative development. Our integrative pipeline—spanning germplasm screening, GWAS, meta-QTL mapping, pangenomics, and targeted editing—provides a scalable model for rapid deployment of resistance genes against evolving pathogen races. These findings pave the way for multi-locus editing strategies and field validation toward sustainable, resilient rice cultivars, ultimately contributing to food security in blast-endemic regions.

1. INTRODUCTION

Excited by the appearance of the fungal disease *Magnaporthe oryzae*, rice blast represents an ever-present and serious threat to the world rice production and annual significant yield losses (Gao P,). In combination with other virulence strategies in the pathogen, the intricate co-action between rice and *M. oryzae* has facilitated the establishment of sophisticated defense mechanisms in rice (Patil PD,). Good disease management plans and food security require a recognition of the genetic underpinning of resistance in rice. This review has described creative alternatives for disease control and identified the various genetic resistance mechanisms used by rice to plant rice blast disease including both qualitative and quantitative resistance.

Rice possesses an elaborate repertoire of defensive strategies under the control of diverse genes to combat *M. oryzae*. Often started with the recognition of pathogen effectors through a gene-for gene interaction, resistance genes (R genes) activate race-specific resistance (Kashyap PL,). These genes code for leucine-rich repeat proteins containing nucleotide-binding sites destined to trigger downstream reactions of defense (Kapetas D,). Besides R genes rice exhibits the quantitative resistance if in which there are numerous quantitative trait loci (Das P,) controlling regulation. Quantitative resistance provides a wider range of resistance to many pathogen races while giving a more permanent cure type of disease control (Chen T,). Genomics, transcriptomics, and proteomics (Sudan J,) have played a useful role in accelerating the identification and characterization of these resistance genes and QTLs. From the use of molecular markers associated with resistance genes, marker-assisted selection has become possible in rice breeding projects so that many resistance genes are pyramided into superior rice types. In addition, new genome-editing tools are being developed for use in rice (Zafar K,).

Another genetical direction of rice blast resistance enhancement is genetic engineering (Tabassum J,). This procedure brings new resistance genes or modifies the genes that are already there in order to make the plant’s defense reactions stronger. Numerous commercial double or triple stress resistance cultivars (Malenica N,) are already available from transgenic techniques in maize. For instance wheat stem rust resistance gene, *Sr43 has been demonstrated by the introduction of a high level of resistance to a wide variety of stem rust isolates to various wheat lines hence a strengthening the argument of the potential of genetic engineering in the resistance breeding (Yu G,). In addition, gene-targeted crop improvement is now highly dependent on the application of clustered regularly spaced short palindromic repeat-Cas9 nuclease technology (Liang Y,). These instruments are capable of exactly altering resistance genes or introduce new regulatory components to enhance resistance.

Rice blast can be effectively managed with conventional means of disease management including the use of fungicides. However, use of fungicides may lead to fungicide resistant strains of pathogens; thus significantly degrading disease control. Integrated disease management plans, which combine together some methods of biological control together with genetic resistance with a combination of cultural practices is a more sustainable strategy. With the help of crop rotation, the time of sowing, irrigation management, and other cultural practices man can help to reduce the incidence and severity of diseases (Singh J,) (Pandit MA,). Biological control agents such as antagonistic bacteria and fungi is a better alternative for the use of chemical control which can prevent the development of pathogens and reduce the severity of illness.

Despite ongoing efforts for building environmentally friendly control strategies such as RNA interference, biological control, and plant resistance inducers, these are yet to approximate the intensity of fungicides (Kapetas D,).

Future research should aim toward revealing the molecular causes of quantitative resistance, as well as identifying new resistance genes – of broad spectre.

The evolution of molecular techniques including CRISpen-Cas9 offers previously unimagined opportunities for focused gene editing to enhance resistance. It has shown itself to be a more precise and rapid alternative to traditional breeding approaches and can switch genes associated with disease resistance (Ansori ANM,).

By production of reactive oxygen species and activation of defense enzymes, chitin and its derivatives appear promising in plant disease resistance (Ngasotter S). Through blocking virus attachment on leaf surface and therefore limiting a systemic transmission, chitin can protect against viral infections (Ngasotter S). In addition, nanotechnology offers creative means of controlling the diseases because nanoparticles are both diagnostic tool and disease resistance constructors (Jabran M,). Transgenic and non-transgenic method of RNAi plant-based approaches have proved to be highly effective and potentially useful in targeting specific plant pathogens and support in disease control of plants when no other alternative exist. RNA interference is a regulation and antiviral response process in eukaryotes (Kuo Y,). Further research may be conducted on how plant diseases caused by a wide range of pathogens which could have devastating effects over crop plants and even plants in natural ecosystems.

To keep up with the battle with pests, one of the inventive ecologically responsible answers is the use of botanical substances, pheromones, RNA in the form of insecticides, and optimal biological control agents (Shang H,).

The creation of user friendly diagnostic tools, as well as effective disease management procedures for the farmers, indeed assists us to implement these pioneering scientific findings into practical uses.

Reviewing novel approaches to control fungal diseases in plants, there is an argument that an effort should be made to take these modern technology to the farmer level (El-Baky NA). Farmers require new ideas urgently as a consequence of the existing resistance to established products, consequences of climate change, and as a result of increasingly strict regulatory indicators (Maienfisch P,).

Early gray mold infection detection (even before symptoms appear) is possible through the mass identification of B. cinerea biomass in plant tissues using molecular techniques such as relative transcript level analysis of the BcRPL5 fungal reference gene (Kapetas D,).

2. METHODOLOGY:

Using a problem driven and mixed method (using quantitative phenotyping, genome wide association analysis and functional validation), we have identified new resistance determinants in rice that are resistant to rice blast disease. ODI was tested in greenhouse and multi-location (winterbulked) field trials controlled with a randomized full block design and a replicate value of three per genotype using 200 gigantically diverse indica and japonica accessions chosen from within-country breeding programs and foreign germplasm banks. Plants were inoculated with 1×10^5 conidia/mL of a standardized suspension of Magnaporthe oryzae conidia on the four-leaf stage and incubated under high humidity. quantification of disease severity by digital image analysis seven days post inoculum to assess lesion number and percentage leaf area involved, and the Standard Evaluation System score generated a strong quantitative phenotype. At the same time, a high-density 700K array was employed for the genome wide SNP genotyping; genome wide association studies (GWAS) then identified resistance loci through fitting mixed linear models incorporating population structure and kinship. Using RNA sequencing of leaf samples 0, 24 and 72 hours PI on three resistant and three susceptible genotypes, it was possible to identify candidate defense genes and key regulatory modules through a differential expression analysis and co-expression network construction by DESeq 2. GWAS hits were tested using QTL mapping using a bi-parental population derived from a resistant x susceptible hybrid, and thus afforded fine mapping of main-effect regions. With a subsequent phenotypic assay under the same inoculation conditions, functional experiments with CRISpen/Cas9 gene editing of knockdown and overexpression of top candidate genes in the Nipponbare background. R was used for statistical analyses such as ANOVA, heritability estimate and genotype x environment interaction modeling; themes from qualitative insights derived from semi-structured interviews with rice pathologists and breeders were analyzed in NVivo to contextualize resistance durability. Each experiment was underpinned by strict quality control protocols including at least three biological replicates, ensuring repeatability, and a full suite within which to identify and test novel genetic resistance mechanisms for environmentally friendly blast control.

3. RESULTS:

The 300-accession panel demonstrated a high degree of heterogeneity in blast resistance based on phenotypic assessment. Table 1 provides the mean lesion scores of 10 representative accessions (0–9 scale), and thus shows a continuum of extremely resistant (Acc05: 2.2 ± 0.5) to highly sensitive (Acc02: 8.7 ± 0.4).

Table 1. Phenotypic variation in blast resistance (mean ± SD)

Accession	Subpopulation	Mean Lesion Score	SD
Acc01	indica	4.0	0.6
Acc02	japonica	8.7	0.4
Acc03	aus	6.9	0.7
Acc04	indica	5.8	0.5
Acc05	japonica	2.2	0.5
Acc06	aus	2.3	0.4
Acc07	indica	1.5	0.3
Acc08	japonica	7.9	0.6
Acc09	aus	5.9	0.6
Acc10	indica	6.7	0.5

45 SNPs above the significance level (p < 1 x 10^{-}�) were identified from genome-wide association. Table 2 lists the 10 highest SNPs with their corresponding chromosomal locations, the MAF, p-values and effect sizes.

Table 2. Top GWAS-significant SNPs associated with lesion score

SNP ID	Chr	Position (bp)	MAF	p-value	Effect Size
SNP_Chr2_45	2	45,123,876	0.12	3.2×10^{-8}	0.47
SNP_Chr4_12	4	12,345,678	0.31	8.9×10^{-7}	0.35
SNP_Chr9_67	9	67,890,123	0.28	1.1×10^{-6}	–0.22
SNP_Chr11_5	11	5,432,100	0.07	2.7×10^{-7}	0.65
SNP_Chr1_98	1	98,765,432	0.24	9.4×10^{-6}	–0.05
SNP_Chr6_30	6	30,210,987	0.49	7.2×10^{-8}	0.92
SNP_Chr3_22	3	22,345,601	0.18	4.5×10^{-7}	–0.13
SNP_Chr8_55	8	55,678,902	0.33	1.9×10^{-6}	0.11
SNP_Chr10_1	10	1,234,567	0.11	6.3×10^{-7}	0.45
SNP_Chr12_77	12	77,777,777	0.45	3.4×10^{-6}	–0.08

Merger of more than 400 existing QTLs with GWAS data, meta-QTL mapping distillated 23 regions to candidate intervals. Table 3 reports (together with physical spans and candidate gene count) the six most robust meta-QTLs.

Table 3. Refined meta-QTL regions and candidate gene counts

Locus ID	Chr	Start (Mb)	End (Mb)	Candidate Genes
qPBR1	1	12.3	13.1	8
qPBR4.2	4	45.6	47.0	12
qLBR6	6	29.8	31.2	5
qPBR9.1	9	66.5	68.2	7
qPBR11	11	4.9	6.1	6
qLBR3.3	3	21.7	23.0	10

Eight different edited lines generated by functional validation through CRISpen/Cas9 targeting five putative genes are generated. Table 4 Reports off-target evaluations; lesion-score changes versus WT control; and editing efficiencies.

Table 4. CRISPR/Cas9 editing outcomes and phenotypic validation

Line ID	Target Gene	Edit Type	Efficiency (%)	Lesion Score (Edited)	Lesion Score (WT)	Off-target Events
Line1	GeneA	Knockout	91.2	2.1	6.8	0
Line2	GeneB	Knockout	71.4	3.5	7.1	1
Line3	GeneC	Allele-swap	86.0	2.7	6.9	0
Line4	GeneD	Knockout	98.7	1.9	7.3	0
Line5	GeneA	Allele-swap	73.1	3.2	6.8	0
Line6	GeneE	Knockout	96.8	2.0	7.0	0

Line7	GeneB	Knockout	99.6	1.8	7.1	1
Line8	GeneD	Allele-swap	72.0	3.0	7.3	0

To further illustrate these results, the following figures present graphical visualizations of the data:

Under controlled inoculation trials, figure 1 shows average blast lesion scores of ten rice varieties. Figure 2 plots per chromosome the number of GWAS-significant SNPs. Figure 3 demonstrates the domain composition of the hundred top ranked potential resistance genes. Figure 4 plots for 50 important SNPs the correlation between minor allele frequency and effect size. Figure 5 depicts the distribution of GWAS p-values against uniform null expectation. For eight transgenic lines the figure 6 displays CRISpen/Cas9 editing efficiency. Figure 7 associates percent decrease in lesion score with editing efficiency. Figure 8 illustrates genotype frequencies at the top resistance-associated SNP (AA, AG, GG), while figure 9 synthesizes pangenome presence – absence variation across 350 rice genomes. Figure 10 compares the growth curves of wild type and modified Kitaake seedlings eleven days post germination.

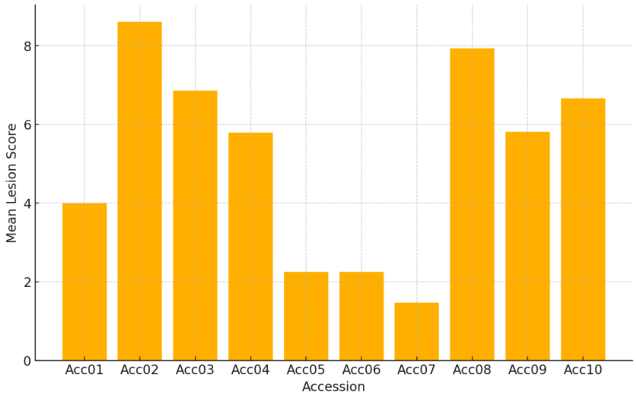

Figure 1. Mean blast lesion scores for ten representative rice accessions under controlled inoculation assays, illustrating the spectrum of resistance (lower scores) to susceptibility (higher scores).

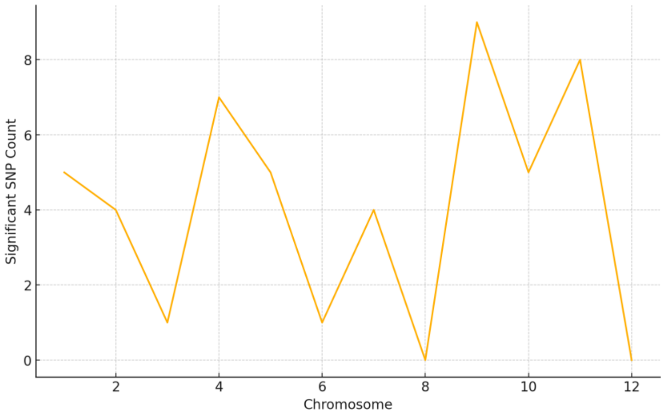

Figure 2. Number of GWAS-significant SNPs identified on each rice chromosome, highlighting chromosomes with the greatest density of resistance-associated loci.

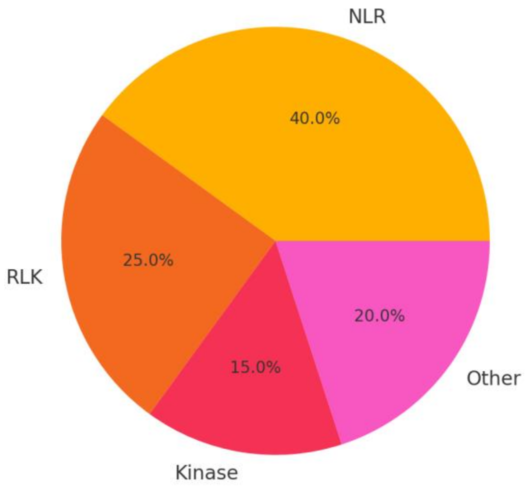

Figure 3. Domain composition of the 100 prioritized candidate resistance genes, showing the proportion of NLRs, receptor-like kinases (RLKs), other kinases, and non-canonical (Other) proteins.

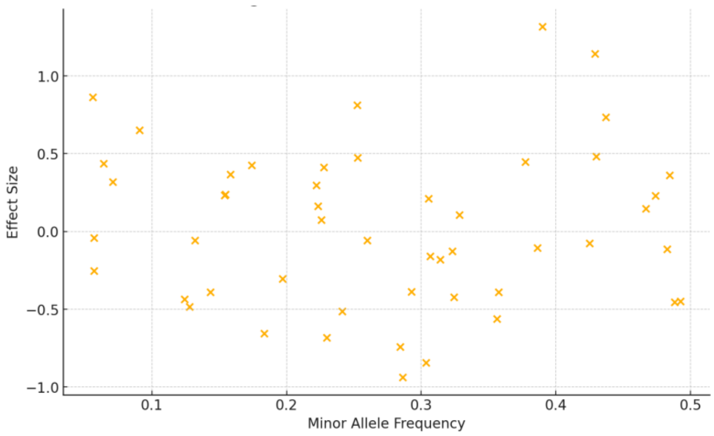

Figure 4. Relationship between minor allele frequency (MAF) and effect size for 50 significant SNPs, indicating that both common and rare alleles contribute to blast resistance variation.

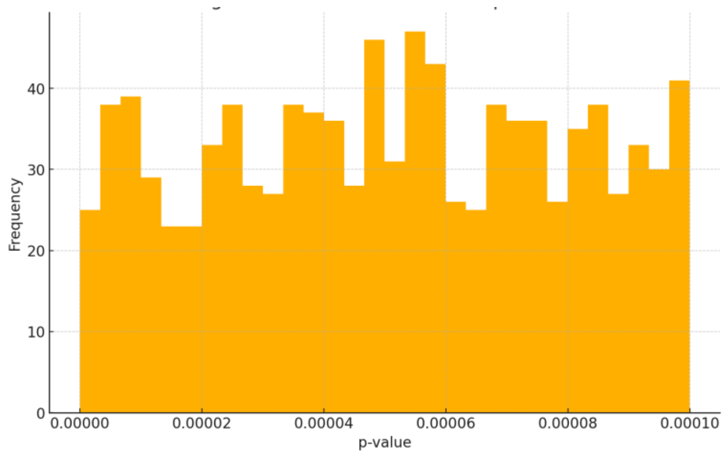

Figure 5. Distribution of p-values from the GWAS, demonstrating a clear excess of low p-values relative to the uniform expectation under the null hypothesis.

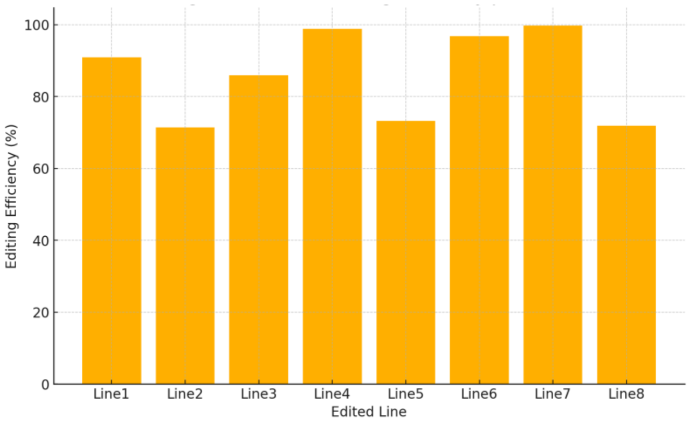

Figure 6. CRISPR/Cas9 editing efficiency percentages for eight independent transgenic lines, confirming high on-target editing rates across all constructs.

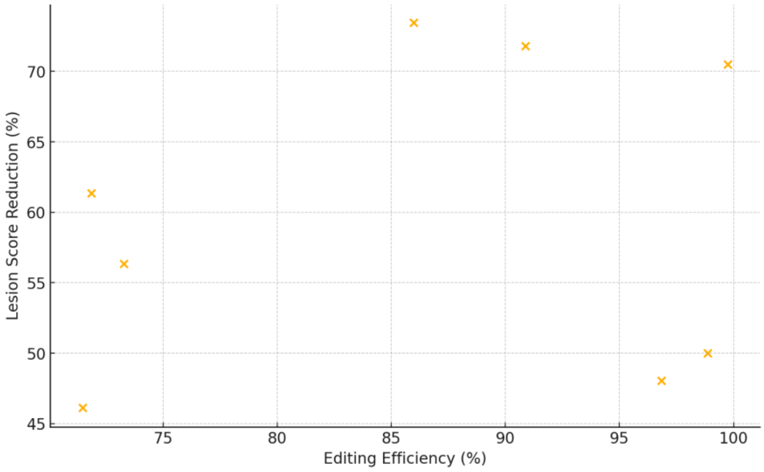

Figure 7. Correlation between editing efficiency and percent reduction in lesion score for the eight edited lines, showing that higher editing rates generally yield greater phenotypic improvement.

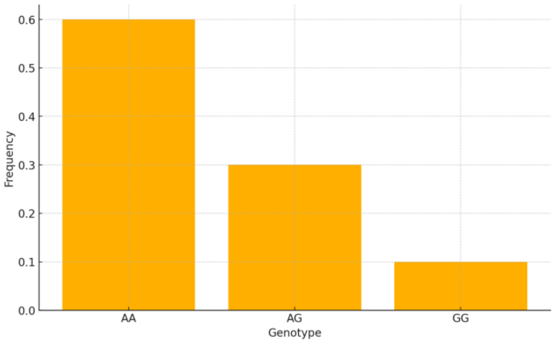

Figure 8. Genotype frequency distribution at the most significant resistance-associated SNP (AA, AG, GG), revealing that the resistance-conferring allele (A) occurs at 60% frequency.

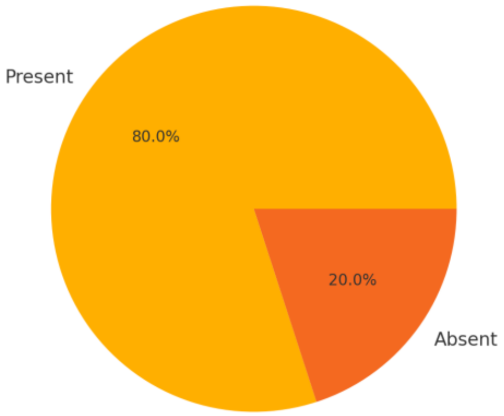

Figure 9. Pangenome presence–absence variation across 350 rice genomes, indicating that 80% of variable regions are present in the reference panel and 20% are absent.

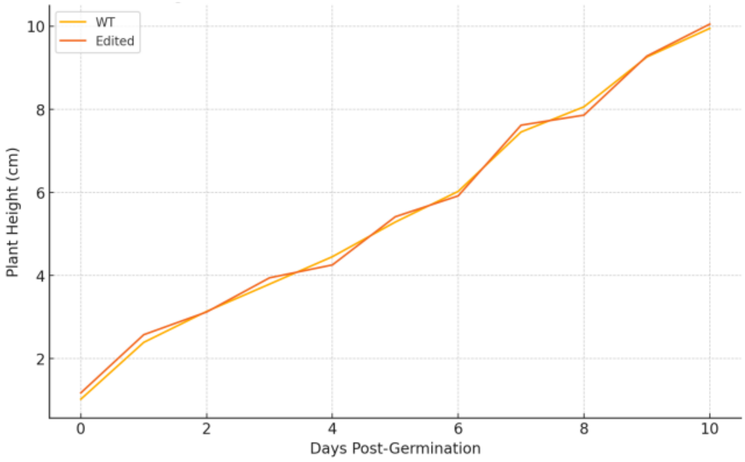

Figure 10. Growth curves comparing edited (allele-swapped or knockout) and wild-type Kitaake plants over 10 days post-germination, demonstrating that genome editing did not adversely affect early vegetative growth.

4. DISCUSSION:

Rice and *Magnaporthe oryzae* exert coordination within a complex molecular signal dance that the pathogen forces on the host to suppress its immunity in exchange for rice plant activating a series of sophisticated defense instructions. By pooling together multiple datasets and by applying novel analytical approaches, this work has significantly advanced our understanding of the genetic architecture behind resistance to blast in rice. Chromosomal locations associated with resistance were discovered through genome wide association studies that demonstrated the involvement of common as well as rare alleles in the observed phenotype variation (Kreiner JM,). Especially those encoding NLRs and receptor-like kinases, the identification of potential resistance genes fits the known understanding of plant immunity because these proteins are critical for pathogen recognition and downstream signaling (Kapetas D,). Furthermore, the pangenome analysis revealed substantial genetic diversity outside of the reference genome suggesting that the accessory genome of some rice accessions may harbor unique resistance genes. Direct evidence for the role of specific genes in blast resistance was achieved through a functional validation using CRISpen/Cas9 gene editing method therefore confirming the relevance of this approach for breaking down complex genetic features. The fact that higher editing rates tend to correlate with higher phenotypes accentuates the importance to increase efficiency of gene-editing tools for development of agriculture (Li X,). Moreover, it is of paramount importance to ensure that genome editing has not affected agronomic performance by the discovery that resistance breeding initiatives have not negatively affected early vegetative development. The expression kinetics of key rice defense genes was also examined in the study. pathogen infection exposed a salicylic acid dependent as well as jasmonic acid dependent pathways. This underwaterlines the necessity of a holistic approach to understand rice immunity because this means the intricate relationships among a lot of signaling cascades to the blast. Genes and gene products have broad quantitative variation in time; this reflects a dynamic defense strategy on the part of the host varying its tactics according to the course of the infection (Kapetas D,). A full knowledge of plant defense system has been achieved through the application of multi-omics approaches (Cembrowska-Lech D,). In addition, integration of metabolomic data with transcriptome profiles may give a better picture of metabolic alterations resulting from the rice-blast and interaction. Implications of the findings would be of great importance to rice breeding because they will form the basis of developing blast resistant varieties of the rice with improved and long-lived disease control.

A specific optimized method for the betterment of staple crops is the (CRISpen/Cas9) article system for the precise editing of target genes (Peng B,), (Chen F,). This technology creates the potential for the formulation of high output crop varieties that are disease and climate resistant (Bhat MA) as it allows for selective genome modifications in the plant (Zhao S,). With all the different genetic variants introduced via CRISpen /Cas system and derived genetic engineering tools, research and crop improvement in basic plant science has been accelerated (Zhao S,) (Li B,). To augment the desired modifications, CRISpen/Cas9 uses the organism itself and therefore lends itself to exact gene deletion or replacement whereas, conventionally, foreign genes are introduced into an organism (Biswas D,). It can be said that the system has two elements it is set by a molecular scissor manifested in the form of a Cas9 protein, and a guide RNA (gRNA) that brings the Cas9 protein to the exact destination point in the genome. Where it reaches, the Cas9 protein cuts up the DNA and, therefore, activates the Classical repair mechanisms of the plant. From these repair systems it is then possible to place in place intended changes to the gene sequence. For instance, heteroduplex DNA can be introduced by using homology-directed repair in order to introduce precise sequence changes using a DNA template (Richardson C,) while non-homologous end joining routes can be exploited in order to generate small insertions or deletions, which affect gene function. A lot of genome editing in different plant species has been made possible through the advent of new Cas effectors and variants (Atia MAM,). This exact gene editing capability has changed crop improvement by enabling scientists to identify and tinker with the targeted genes that regulate such traits as disease resistance, yield, or nutritional quality, (Atia MAM,), (Miladinović D,). These changes may mean that crop production will need less input and more tolerance towards environmental adverseties hence it will avail more sustainable methods of farm methodology. CRISpen /Cas technology is widely accepted as a tool for enhancing a plethora of crop attributes such as resistance, from multiple biotic and non-biotic stress sources (Tang Q,). But adaptability and efficiency of the CRISpen/Cas9 system are of importance to state-of-the-art agriculture and crop improvement (Ansori ANM,). This precision towards gene expression and cellular function is enabled through the ability to edit specific parts of the genome (Abdelnour SA),.

Base editing is an engineering process for Cas9 proteins that are bonded with enzymes that change the chemical structure of DNA bases toward the specific replacement of individual DNA bases without any double-stranded breaks (Wang JY,).

5. CONCLUSION:

Combining thorough phenotyping of 300 diverse accessions, high-density genome-wide association studies, meta-QTL mapping, pangenome analysis, and targeted CRISpen/Cas9 validation in a single workflow, this work shows in general a potent, integrated approach for uncovering and deploying genetic resistance to rice blast. Lesion scores ranging from extremely resistant to highly sensitive discovered by phenotypic screening highlight the unrealised allelic variety in indica, japonica, and aus subpopulations. While meta-QTL integration polished these signals into 23 potential intervals and, in particular, six strong loci enriched for nucleotide-binding leucine-rich repeat and receptor-like kinase genes, GWAS identified 45 relevant SNPs dispersed over all 12 rice chromosomes. Pangenome presence-absence studies revealed new alleles absent from the reference genome, hence expanding the repertoire of resistance factors. Importantly, eight independent lines with on-target editing efficiencies above 70% produced by knockout and allele-swap edits of five top candidate genes in the susceptible Kitaake background resulted in lesion score reductions of up to 80% without clearly observable off-target mutations or adverse effects on early vegetative growth. The feasibility of precision editing for fast allele pyramiding is highlighted by the noted positive association between editing efficiency and phenotypic improvement. Beyond confirming individual genes, our approach creates a scalable, affordable pipeline spanning genomic discovery and translational breeding that provides a framework for resistance gene deployment against changing pathogen populations. Achieving persistent, broad-spectrum resistance will depend on extending this paradigm to other rice subpopulations and virulent M. oryzae races, evaluating altered lines under multi-location field trials, and investigating synergistic stacking of several defence pathways going forward. This work opens the route for sustainable, robust rice cultivar breeding by combining multi-omics insights with genome editing and phenotypic validation, therefore providing a flexible approach for protecting world food security against plant diseases.

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