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Evaluation of Blast Disease Resistance in Rice Plants (*Oryza sativa)* Resulting from *BSR-D1* Gene Editing Through In-Planta Transformation With CRISPR/Cas9 Technology

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Abstract

Rice blast disease, caused by the fungus Magnaporthe oryzae, poses a significant threat to rice productivity worldwide. To enhance rice resistance to this disease, CRISPR/Cas9 gene-editing technology has been used to modify the Bsr-d1 gene, which is known to regulate plant resistance to fungal infections negatively. This study aims to evaluate the blast disease resistance of the T2 generation of the Bsr-d1 gene-edited rice variety MR 297. The transformed plants were inoculated with a P. oryzae isolate (Isolate MR297 3.1), and disease symptoms were assessed nine days post-inoculation using the IRRI scale (0–9). The results demonstrated that several gene-edited plant lines exhibited significantly milder disease symptoms compared to susceptible control plants. These findings indicate that editing the Bsr-d1 gene effectively enhances rice resistance to blast disease without compromising key agronomic traits. By eliminating the need for tissue culture, this protocol offers a simpler and more efficient approach with the potential for widespread adoption in disease-resistant rice breeding programs. The primary advantage of this study is the application of CRISPR/Cas9 technology to specifically target the Bsr-d1 gene, a negative regulator of blast resistance. This method enhances rice plant resistance without introducing foreign genes, increasing its likelihood of regulatory approval and acceptance in field applications. Resistance testing using local isolates of Magnaporthe oryzae enhances the relevance of the findings to local agroecosystem conditions. Furthermore, the study demonstrates that increased resistance does not compromise key agronomic traits, supporting the development of superior rice varieties that are both high-yielding and disease-resistant. It is recommended that users conduct multi-location and multi-season trials to evaluate the stability of gene-edited plant resistance under varying environmental conditions and pathogen pressures. Additionally, a more in-depth analysis of the impact of the Bsr-d1 gene on the expression of other genes related to plant growth and yield is necessary.

Keywords: Agronomy, *Magnaporthe oryza* Fungus, Environment, Tissue Culture

1. Introduction

Rice (*Oryza sativa*) is a major food crop, serving as a primary source of carbohydrates for a large portion of the world's population, particularly in Asia. The MR 297 rice variety is a superior cultivar developed for optimal yields and resistance to various environmental stresses. However, rice productivity is often threatened by several fungal diseases, including rust and blast. According to Safrizal et al. (2023), oryzae disease causes severe damage to leaves, stems, and rice panicles, leading to reductions in crop yields.

Efforts to enhance rice plants' resistance to blast disease have become a primary focus in plant breeding programs. According to **Error! Reference source not found.**blast disease significantly reduces crop yields by causing incomplete grain filling in panicles, thereby

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substantially diminishing rice quality. Damage from blast attacks can lead to yield losses of up to 30%. **Error! Reference source not found.** report that CRISPR/Cas9-based gene-editing technology has emerged as an innovative tool that enables precise gene modification in a shorter time frame than conventional methods. This technology allows altering genes involved in plant resistance to enhance defence responses without compromising other important agronomic traits.

One gene that has received attention in the context of blast resistance is the Bsr-d1 gene, which functions as a negative regulator in the plant defense system against M. oryzae fungal infection. In a study **Error! Reference source not found.**modification of the Bsr-d1 gene using CRISPR/Cas9 can suppress its function, thereby increasing resistance to blast disease in rice plants.

To support the implementation of this gene editing technology, an efficient and practical transformation method is essential (**Error! Reference source not found.**transformation is a promising approach because it allows the introduction of genetic material directly into plant tissue without the complex, time-consuming tissue culture steps. This method can accelerate the development of disease-resistant varieties and increase farmers' adoption of technology.

This study aims to evaluate resistance to blast disease in rice plants of the MR 297 variety that have undergone Bsr-d1 gene editing through in planta transformation using the CRISPR/Cas9 system. Furthermore, this study assesses the agronomic characteristics of the edited plants to ensure that genetic modification does not negatively affect plant quality or yield. It is hoped that the results of this study can contribute to the development of more efficient and sustainable disease-resistant rice varieties, thereby supporting national food security.

The application of the CRISPR/Cas9 system in plant genome editing generally involves four main stages: (1) determining the target DNA sequence, (2) designing gRNA and Cas9 components, (3) delivering the target into plant cells, and (4) analyzing the editing results. Of these four stages, the delivery method is one of the most crucial aspects and often determines the success of the entire process. The most commonly used delivery methods currently include Agrobacterium tumefaciens, particle bombardment, and PEG-mediated delivery (**Error! Reference source not found.**However, these approaches usually require complex, time-consuming tissue culture procedures and are not necessarily effective for all rice varieties, especially recalcitrant ones.

In recent years, the in planta transformation approach has gained attention as a promising alternative. In planta transformation is a method of directly introducing genetic material into plant tissue, bypassing the tissue culture regeneration process. Commonly used plant parts include shoot tips, flowers, fruits, embryos, and roots. This approach not only reduces reliance on aseptic techniques but also speeds up the overall transformation process.

Several successful in planta transformations with the CRISPR/Cas9 system have been reported in cotton, wheat, sorghum, and several other dicotyledonous plants, both via Agrobacterium-mediated transformation and biolistic transformation. However, the application of this method to rice plants, especially local Malaysian varieties such as MR 219, has not been widely studied. **Error! Reference source not found.** have reported protocols for in planta transformation in rice, but they have not yet included applications of the CRISPR/Cas9 system. Several other studies have attempted similar approaches, but these were limited to floral or embryonic structures and did not focus on stable transformation using genome editing systems.

This study reports for the first time. This study continues the research of **Error! Reference source not found.**They conducted a study on In planta genetic transformation: a concise technique for delivering the CRISPR/Cas 9 system into cells for the purpose of editing the rice genome, which obtained the necessary results to conduct continuous research on how successful Agrobacterium-based in planta transformation is in delivering the CRISPR/Cas9 system into rice *(Oryza sativa subsp. indica). cv. MR 219),* by targeting the apical meristem tissue open by cutting the coleoptile of germinating seeds. This study did not focus on the results of editing the target sequence, but rather on the successful integration and expression of the Cas9 gene in the transformant plants. **Error! Reference source not found.**conducted research on how to deliver the CRISPR/Cas9 system into plant cells using *Agrobacterium* as an intermediary for application to rice plants (*Oryza sativa*). In addition, blast disease resistance is needed, so the BSR-D1 gene was added to protect rice plants from blast disease.

By developing this approach, it is hoped that it will pave the way for the application of simpler, more efficient, and tissue culture-free genome editing techniques in rice and other food crops, especially in tropical regions such as Malaysia.

To address this issue, developing rice varieties resistant to blast disease has become a key strategy in crop improvement programs. Currently, CRISPR/Cas9-based gene editing technology has emerged as a highly promising tool in plant breeding. This technology enables precise gene modification in a significantly shorter timeframe than conventional methods. One gene identified as playing a crucial role in blast resistance is Bsr-d1, which functions as a negative regulator of plant defence against Magnaporthe oryzae infection. Using CRISPR/Cas9 technology, the Bsr-d1 gene can be edited to enhance rice disease resistance without compromising other agronomic traits.

This study aims to evaluate resistance to blast disease in MR 297 rice plants that have undergone Bsr-d1 gene

editing via in planta CRISPR/Cas9 transformation. Additionally, the study assesses the agronomic characteristics of the edited plants to ensure that genetic modification does not adversely affect plant quality or yield. The results are expected to contribute to the development of more efficient and sustainable disease-resistant rice varieties, thereby supporting national food security.

2. Material and Methods

2.1. Place and Time of Research

This research was conducted in several integrated facilities, namely the Plant Transformation and Tissue Culture Laboratory at the Horticulture Research Centre (HR1), MARDI Kuala Lumpur, Malaysia. Planting and resistance testing were conducted at the Horticulture Research Centre (HR1) at MARDI, Kuala Lumpur, Malaysia. Coordinates are 3.034° N, 101.693° E, +30 masl.

Research activities were carried out from February to August 2025 and included genetic transformation, plant regeneration, molecular confirmation, and evaluation of resistance to blast disease (*Magnaporthe oryzae*).

2.2. Materials and tools

2.2.1. Material

The materials used in this study include:

a) Culture Media: Murashige and Skoog (MS) medium (3.15 g), Glucose (10 g) and Maltose (10 g), Amino acids: Arginine, Asparagine, Glutamine (0.1 g each), Plant growth regulators: 2,4-D (0.5 mL) and NAA (10 mL), Acetosyringone (45.05 mg). Additional media: Gamborg B5 (BE) medium

b) Transformation Materials and Molecular Analysis: Construction of CRISPR/Cas9 plasmid with BSR-D1 target gRNA *Agrobacterium tumefaciens strain* LBA4404 or GV3101, Virulent M *agnaporthe oryzae* spores, DNA extraction materials: lysis buffer, ethanol, RNase (optional). PCR materials: PCR reaction buffer (5 µL), High GC Enhancer (5 µL), dNTP (0.5 µL), Forward and Reverse Primer (1.25 µL each), DNA Template (1 µL), Taq Polymerase, Distilled water (10.75 µL), Agarose gel and Ethidium bromide

c) Supporting Materials: Rice seeds of blast-prone varieties (e.g. IR64 or Nipponbare), nutrient solution, base fertilizer, sterile water, pesticide (if needed), liquid nitrogen, Falcon tube, scotch bottle, and sterile flask.

2.2.2. Tools

The main equipment used in this study includes: Laminar Air Flow Cabinet, Autoclave, Centrifuge, PCR Thermal Cycler, Gel Electrophoresis Set, Gel Documentation System, Hot Plate Stirrer, PH meter Spectrophotometer / Chlorophyll Meter, Micropipette and sterile pipette tips, Mortar and pestle, Bunsen burner, Forceps and scalpel, Incubator / Growth chamber, Tissue culture rack, Vacuum pump, Sprayer (for inoculation), Transgenic Greenhouse and Digital camera for documenting disease symptoms.

2.2.3. Research Stages

This research consists of several main stages, namely:

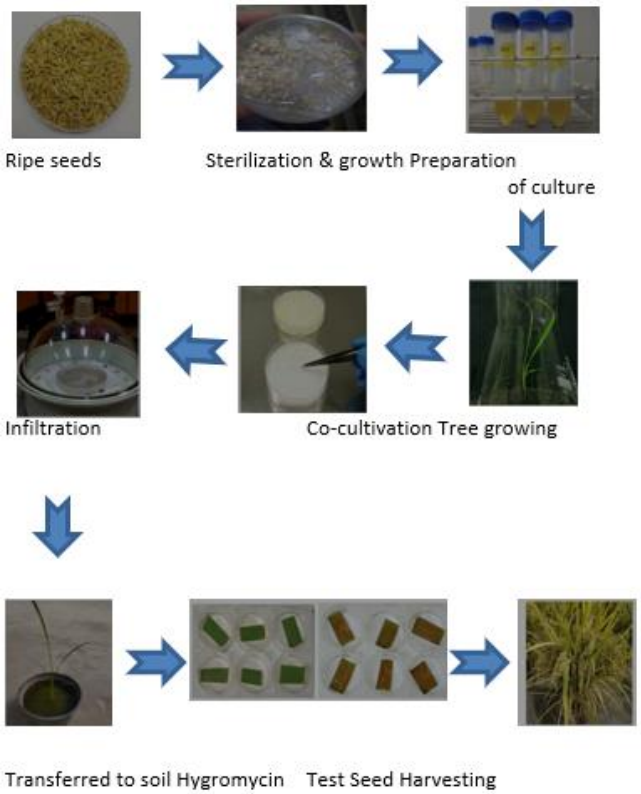

Figure 1. Research flow diagram

a) **Design and Construction of the CRISPR/Cas 9 System**: The initial stage involved identifying and designing gRNA specific to *the BSR-D1 target gene*. The gRNA construct and *Cas9 sequence* were inserted into a plasmid vector and introduced into *Agrobacterium tumefaciens* via transformation.

b) **In Planta Transformation**: Transformation is carried out using the *in planta method*, namely direct infiltration into plant tissue using Agrobacterium *containing* the CRISPR/Cas9 system. Rice seedlings are prepared at a specific stage, and after infiltration, they are planted in culture medium for regeneration.

c) **Plant Regeneration and Selection**: Transformed plants were grown on selection media and acclimatized in a greenhouse. Selection was based on target gene expression, and PCR confirmed successful BSR-D1 gene editing.

d) **Blast Disease Resistance Test**: Edited and control plants were planted separately in a greenhouse. Magnaporthe *oryzae inoculation* is performed during the plant's vegetative phase using a spray method. Symptom evaluation is based on standard blast

resistance scoring, with lesions observed for intensity and distribution on the leaves.

e) Data Analysis: The data obtained were analyzed descriptively and quantitatively. Statistical tests were performed using ANOVA to compare the resistance between edited and control plants. Correlation analysis between mutations in *the BSR-D1 gene* and resistance levels was also performed if necessary.

2.3. Plant Material

The MR 297 rice seeds used in this study were obtained from MARDI Seberang Perai, Pulau Pinang. In addition, the MR 211 and MR 84 varieties were used as controls.

2.4. Seed Preparation and Sterilization

MR 297 seeds were manually picked, then surface sterilized by gradual immersion using 100% (v/v) ethanol for 1 minute, followed by immersion in 1% (v/v) Virkon solution for 30 minutes, and continued by immersion in 70% (v/v) sodium hypochlorite (NaOCl) solution containing 0.25% Tween 20 for 30 minutes. After that, the seeds were washed 4 times with sterile distilled water to remove residual NaOCl, then dried on sterile filter paper for 1 hour.

2.5. In Planta Transformation with Agrobacterium

The dried sterile seeds were then soaked in sterile distilled water and allowed to germinate in the dark for 7 days. After coleoptile emergence, a portion of the coleoptile was cut using a sterile knife. The prepared seeds were then infiltrated with a suspension *of Agrobacterium tumefaciens* carrying the CRISPR/Cas9 plasmid, with an optical density (OD600) of 0.6. Infiltration was performed using a vacuum pump at 80 kPa for 15 minutes to increase contact between Agrobacterium and coleoptile tissue.

2.6. Propagation and Planting

After infiltration, the seeds were transferred to MS liquid medium (Murashige and Skoog, 1962) to facilitate shoot and root growth. After root and shoot development, the plants were transferred to a vermiculite-soil mixture and maintained in a growth chamber before being transplanted into potted rice paddy soil to grow to maturity.

2.7. Blast Disease Resistance Testing

T_2 generation plants resulting from the transformation were tested against the pathogen Pyricularia oryzae (the cause of rust disease). The pathogen isolate MR 297 3.1 was grown on Oat Meal Agar for 7 days, and sporulation was induced under fluorescent lighting for 4–7 days. A spore suspension with a concentration of 1 × 10^5 spores/mL was sprayed onto the plants at the 3–4 leaf stage, then covered. overnight to increase inoculation success.

2.8. Assessment of Disease Symptoms

Symptoms of rust disease were assessed on the 9th day after inoculation using the IRRI Standard Evaluation System (SES) scale, with values ranging from 0 (very susceptible) to 9 (very susceptible).

2.9. Molecular Analysis

Genome extraction and DNA sequencing was performed on transformed plant leaves. The presence of *the Cas9* and *hpt* II genes was analyzed using PCR. Next, *the Bsr-d1 region* was analyzed by DNA sequencing to verify the mutations generated by CRISPR/Cas9 and to link them to the rust resistance phenotype.

2.10. Data Analysis

Disease resistance data were analyzed using descriptive statistics and ANOVA to identify significant differences between mutant and control lines. Genetic analysis was performed to confirm the presence and type of mutations in the target genes. The tools used for data analysis were an automated micropipette and the SnapGene Vx.XX application, and SPSS Statistics v28.0 data processing software.

3. Results and Discussion

3.1. Table of Chlorophyll Content of Rice Leaves MR 2 97 on October 8, 2025

Based on the table data, the chlorophyll content in the leaves of the rice variety MR 297 was measured on October 8, 2025. Measurements were made across several rows of plants, including the control and several genetically engineered lines (e.g., G20, G23, and others). The chlorophyll values in the control group were relatively consistent, with an average of 36.7-38.8. The highest reading in the control group was 40.8, and the lowest was 26.5, indicating variations that environmental factors or measurement techniques may cause.

Some genetically engineered lines, such as G23 (L2) and G20 (L1), showed chlorophyll values comparable to those of the control. For example, G23 (L2) had an average value of 34.9 ± 3.7, while G20 (L1) recorded 31.8 ± 2.4. According to (2024), the relatively small standard deviation indicates that the variation between repetitions within a line is quite low. Overall, most test lines, including those genetically modified, showed chlorophyll levels within the normal range for healthy rice leaves, suggesting that photosynthetic activity was not disturbed.

3.2. Soil pH in Rice Cultivation Experiment Plots and Trays

Soil pH measurements across all concrete rice field plots ranged from 5.6 to 5.8, within the optimal range for rice cultivation (5.5–6.5). This finding is important because ideal soil pH supports the availability of essential nutrients,

such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), which significantly impact rice plant growth and productivity.

Table 1. Chlorophyll Content of Rice Leaves MR 297 on October 8, 2025 Rice Leaf Chlorophyll (MR 297)

Treatment	Test	Reading 1	Reading 2	Reading 3
Control	1	24.8	3.8	36.2
Control	2	37.2	37.8	37.2
Control	3	37.5	38.6	38.8
Control	4	34.1	40.2	36.7
Control	5	26.8	40.3	35.2
Control	6	40.8	38.7	38.8
Control	7	38.2	37.6	40.1
Control	8	34.2	38.6	42.0
G23 (L2)	1	30.3	35.7	34.1
G23 (L2)	2	32.5	35.1	39.1
G23 (L2)	3	35.5	35.7	38.0
G23 (L2)	4	35.2	37.0	36.8
G23 (L2)	5	36.9	34.8	36.1
G23 (L2)	6	32.4	34.6	30.9
G23 (L2)	7	29.4	32.4	41.2
G23 (L2)	8	35.7	34.6	36.4
G23 (L2)	9	29.5	34.7	40.1
G23 (L2)	10	32.5	33.3	39.0
G20 (L1)	1	30.8	32.0	30.6
G20 (L1)	2	30.9	32.7	35.6
G20 (L1)	3	31.8	30.6	33.6
G20 (L1)	4	33.0	35.2	31.3
G20 (L1)	5	32.2	29.8	33.8
G20 (L1)	6	26.7	0	26.0
G20 (L1)	7	29.4	30.9	34.7
G20 (L1)	8	30.1	33.1	34.7
G20 (L1)	9	49.4	29.4	30.7
G20 (L1)	10	27.2	26.1	35.2

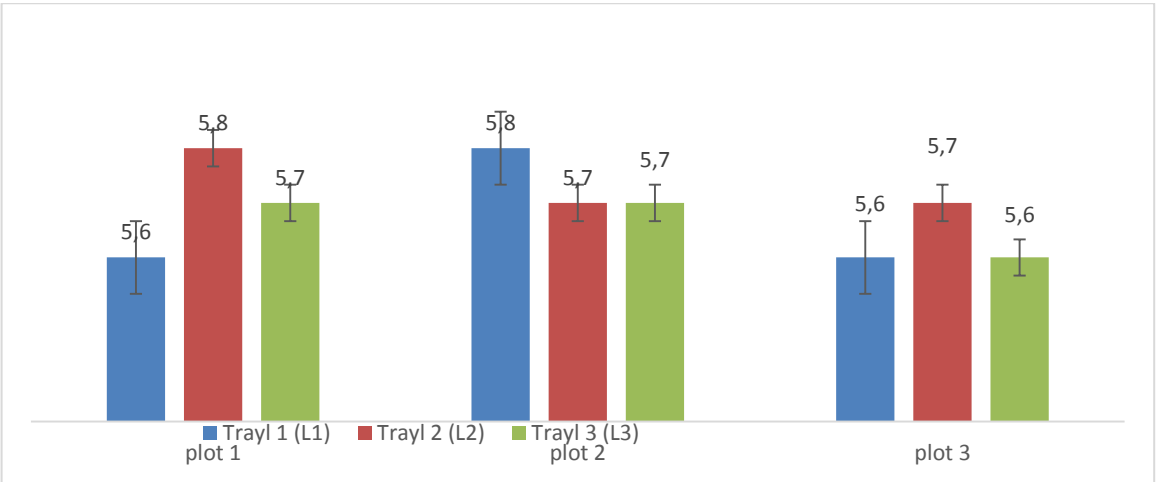

Figure 2. Soil pH measurements

Soil that is too acidic (pH < 5.5) is at risk of experiencing: toxicity of iron (Fe) and aluminum (Al), decreased phosphorus availability, inhibition of root growth and decreased yield. On the other hand, soil that is too alkaline (pH > 7.0) can cause: micronutrient deficiencies such as Zn, Fe, and Mn, chlorosis and plant development disorders.

In this study, the soil pH in the MR 297 experimental tray also ranged from 5.6 to 5.9, within the optimal range. According to **Error! Reference source not found.**), this indicates that not only the rice field plot, but also the growing medium in the tray, has been well managed to maintain the efficiency of nutrient uptake by plants.

Stable, optimal soil pH values across all experimental

sites (plots and trays) provide a healthy environmental foundation for rice growth. This finding is also a contributing factor in evaluating plant resistance to diseases such as blast, as soil stress (including pH imbalance) can exacerbate plant susceptibility to infection.

3.3. Disease Screening in Rice Plants MR 297

Disease screening was conducted on several plant lines, including MR 297, G23, and G20, on October 1, 2025, based on observations from August 10, 2025, with a planting date of April 12, 2025. The collected data showed the level of disease attack across various plant lines as disease scores (scale 0–3) and the frequency of occurrence (shown as "score/frequency").

The data are processed to identify each line's resistance or susceptibility to disease based on the total score and the frequency of attacks. The higher the score and frequency, the more susceptible the line is to disease.

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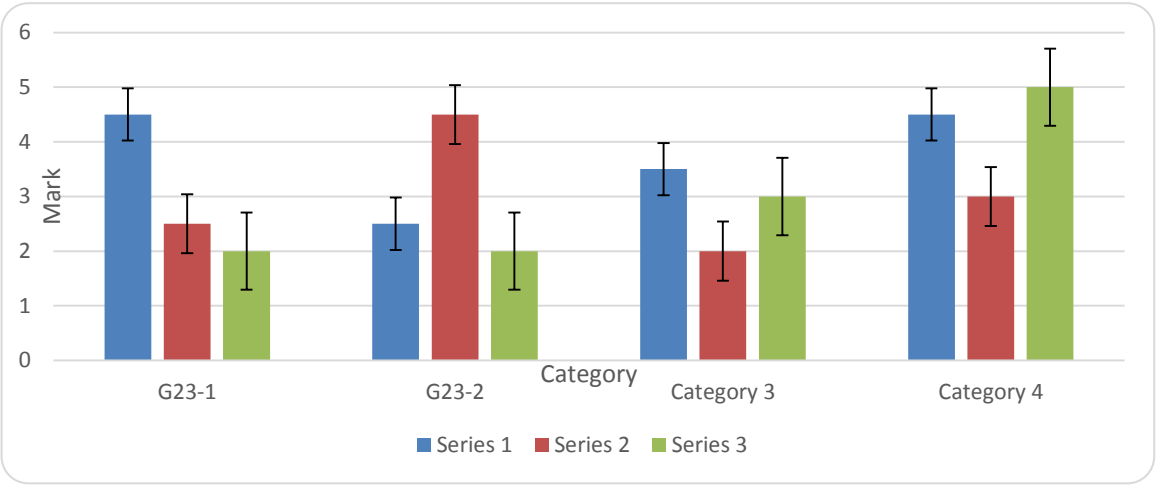

Figure 2. Comparison of Values Between Categories. Endurance Category (estimated): 0–10: Very Resistant; 11–20: Hold; 21–30: Moderate; >30: Vulnerable.

In disease resistance testing, the G20-3 plant line achieved a total disease score of 28, placing it in the "Moderate" category. Based on this score, G20-3 demonstrated adequate resistance, but not optimal compared with other lines that have shown higher levels of resistance. Compared to other plant lines, MR 284, the comparison variety, had the lowest score of 10 and was categorized as "Very Resistant". The G20-2 line from the same group (G20) recorded a score of 13 with a "Hold" category, indicating better performance than the G20-3. On the other hand, G20-4 scored 34 and was classified as "Vulnerable", indicating that G20-3 is still in a middle position in its group.

In general, a score of 28 indicates that G20-3 is still facing quite high disease pressure and that further evaluation is needed, especially regarding the dominant disease types and the stability of resistance under different environmental conditions.

The G20-3 plant line, while not yet classified as resistant or highly resistant, still has potential for improvement through further breeding or integration with integrated pest and disease management (IPM) technologies. In the context of initial selection, G20-3 could still be considered for further evaluation if it has other advantages (such as yield, maturity, or grain quality), but in terms of resistance disease, still requires special attention before it can be widely recommended.

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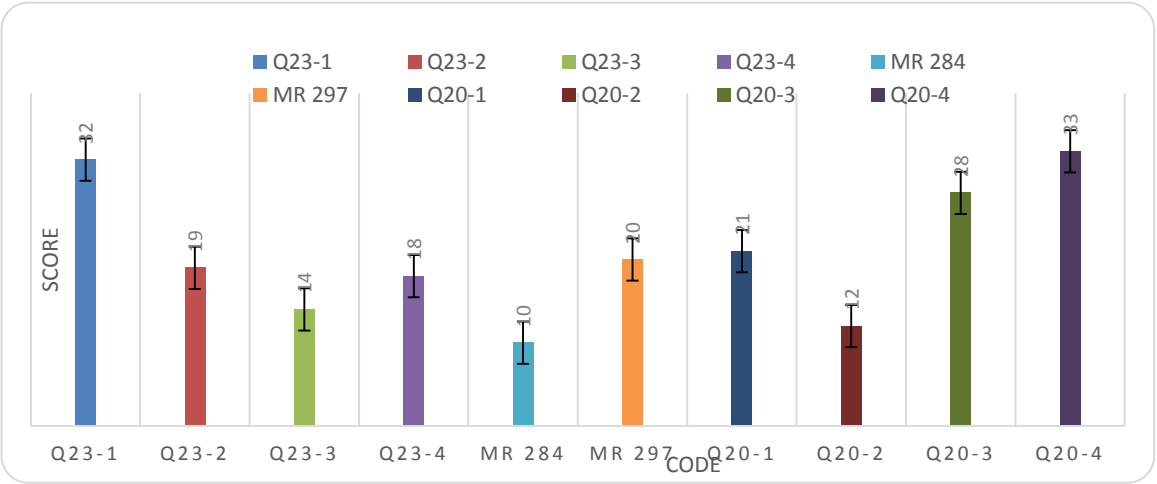

Figure 3. Total Disease Score per Plant Line. Endurance Category (estimated): 0–10: Very Resistant; 11–20: Hold; 21–30: Moderate; >30: Vulnerable.

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In general, a score of 28 indicates that G20-3 is still facing quite high disease pressure and that further evaluation is needed, especially regarding the dominant disease types and the stability of resistance under different environmental conditions.

The G20-3 plant line, although not yet classified as resistant or highly resistant, still has potential to be improved through further breeding or technological integration.

Integrated pest and disease management (IPM). In the context of initial selection, G20-3 could still be considered for further evaluation if it has other advantages (such as yield, maturity, or grain quality), but in terms of disease resistance, it still requires special attention before it can be widely recommended.

3.5. In-Planta Transformation of MR 297 Variety Using CRISPR/Cas9

In planta transformation was successfully carried out in the MR 297 rice variety using the partial coleoptile excision method and vacuum infiltration with Agrobacterium tumefaciens strain EHA105 carrying the CRISPR/Cas9 binary vector targeting the *Bsr-d1 gene*. Of the 200 transformed seeds, 30 plants successfully grew and survived to maturity.

PCR analysis to determine the presence of the Cas9 and hptII genes showed that 3 out of 30 plants (G20, G23, and G14) contained these genes. *O*f the 200 MR 297 rice seeds transformed using the in planta method, 30 plants grew to maturity. PCR analysis showed that 3 of 200 plants carried the Cas9 and hptII genes. Thus, the stable transformation efficiency obtained in this study was approximately 1.5–2%.

Meanwhile, if calculated based on the number of plants that survived to adulthood, the proportion of plants confirmed positive was around 9–10%.

3.6. Genetic Analysis of Transformed Plants

PCR amplification results showed specific bands at around 1000 bp for the *Cas9 gene* and 700 bp for *hpt* II in positive plants. Furthermore, DNA sequencing results at the Bsr-d1 target locus confirmed the presence of deletions around the PAM site, indicating that the CRISPR/Cas9 system successfully induced gene editing in MR 297 rice plants. DNA sequencing results showed a deletion in the Bsr-D1 target gene, confirming the success of gene editing and disrupting plant gene function.

This mutation is thought to disrupt *Bsr-d1 function*, which is consistent with the increased resistance to blast disease observed in phenotypic assays. Absorption of the Bsr-d1 gene makes rice plants more resistant to blast disease by enhancing the antioxidant defense system and controlling oxidative stress without compromising plant

growth.

3.7. Phenotypic Test of Resistance to Blast Disease

Rust disease symptoms at 9 days after inoculation with virulent isolates *of P. oryzae* showed that the two mutant lines (G20 and G23) had disease scores of 1–3, much lower than those of the control lines MR 297 and MR 211, which served as susceptible controls (scores of 7–9). In contrast, these results were close to those of MR 84 and MR 315 (resistant controls), which had scores of 1–3. 0, which means the plant shows no symptoms of disease and is completely resistant to infection.

This finding shows that editing *the Bsr-d1 gene* significantly increases plant resistance to rust disease without causing other visually visible abnormal phenotypes.

3.8. General Agronomic Observations

$_{T2}$ plants (especially G20 and G23) grew normally, with a morphology similar to that of the control MR 297 plants. No significant differences were found in plant height, tiller number, or flowering time. This indicates that *Bsr-d1 gene editing* did not negatively impact key agronomic traits in this generation.

This study shows that CRISPR/Cas9 technology, combined with an in planta transformation method, can be used effectively to produce rice variety MR 297 plants that are more resistant to rust disease. According to **Error! Reference source not found.** by editing the *Bsr-d1* gene, a negative regulator of plant defence responses, plant lines were produced that showed higher resistance to Magnaporthe oryzae infection compared to susceptible controls.

Gene Bsr-d1 plays a role in suppressing the production of Reactive Oxygen Species *(ROS),* especially hydrogen peroxide (H_2O_2), which is known to be important in plant defence mechanisms. By inactivating this gene through CRISPR/Cas9, ROS production remains high during pathogen infection, strengthening cell walls and inhibiting pathogen spread. The results of disease symptom assessment using the IRRI scale (0–9) showed that certain mutant lines, such as G20 and G23, had lower disease symptom scores than MR 297 (susceptible control), indicating the success of gene editing in increasing resistance.

The in planta transformation method used in this study offers a faster, simpler approach than conventional tissue culture-based transformation techniques, according to Andriyani, Y., & Wiyono, S. (2021). Pola teknik budi daya dan sifat kimia tanah yang berhubungan dengan penyakit blas pada padi sawah. *Jurnal Fitopatologi Indonesia, 17*(2), 76–82. https://doi.org/10.14692/jfi.17.2.76-82

Efiddin, A., Sutrawati, M., Bustamam, H., Ganefianti, D. W., & Sipriyadi, S. (2021). Penyakit tungro pada tanaman padi (*Oryza sativa* L.) di Kecamatan Taba Penanjung: Insidensi penyakit dan deteksi virus secara molekuler. *Jurnal Ilmu-Ilmu Pertanian Indonesia, 23*(1), 37–45. https://doi.org/10.31186/jipi.23.1.37-45

Kurrata, G., Kuswinanti, T., & Nasruddin, A. (2021). Keparahan penyakit blas *Pyricularia oryzae* dan analisis gen virulensi menggunakan metode sequence characterized amplified region. *Jurnal Fitopatologi Indonesia, 17*(1), 19–27. https://doi.org/10.14692/jfi.17.1.19-27

Novantara, P., Firmansyah, R. L., & Arismawati, M. (2025). Deteksi hama penyakit daun padi dengan menggunakan teknik optimasi deep learning convolutional neural network. *Bit-Tech, 7*(3), 975–983. https://doi.org/10.32877/bt.v7i3.2284

Yusliana, R., Wahyuni, T., & Universitas Muhammadiyah Makassar. (2024). Sistem pakar tentang mendiagnosa penyakit pada tanaman padi menggunakan metode forward chaining. *Arus Jurnal Sains dan Teknologi (AJST), 2*(2). http://jurnal.ardenjaya.com/index.php/ajst

Ramadhan, S., Afifah, L., Adhi, S. R., & Irfan, B. (2023). Intensitas penyakit tanaman padi (*Oryza sativa* L.) varietas Ciherang pada aplikasi beberapa teknik pengendalian. *Jurnal Agrotech, 13*(2).

Safrizal, D., Handayani, R. S., Yusuf, M., & Fuzari, R. (2023). Pengaruh lama genangan dan varietas terhadap pertumbuhan dan hasil padi (*Oryza sativa* L.). *Jurnal Ilmiah Mahasiswa Agroekoteknologi, 2*(3), 61–66. https://doi.org/10.29103/jimatek.v2i3.15454

Septani, P., Yasin, F., & Dewi, C. (2025). Pengembangan aplikasi website rule-based diagnosa dan pengobatan pada tanaman padi menggunakan framework Laravel. *Jurnal Pengembangan Teknologi Informasi dan Ilmu Komputer, 9*(10). http://j-ptiik.ub.ac.id

Sukmawati, C. E., Pratama, A. R., Fitri, A., & Masruriyah, N. (2023). Prediksi penyakit blas pada tanaman padi dengan model spatial multi-criteria evaluation (SMCE) berbasis mobile. *Syntax: Jurnal Informatika, 12*(2).

Zhang, Y., Lin, X., Li, L., Piao, R.-H., Wu, S., Song, A., Gao, M., & Jin, Y. (2024). CRISPR/Cas9-mediated knockout of *Bsr-d1* enhances blast resistance of rice in Northeast China. https://doi.org/10.21203/rs.3.rs-3920499/v1

Multazam, Z. (2023). Kajian nilai pH tanah pada berbagai toposekuen dan kelas lereng yang berbeda pada lahan perkebunan karet rakyat di Kecamatan Pelepat Ilir, Kabupaten Bungo, Jambi. *Jurnal Riset Rumpun Ilmu Teknik, 2*(2), 179–188. https://doi.org/10.55606/jurritek.v2i2.2709

Zulkarnain, A., Nurhafitri, A., Tjong, D. H., & Idris, M. (2025). A literature review on transgenic crops in Indonesia and ASEAN countries: Transgenic research development and future prospects for food sovereignty. *Jurnal Biologi Tropis, 25*(4a), 574–582. By targeting coleoptile meristem tissue through partial wounding and Agrobacterium infiltration, and utilizing vacuum assistance to enhance infection efficiency, the CRISPR/Cas9 system can be delivered directly into plant tissue without requiring callus formation or in vitro regeneration. This protocol not only saves time and resources but is also better suited to local varieties that are often difficult to transform via tissue culture.

PCR analysis and sequencing of the *Bsr-d1 locus and marker genes (Cas9, hptII)* confirmed the presence of mutations in several transformed plants. This finding indicates that the CRISPR/Cas9 system successfully integrated and produced genetic modifications in the T2 generation.

These results support previous findings that genetic editing of Bsr-d1 can increase resistance to blast disease without disrupting important agronomic traits such as yield and grain quality. **Error! Reference source not found.**Therefore, this technique has great potential to be

integrated into a broader disease-resistant rice breeding program, especially for local varieties such as MR 297, which have important economic value but are susceptible to disease.

However, the obtained transformation efficiency (2%) is still moderate and requires further optimization. Several factors that can be improved include cocultivation conditions, infiltration duration, and Agrobacterium concentration. Additionally, Further evaluation of field resistance and other agronomic performance is necessary in subsequent generations (T3, T4) to ensure the stability of desired traits and their commercialization potential.

Figure 4. Transformed T2 plants (especially G20 and G23)

Overall, this study confirms that the CRISPR/Cas9-based gene-editing approach via in planta transformation is an effective, efficient, and applicable strategy for developing blast-resistant rice varieties that are free of tissue culture dependence.

4. Conclusion

This study demonstrates the superiority of Bsr-d1 gene editing in the rice variety MR 297 using the CRISPR/Cas9 system via Agrobacterium-mediated in planta transformation. This method is effective, achieving a transformation efficiency of 9%, and does not require tissue culture, making it simpler, faster, and more resource-efficient. Consequently, several mutant lines—particularly G20 and G23—exhibited significantly enhanced blast resistance compared to susceptible controls, without compromising plant growth or agronomic traits.

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References

Andriyani, Y., & Wiyono, S. (2021). Pola teknik budi daya dan sifat kimia tanah yang berhubungan dengan penyakit blas pada padi sawah. *Jurnal Fitopatologi Indonesia, 17*(2), 76-82. https://doi.org/10.14692/jfi.17.2.76-82

Efiddin, A., Sutrawati, M., Bustamam, H., Ganefianti, D. W., & Sipriyadi, S. (2021). Penyakit tungro pada tanaman padi (*Oryza sativa* L.) di Kecamatan Taba Penanjung: Insidensi penyakit dan deteksi virus secara molekuler. *Jurnal Ilmu-Ilmu Pertanian Indonesia, 23*(1), 37–45. https://doi.org/10.31186/jipi.23.1.37-45

Kurrata, G., Kuswinanti, T., & Nasruddin, A. (2021). Keparahan penyakit blas *Pyricularia oryzae* dan analisis gen virulensi menggunakan metode sequence characterized amplified region. *Jurnal Fitopatologi Indonesia, 17*(1), 19-27. https://doi.org/10.14692/jfi.17.1.19-27

Novantara, P., Firmansyah, R. L., & Arismawati, M. (2025). Deteksi hama penyakit daun padi dengan menggunakan teknik optimasi deep learning convolutional neural network. *Bit-Tech, 7*(3), 975-983. https://doi.org/10.32877/bt.v7i3.2284

Yusliana, R., Wahyuni, T., & Universitas Muhammadiyah Makassar. (2024). Sistem pakar tentang mendiagnosa penyakit pada tanaman padi menggunakan metode forward chaining. *Arus Jurnal Sains dan Teknologi (AJST), 2*(2). http://jurnal.ardenjaya.com/index.php/ajst

Ramadhan, S., Afifah, L., Adhi, S. R., & Irfan, B. (2023). Intensitas penyakit tanaman padi (*Oryza sativa* L.) varietas Ciherang pada aplikasi beberapa teknik pengendalian. *Jurnal Agrotech, 13*(2).

Safrizal, D., Handayani, R. S., Yusuf, M., & Fuzari, R. (2023). Pengaruh lama genangan dan varietas terhadap pertumbuhan dan hasil padi (*Oryza sativa* L.). *Jurnal Ilmiah Mahasiswa Agroekoteknologi, 2*(3), 61-66. https://doi.org/10.29103/jimatek.v2i3.15454

Septani, P., Yasin, F., & Dewi, C. (2025). Pengembangan aplikasi website rule-based diagnosa dan pengobatan pada tanaman padi menggunakan framework Laravel. *Jurnal Pengembangan Teknologi Informasi dan Ilmu Komputer, 9*(10). http://j-ptiik.ub.ac.id

Sukmawati, C. E., Pratama, A. R., Fitri, A., & Masruriyah, N. (2023). Prediksi penyakit blas pada tanaman padi dengan model spatial multi-criteria evaluation (SMCE) berbasis mobile. *Syntax: Jurnal Informatika, 12*(2).

Zhang, Y., Lin, X., Li, L., Piao, R.-H., Wu, S., Song, A., Gao, M., & Jin, Y. (2024). CRISPR/Cas9-mediated knockout of *Bsr-d1* enhances blast resistance of rice in Northeast China.

https://doi.org/10.21203/rs.3.rs-3920499/v1

Multazam, Z. (2023). Kajian nilai pH tanah pada berbagai toposekuen dan kelas lereng yang berbeda pada lahan perkebunan karet rakyat di Kecamatan Pelepat Ilir, Kabupaten Bungo, Jambi. *Jurnal Riset Rumpun Ilmu Teknik*, *2*(2), 179–188. https://doi.org/10.55606/jurritek.v2i2.2709

Zulkarnain, A., Nurhafitri, A., Tjong, D. H., & Idris, M. (2025). A literature review on transgenic crops in Indonesia and ASEAN countries: Transgenic research development and future prospects for food sovereignty. *Jurnal Biologi Tropis, 25*(4a), 574–582.