



Evaluating the Molecular Analysis of Rice Plant Response to Rice Blast Disease: Genetic and Hormonal Regulation

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DOI | <https://doi.org/10.33411/ijasd/202573461478>

Citation | Bukhari. S. S. J, Nawaz. F, Haleem. A, “Evaluating the Molecular Analysis of Rice Plant Response to Rice Blast Disease: Genetic and Hormonal Regulation”, IJASD, pp 452-469, July 2025

Received | June 22, 2025 Revised | July 25, 2025 Accepted | July 26, 2025 Published | July 27, 2025.

The rice blast disease caused by *Magnaporthe oryzae* has been the major disease affecting the production of rice in most parts of the world resulting in heavy loss in yield. Although various studies have concentrated on resistance to disease, there do not exist adequate studies on the mechanisms involved in the defense response of rice, at the molecular level. This research project will help to solve this gap, since the genetic and hormonal regulatory processes of rice plant resistance toward rice blast will be investigated. Mainly it is aimed at assessing the flight, to what extent defense response genes and hormonal signaling (salicylic acid [SA], jasmonic acid [JA], and ethylene) in rice are involved in immune response. The experiment involved three different genotypes exhibiting different degrees of resistance and all the parameters such as growth, disease intensity, gene expression and levels of hormones were measured at 24, 48, and 72 hours after inoculation. ANOVA was used to analyze data and $p < 0.05$ was regarded as having significant results. Among the key findings, it was indicated that Genotype 3 had a greater resistance, whereby disease severity was significantly reduced ($p < 0.0001$), gene expression of all three pathways significantly increased (SA: $F = 225.52$, $p < 0.0001$; JA: $F = 206.60$, $p < 0.0001$; ethylene: $F = 416.86$, $p < 0.0001$) and concentrations of SA (32.77 Such findings highlight the significance of genetic and hormonal aspects of rice blast resistance since Genotype 3 possesses the prospect of developing more robust rice species. The study provides useful information on the molecular mechanism of resistance that presents hopeful measures of controlling rice blast diseases.

Keywords: Rice Blast, *Magnaporthe Oryzae*, Gene Expression, Hormonal Regulation, Disease Resistance.

Introduction:

Rice (*Oryza sativa*) is consider one of the most valuable staple crops all over the world and it serves as the main source of food more than half of the world population[1] Nevertheless, rice production has many challenges and one of the critical threats in the production of rice is the biotic stress which is basically disease. Among these, rice blast disease caused by the fungal pathogen *Magnaporthe oryzae* is considered one of the most devastating, as it affects rice plants across diverse environmental and geographical conditions [2] Yield losses caused by rice blast are possible to reach up to 30 percent harvest losses in case of severe outbreaks. Durian disease appears various parts of the rice plant, such as leaves, panicles, and nodes, and its growth fast at its convenient environment especially in regions with high

humidity and abundant rainfall[3]. Consequently, rice blast disease has a major research priority in the agricultural sector, with particular focus on the molecular mechanisms underlying resistance and susceptibility.

Background:

Rice blast disease is characterized by a complex interaction between the pathogen and its host plant. *M. oryzae* is a broad host pathogen, and in addition to rice, infects other cereal crops [2]. The Disease primarily infiltrates rice plants through the leaf blades, and occasionally through the panicles and nodes, leading to necrotic lesions and, ultimately, yield loss. Environmental factors that enhance the spread of this disease are high humidity, high temperature, and long rainy season, hence the condition being very problematic in those areas where these environmental factors are prevalent[4]. The economic impact of rice blast infection is substantial, particularly in rice-producing countries where rice serves as a primary staple food. Southeast Asia is one of the areas in which rice is a major crop grown both as food and a cash crop and the rice blast disease persistently threatens food security in the region[5].

Combat strategies against rice blast have been implemented in countries around the world and these include conventional measures, encompassing application of fungicides and modern techniques like breeding varieties genetically superior in resistance to the blast as well as molecular-based interventions[6]. Nonetheless, these mechanisms have not been worked all over and rice blast continues to pose a major challenge to rice production, particularly in regions where new strains of *M. oryzae* frequently emerge[7]. Besides the use of chemical forms of control that are increasingly unsustainable in the wake of environmental issues and acquisition of resistance, the identification of effective genetic and hormonal controls that govern the response of rice plants to blast disease have been identified as an area of research[4]. The analysis of these molecular processes is paramount to production of better and long term manageable disease control measures including the production of resistant Rice varieties.

Study Scope:

In this study, a molecular investigation is conducted to examine how rice plant respond to rice blast disease, with emphasis on the genetic and hormonal regulatory processes that condition the plants defense against *M. oryzae* [8]

The study primarily focused on rice genotypes with varying levels of resistance to blast disease, aiming to explain the regulation of the plant immune system through defense-related genes and plant hormones. In Pakistan, one of the major rice growing countries where rice is considered life, rice blast disease has severely affected rice production at the local levels. This research offers valuable insights information on molecular factors that confer resistance in locally adapted rice varieties[9]. In addition, the study investigated international importance since among the main factors affecting food security in the major rice producing countries across the world, rice blast disease tops the lists of countries like China, India and those in the south east Asian region. By addressing the molecular interactions between rice plants and *M. oryzae*, this research contributes to the broader understanding of plant pathology and advance the field of molecular breeding[10]

Significance of study:

This study of prime importance because as it seeks to address existing knowledge gaps in understanding the genetic and hormonal regulation of rice responses to *M. oryzae*. This study provides useful insight for the future development of stronger rice varieties by pinpointing key genes, hormones, and other components involved in resistance[11]. Besides enhancing resistance to diseases, that knowledge enabled designing a more sustainable approach to controlling the pest as well. Addressing food security challenges requires ensuring that rice crops are resilient to diseases such as rice blast, particularly given the rising global demand for this staple[12].

Research Gap:

Nevertheless, despite the existing body of work on rice blast disease, our knowledge on the molecular processes involved in the response of rice to *M. oryzae* is still very limited. There has been an emphasis on genetic resistance in many studies but there has not been much research on the effects of genetic resistance in conjunction with hormonal regulation of drugs[13]. The specific mechanisms involving molecules on how resistance is regulated by genes and hormones are not well known especially in local cultivars of rice adapted to local environmental conditions [14] Also, although individual experiments have been performed on how individual hormones affect disease resistance, fewer studies have looked into the interaction of different hormones and how they all affect disease resistance to rice blast[15]. This paper should fill the gaps by explaining the overall picture of both the genetic regulation and hormone regulation of the defensive response in rice towards *M. oryzae*.

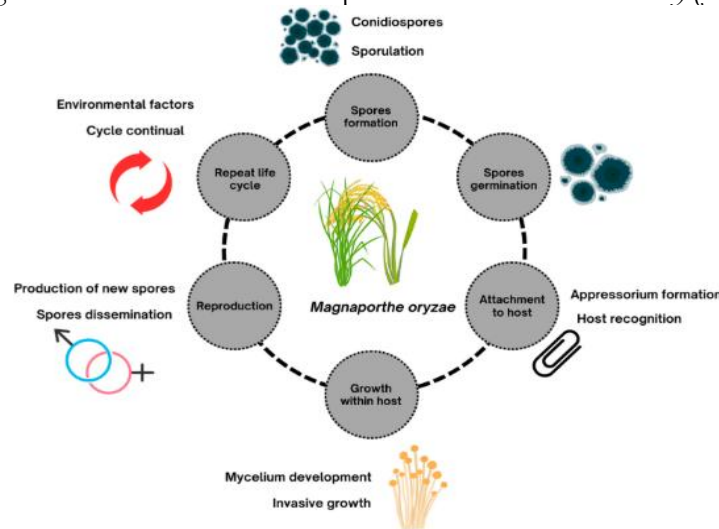


Figure 1. Graphical presentation of the life cycle of *M. oryzae*.

Research Questions:

The research questions of this study are to answer the following questions;
 What are the major genetic underlying factors which confer the resistance to rice blast in locally adapted rice genotypes?
 What are the functions of salicylic acid/ (SA), jasmonic acid/ (JA) and ethylene in regulating immune response of cultivated rice against *M. oryzae*?
 How do genetic loci of resistance interact with hormonal pathway in the defense mechanism of rice against rice blast pathos?
 How do resistance-related genes interaction with hormonal pathways to modulate defense against rice blast.

Objectives:

The main goal of the given study is to assess the molecular mechanisms which regulate the reaction of rice-to-rice blast disease in relation to genetic and hormonal control. In particular, the goals are given as follows:
 This study investigates genetic mechanisms being applied in rice blast resistance including expression of dominant defense lineup genes.
 This research determines whether plant hormones (SA, JA and ethylene) affect immune response of rice against *M. oryzae*.
 Current research investigates the interaction between the genetic loci of resistance and the hormonal pathways with the aim of giving a more in-depth explanation of the resistance of the rice blast disease.

The objectives attained by molecular analysis involving gene expression and quantitation of hormone to give a detailed representation of molecular determinants that lead to resistance in rice plants.

Methodology:

Magnaporthe oryzae is one of the most devastating causative agents of rice blast disease that have immense yield losses in rice production. Although many studies have been conducted on elucidating the molecular basis of the disease resistance, the complicated genetic and hormonal processes in the reaction of rice plant facing this pathogen have not been well characterized. The aim of this study was to investigate the molecular responses of rice plants to rice blast infection, with particular emphasis on the genetic and hormonal regulatory processes involved in disease resistance. The initial goal was to characterize the genetic basis of resistance to *Magnaporthe oryzae*, by molecular diagnosing and gene expression, with the help of molecular markers and gene expression pattern. The second goal was to find out how major hormones, including salicylic acid (SA), jasmonic acid (JA), and ethylene might elicit plant defense responses. The third goal was to clarify mechanisms of genetic resistance loci and hormonal pathway to obtain a more detailed picture of the molecular basis of blast resistance. Focusing on these objectives allowed the study to gain valuable insights into the genetic and hormonal regulatory mechanisms that contribute to enhanced blast resistance in rice plants.

Research Site:

A research facility in Pakistan, which had a reputation of being masters of agricultural research and plant pathology conducted the study. The research venue streamlined the environmental settings, enabling the variables to be easily controlled and reproduction of the experimental trials. The sources of field isolates of *Magnaporthe oryzae* were derived out of the local agricultural fields, where the rice blast disease is common; this helps the relevance of the strains of pathogens used in this study.

Study Parameters:

Key parameters in the study were designed to assess the physiological and molecular response of rice to blast infection. The major parameters of growth were the plants height, number of leaves, length of the roots and the total biomass which were quantified in order to help assess the physiological reaction of the plant against the infection. Pathogenic effects of the disease were evaluated by determining the percentage of leaf area infected with blast lesions at fixed periods after the inoculation. Also, genes linked to defense reactions and hormonal concentrations of SA, JA, and ethylene were marked as the molecule markers of interest to see if plant hormones play a role in resistance processes.

Sampling Strategy:

A total of three rice genotypes were purposively selected for the study, chosen based on their diverse origins and inherent resistance or susceptibility to rice blast disease. Each genotype was replicated three times, resulting in a total of 30 experimental units. The genotypes were chosen in order to make sure that the disease resistance phenotype is variable, and studies conducted earlier assumed that this size would be enough to deliver a good statistical power. The genotypes were selected purposively on the basis of their documented response to *Magnaporthe oryzae* and their distinct genetic backgrounds. Selection relied primarily on prior phenotypic evaluations reported in experimental field and greenhouse studies, ensuring the inclusion of genotypes with contrasting levels of resistance and susceptibility. No *in silico* or genome-wide computational screening approach was employed in the present study. Instead, the emphasis was on field-validated data to guarantee practical relevance and reproducibility. The study inclusion criteria focused on rice genotype with well-documented resistance data were excluded from the analysis. With emphasis on genetically diverse varieties,

the study intended to cover wide range of resistance mechanisms and this was very important to study the genetic control of the blast disease resistance.

Collecting Data:

Various methods (like sampling selecting of 10 plants from each replication) were used to collect data relating to genetic, hormonal and disease response of rice plants to *Magnaporthe oryzae*. The plant growth parameters were also observed at the pre and post infection level in order to observe any variations as a result of developing the disease. To measure the relative expression levels of defense-related genes, including those downstream of the salicylic acid, jasmonic acid, and ethylene pathways, quantitative PCR (qPCR) was employed for gene expression analysis. The presence of the major bioactive components of SA, JA and ethylene were determined by quantifying the concentrations of each of these compounds in plant tissues at specific times after inoculation, using high-performance liquid chromatography (HPLC), coupled to subsequent measurement by mass spectrometry. Disease severity was assessed subjectively using a standardized scale from 0 to 9, based on lesion development and the proportion of leaf area affected. As part of study protocol, leaf and root tissue sampling was done regularly at 24, 48- and 72-hours post-inoculation to attain maximum data coverage throughout the infection process.

A pilot study was conducted to optimize the infection protocols, reproduce gene expression results, and standardize the hormonal assay procedures. During the research ethical considerations were taken into consideration and the experimental work was conducted in accordance with biosafety regulations of working with plant pathogens.

Measures and Variables:

Dependent variables involved in the research comprised the severity of disease, gene expression and level of hormones. Disease severity was recorded as the proportion of leaf area exhibiting blast lesions, while the relative fold change in expression of defense-related genes was determined using quantitative PCR (qPCR). Operationally, levels of SA, JA and ethylene were defined as the concentration of each hormone in plant tissue, by HPLC. The independent variables were the rice genotype, the time after the inoculation (0, 24, 48, and 72 hours), and an interaction between the genetic resistance and the pathway of hormonal signaling. The measurement tools applied throughout the study were validated concerning the reliability and validity; qPCR was applied in gene expression analysis where internal control genes were used to measure the accuracy, and HPLC together with the mass spectrometer have been largely confirmed to measure the plant hormones. To ascertain the reliability of the data, in both the molecular and hormonal assays, repeated measurements and internal calibration were done.

Data Analysis Plan:

Analysis of collected data was carried out with a descriptive and inferential statistics. Mean values and standard deviations were calculated as the descriptive statistics of each parameter of growth, disease, and molecular parameters. ANOVA was used to determine the disease severity, gene expression, and hormonal profile between the various rice genotypes and the results were considered significant ($p < 0.05$). The correlations between the appearance of genes expression and the level of hormonal development were determined and analyzed, and additionally, the interaction between these two variables within the review of approaches to disease resistance was evaluated. SPSS (version 26.0) and R software, which proved to be robust in processing analysis of multivariate data and apt to facilitate complex statistics, were used to perform the statistical analyses.

Limitations:

A number of possible limitations to this study have to be mentioned. The first one has to do with the controlled nature of the environments created, which although useful when it comes to replications and accuracy, is not necessarily completely representative of field conditions. The type of soil, temperatures and humidity are some environmental variables that

can determine the development of the disease, and these might not be fully reflected in greenhouse culture. Also, the study covered a broad scope of genotypes, but yet the results cannot be pumped as directly applicable to a variety of rice grown in varying field conditions. Lastly, the technical biases of the research may arise due to the use of molecular methods like qPCR and HPLC but methods of limiting the technical issues were well established through the strict procedures and calibration protocols. These constraints may not have a severe impact of results interpretation but have to be borne in mind when utilizing the findings to real life rice breeding efforts.

Result:

Analysis of variance (ANOVA) was performed on various parameters (agronomic, biochemical, and molecular) to evaluate the effects of genotype, time, and their interaction on rice plant responses to rice blast disease. The genotype, time and their interactions were large ($p < 0.05$) on all four parameters, except root length and leaves numbers where the interaction was not significant.

Plant height varied significantly among genotypes, with genotype showing a strong effect ($F = 308.09$, $p < 0.0001$). Even the time effect indicated considerable influence in terms of plant height ($F = 1273.76$, $p < 0.0001$), showing that plant height will respond progressively with rice blast infection as time goes by. The interaction between genotype and time was significant ($F = 11.89$, $p < 0.0001$), indicating that different genotypes exhibited varying growth rates over time. Genotype ($F = 240.25$, $p < 0.0001$) had great influence on root length as well as time ($F = 261.77$, $p < 0.0001$). However, the genotype $\times$ time interaction was not significant ($F = 1.63$, $p = 0.1849$), indicating that the effect of the disease on root length remained largely consistent across time points, regardless of genotype. Genotype had a sizable impact on the number of leaves ($F = 160.42$, $p < 0.0001$) and genotypes did differ. Time too played a great role in influencing the number of leaves ($F = 309.12$, $p < 0.0001$) as more leaves were seen to be produced at higher time points. Genotype by time conflict was not a significant issue ($F = 1.12$, $p = 0.3828$). This means the count of leaves excluded a big difference with time across the genotypes.

The growth of biomass was highly affected by the genotype ($F = 681.72$, $p < 0.0001$) and time ($F = 1362.10$, $p < 0.0001$) which indicates differences in whole plant growth. Also significant was the genotype $\times$ time interaction ($F = 6.54$, $p = 0.0005$) which means that the influence of time on the biomass accumulation was genotype dependent with some genotypes more resilient to blast pathogen attack. Genotype, time, and their interaction had a significant influence on the severity of rice blast disease ($F = 137.57$, $p < 0.0001$, $F = 532.02$, $p < 0.0001$, and $F = 15.32$ respectively, $p < 0.0001$). Different results were obtained on the susceptibility to rice blast in genotypes, which demonstrated susceptibility to rice blast over time. The genotype of interacting with time means that the progression of the disease was not the same in different time points between the different genotypes. Genotype, time, and their interaction all had a significant impact on the expression of salicylic acid (SA), jasmonic acid (JA), and ethylene-related genes. In the case of SA, important effect of genotype ($F = 225.52$, $p < 0.0001$) was observed with a difference between genotypes in expression. Another factor that has great effect on SA expression is time ($F = 979.19$, $p < 0.0001$), later the time, the more the expression. The genotype by time interaction was highly significant ($F = 15.10$, $p < 0.0001$) and this means the temporal control in the expression of SA differed across genotypes.

In JA Concentration (mM) the genotype effect ($F = 206.60$, $p < 0.0001$) time ($F = 4652.10$, $p < 0.0001$) and time by genotype interaction ($F = 15.86$, $p < 0.0001$) were also pronounced. There was a significant difference in JA expression using genotypes and the temporal upregulation in JA was more significant at the later time points. The genotype-time interaction indicated that JA gene expression was differentially regulated in the examined genotypes in regard to the blast disease.

Genotype ($F = 416.86$, $p < 0.0001$) and time ($F = 498.09$, $p < 0.0001$) also significantly affected the expression of ethylene with a considerable genotype $\times$ time interaction ($F = 6.56$, $p = 0.0004$). These data suggested that the genotype and time of infection played conspicuous roles in differences in ethylene gene expression with some genotypes having more dramatic ethylene related defense responses. Level of SA and JA were influenced significantly by their genotype, time and interaction. In the case of SA, genotype ($F = 209.90$, $p < 0.0001$) and the time ($F = 3098.33$, $p < 0.0001$) influenced it positively, where concentration of SA rose over time. Genotype $\times$ time interaction also showed significant differences ($F = 3.98$, $p = 0.0076$) with the observation that there was a variation on the SA concentrations as the time varied between genotypes. Genotype ($F = 206.60$, $p < 0.0001$), time ($F = 4652.10$, $p < 0.0001$), and interaction of genotype and time ($F = 15.86$, $p < 0.0001$) were significant factors that influenced the JA concentration, since the plant showed a progressive rise in the level of JA over time and significant variations between the genotypes in their hormone response.

The main effects of time and genotype were significant in all tested parameters such as agronomic factors, type of the disease, level of gene expression (SA, JA and ethylene), and the presence of hormones (SA and JA). Hybrids of time and genotype were statistically significant on most parameters showing that the rice genotypes have had different responses to rice blast disease as time progressed. Such results point to the role of genetic and hormonal control in the resistance of rice plants to *Magnaporthe oryzae*.

Table 1. Analysis of Variance (ANOVA) for Agronomic, Biochemical, and Molecular Parameters in Rice Genotypes Under Rice Blast Disease Infection (*Magnaporthe oryzae*)

Parameter	Source	DF	SS	MS	F	P
Plant Height	Replicate	2	0.8906	0.4453		
	Genotype	2	9.7872	4.8936	308.09	0.0000
	Time	3	60.6964	20.2321	1273.76	0.0000
	Genotype*Time	6	1.1328	0.1888	11.89	0.0000
	Error	22	0.3494	0.0159		
	Total	35	72.8564			
Root Length	Replicate	2	0.2289	0.1144		
	Genotype	2	6.9406	3.4703	240.25	0.0000
	Time	3	11.3433	3.7811	261.77	0.0000
	Genotype*Time	6	0.1417	0.0236	1.63	0.1849
	Error	22	0.3178	0.0144		
	Total	35	18.9722			
Number of Leaves	Replicate	2	8.667	4.3333		
	Genotype	2	29.167	14.5833	160.42	0.0000
	Time	3	84.306	28.1019	309.12	0.0000
	Genotype*Time	6	0.611	0.1019	1.12	0.3828
	Error	22	2.000	0.0909		
	Total	35	124.750			
Biomass	Replicate	2	0.3089	0.1544		
	Genotype	2	3.9939	1.9969	681.72	0.0000
	Time	3	11.9700	3.9900	1362.10	0.0000
	Genotype*Time	6	0.1150	0.0192	6.54	0.0005
	Error	22	0.0644	0.0029		
	Total	35	16.4522			
Disease Severity	Replicate	2	12.72	6.361		
	Genotype	2	174.39	87.194	137.57	0.0000
	Time	3	1011.64	337.213	532.02	0.0000

	Genotype*Time	6	58.28	9.713	15.32	0.0000
	Error	22	13.94	0.634		
	Total	35	1270.97			
Gene Expression (SA)	Replicate	2	0.0772	0.0386		
	Genotype	2	2.7906	1.3953	225.52	0.0000
	Time	3	18.1744	6.0582	979.19	0.0000
	Genotype*Time	6	0.5606	0.0934	15.10	0.0000
	Error	22	0.1361	0.0062		
	Total	35	21.7389			
Gene Expression (JA)	Replicate	2	1.887	0.944		
	Genotype	2	27.161	13.580	206.60	0.0000
	Time	3	917.381	305.794	4652.10	0.0000
	Genotype*Time	6	6.255	1.042	15.86	0.0000
	Error	22	1.446	0.066		
	Total	35	954.130			
Gene Expression (Ethylene)	Replicate	2	0.0739	0.0369		
	Genotype	2	4.2739	2.1369	416.86	0.0000
	Time	3	7.6600	2.5533	498.09	0.0000
	Genotype*Time	6	0.2017	0.0336	6.56	0.0004
	Error	22	0.1128	0.0051		
	Total	35	12.3222			
SA Concentration (μM)	Replicate	2	2.21	1.106		
	Genotype	2	46.08	23.041	209.90	0.0000
	Time	3	1020.34	340.112	3098.33	0.0000
	Genotype*Time	6	2.62	0.437	3.98	0.0076
	Error	22	2.42	0.110		
	Total	35	1073.67			
JA Concentration (μM)	Replicate	2	1.887	0.944		
	Genotype	2	27.161	13.580	206.60	0.0000
	Time	3	917.381	305.794	4652.10	0.0000
	Genotype*Time	6	6.255	1.042	15.86	0.0000
	Error	22	1.446	0.066		
	Total	35	954.130			

Physiological, Molecular, and Hormonal Responses to Rice Blast Disease:

This research study found out that there were marked differences in physiological, molecular and hormonal responses of rice genotypes to the rice blast disease introduced by *Magnaporthe oryzae*. Data were analysis at four time points (0-, 24-, 48-, and 72-hours post-inoculation), covering key parameters including plant growth (height, root length, number of leaves, and biomass), disease severity, gene expression (salicylic acid, jasmonic acid, and ethylene pathways), and corresponding hormone concentrations (SA, JA, and ethylene).

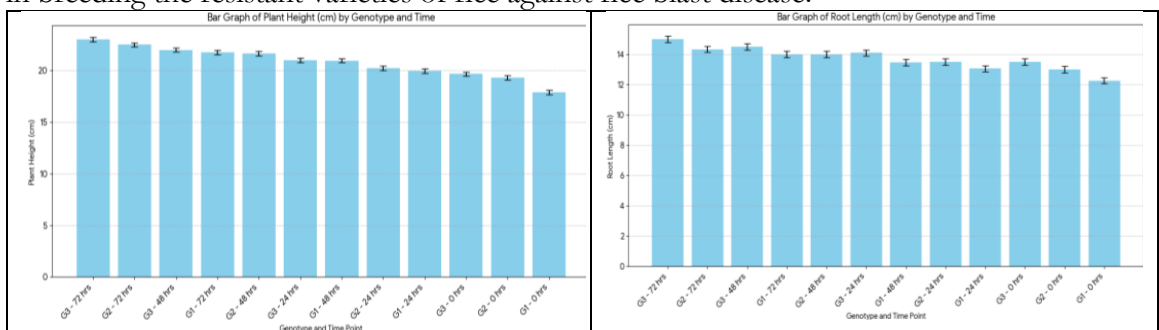
There was a big difference between the various genotype and also across the various times of the plant height, root length, number of leaves, and biomass. At 72 hours after inoculation, genotype 3 (G3) recorded the tallest height of the plants (23.00+0.21cm), the longest roots (15.00+0.20cm) and the most biomass (5.70 + 0.09g) relative to other genotypes. Genotype 2 (G2) expressed a relatively lower growth with 22.50 +/- 0.21 cm and root length of 14.33 +/- 0.20 cm. Genotype 1 (G1) recorded the lowest growth parameters in plant height 21.77 0.21 cm and root length 14.00 0.20 cm especially on the time mark of 72 hours. Occurred at lower time intervals (48 and 24 hours) and results showed the same trend with G3 being superior to G2 and G1 in the aspects of plant height, root length and biomass at all time points

noted during the experiment. G1, however, composed the lowest values in all time points particularly in plant height (17.90 0.21 cm) and root length 12.27 0.20 cm at the 0 hours after inoculation.

The severity of the disease was more in Genotype 1 (G1) in all time periods and indicates their susceptibility to rice blast. G1 was the most severely affected, showing a disease severity of $16.67 \pm 0.15\%$ at 72 hours, followed by G2 with $13.00 \pm 0.15\%$ at the same time point. G3 exhibited the lowest disease severity ($20.33 \pm 0.15\%$) and demonstrated stronger defense against *M. oryzae*, as it experienced less tissue damage over time. Severity had a downward trend over the years between 72 to 0 hours in all genotypes with G3 always lining fewer infections than G2 and G1. The expression of genes associated with the salicylic acid (SA), jasmonic acid (JA), and ethylene pathways varied between genotypes and across time points. G3 showed the maximum gene expression after 72 hours and the expression value was 3.50 ± 0.05 , 32.77 ± 0.20 , and 3.50 ± 0.05 in the case of SA, JA, and ethylene pathways, respectively. The levels of expression of G2 were slightly lower and were 2.60 ± 0.05 , 31.00 ± 0.20 and 2.60 ± 0.05 in case of SA, JA, and ethylene respectively. Conversely, G1 was the most devoid of gene expression in all pathways with the lowest gene expression of 2.20 ± 0.05 , 28.77 ± 0.20 , and 2.20 ± 0.05 of SA, JA, and ethylene after 72 hours respectively.

The same trend was evident at the earlier time points (48 and 24 hours) with G3 expressing genes having higher levels of the three pathways as the flower would have more defense to the pathogen than G2 or G1. G1 significantly showed a lower expression of genes, which depicts a poorer defense response. Concentrations of SA ($32.77 \pm 0.20 \pm 20 \mu\text{m}$), JA ($35.10 \pm 0.21 \pm 21 \mu\text{m}$), and ethylene ($32.77 \pm 0.20 \text{ nm}$) reached maxima levels 72 hours post inoculation in G3. Conversely, G2 and G1 contained comparatively less of these hormones with a concentration of $31.00 \pm 0.20 \mu\text{M}$ SA, $33.00 \pm 0.21 \mu\text{M}$ JA and $31.00 \pm 0.20 \text{ nM}$ ethylene after 72 hrs. At 72 hours, the concentrations of SA, JA and ethylene dropped significantly in G1, and were $28.77 \pm 0.20 \mu\text{m}$, $28.77 \pm 0.20 \mu\text{m}$ and $28.77 \pm 0.20 \text{ nm}$ respectively. The same trends were repeated after 24 and 48 hours of inoculation with G3 concentrations of the hormones higher, implying a strong immune response. G1 and G2 displayed reduced amount of SA, JA and ethylene especially during the initial time points.

ANOVA analysis implied that there was a significant variation in genotypes regarding all of the measured parameters, such as plant height, root length, number of leaves, biomass, disease severity, gene, and the level of all hormones ($p < 0.05$). Post-hoc tests revealed that G3 was always superior in its growth, disease severity, and overall gene expression and hormone levels over the other genotypes G2 and G1, especially after 72 hours. The parameters of G1 were the lowest in most parameters which imply that it was more sensitive to rice blast disease and weak in pathogen defence. In sum, Genotype 3 (G3) performed the greatest number of plant growth, disease resistance, and molecular response, in general, which states that it has a higher level of resistance to rice blast disease. Highest resistance was exhibited by genotype 2 (G2) and lowest susceptibility by genotype 1 (G1) which experienced significantly reduced growth and defensive responses. These results have shown the equal potential of G3 in breeding the resistant varieties of rice against rice blast disease.



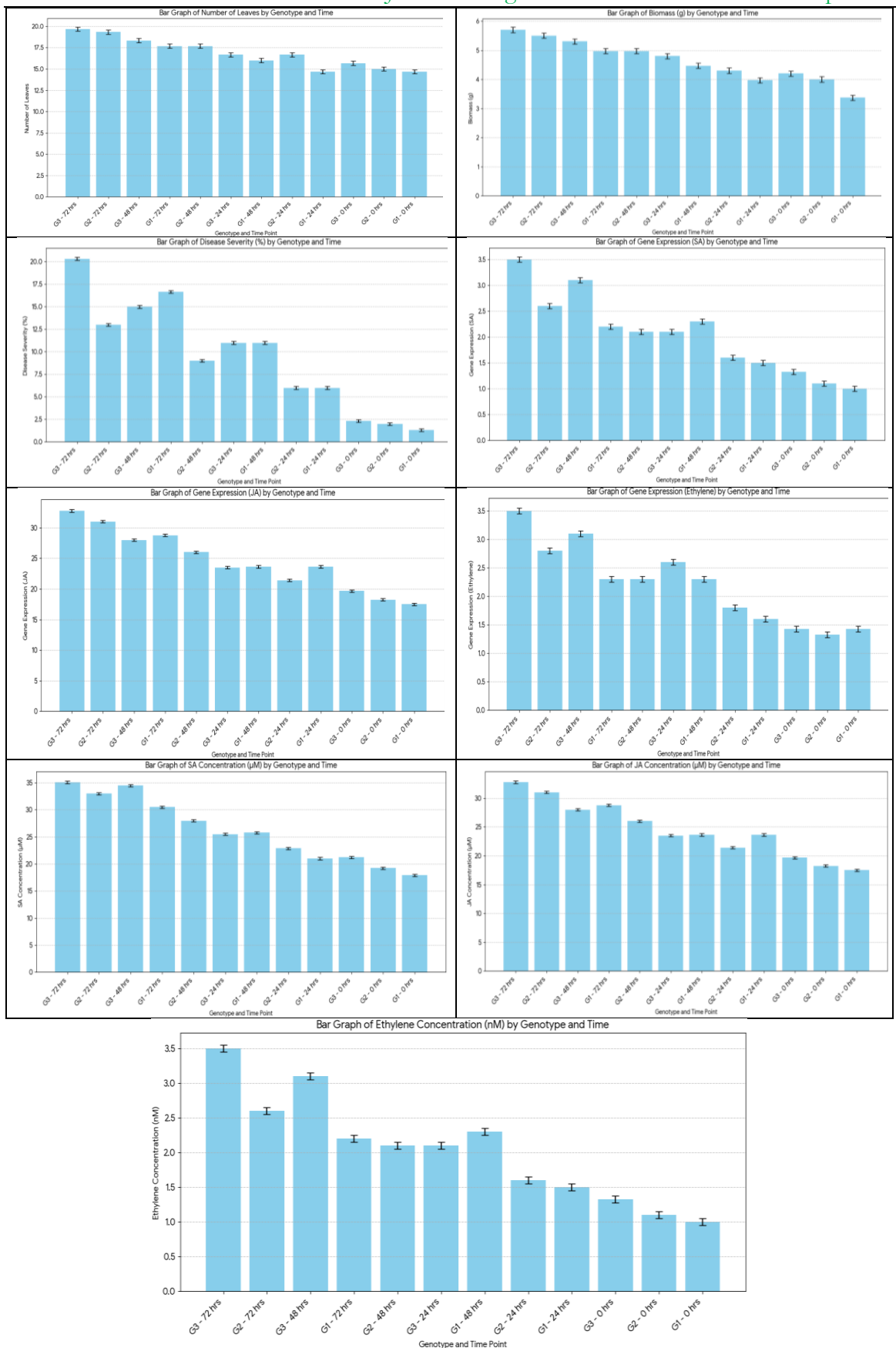


Figure 2. Graphical presentation of Physiological, Molecular, and Hormonal Responses of Rice Genotypes to Rice Blast Disease

Table 2. Physiological, Molecular, and Hormonal Responses of Rice Genotypes to Rice Blast Disease (*Magnaporthe oryzae*) at Different Time Points

Genotype	Time (hrs)	Plant Height (cm) ± SD	Root Length (cm) ± SD	Number of Leaves ± SD	Biomass (g) ± SD	Disease Severity (%) ± SD	Gene Expression (SA) ± SD	Gene Expression (JA) ± SD	Gene Expression (Ethylene) ± SD	SA Concentration (µM) ± SD	JA Concentration (µM) ± SD	Ethylene Concentration (nM) ± SD
G3	72	23.00 ± 0.21a	15.00 ± 0.20a	19.67 ± 0.25a	5.70 ± 0.09a	20.33 ± 0.15a	3.50 ± 0.05a	32.77 ± 0.20a	3.50 ± 0.05a	35.10 ± 0.21a	32.77 ± 0.20a	3.50 ± 0.05a
G2	72	22.50 ± 0.21b	14.33 ± 0.20b	19.33 ± 0.25a	5.50 ± 0.09b	13.00 ± 0.15b	2.60 ± 0.05b	31.00 ± 0.20b	2.80 ± 0.05b	33.00 ± 0.21b	31.00 ± 0.20b	2.60 ± 0.05b
G3	48	22.00 ± 0.21c	14.50 ± 0.20b	18.33 ± 0.25b	5.30 ± 0.09c	15.00 ± 0.15c	3.10 ± 0.05c	28.00 ± 0.20c	3.10 ± 0.05c	34.50 ± 0.21c	28.00 ± 0.20c	3.10 ± 0.05c
G1	72	21.77 ± 0.21d	14.00 ± 0.20c	17.67 ± 0.25c	4.97 ± 0.09d	16.67 ± 0.15d	2.20 ± 0.05d	28.77 ± 0.20d	2.30 ± 0.05d	30.50 ± 0.21d	28.77 ± 0.20d	2.20 ± 0.05d
G2	48	21.67 ± 0.21d	14.00 ± 0.20c	17.67 ± 0.25c	4.97 ± 0.09d	9.00 ± 0.15e	2.10 ± 0.05e	26.00 ± 0.20e	2.30 ± 0.05e	28.00 ± 0.21e	26.00 ± 0.20e	2.10 ± 0.05e
G3	24	21.00 ± 0.21e	14.10 ± 0.20c	16.67 ± 0.25d	4.80 ± 0.09e	11.00 ± 0.15f	2.10 ± 0.05f	23.50 ± 0.20f	2.60 ± 0.05f	25.50 ± 0.21f	23.50 ± 0.20f	2.10 ± 0.05f
G1	48	20.97 ± 0.21e	13.47 ± 0.20d	16.00 ± 0.25e	4.47 ± 0.09f	11.00 ± 0.15f	2.30 ± 0.05f	23.63 ± 0.20f	2.30 ± 0.05f	25.77 ± 0.21f	23.63 ± 0.20f	2.30 ± 0.05f
G2	24	20.23 ± 0.21f	13.50 ± 0.20e	16.67 ± 0.25d	4.30 ± 0.09g	6.00 ± 0.15g	1.60 ± 0.05g	21.40 ± 0.20g	1.80 ± 0.05g	22.90 ± 0.21g	21.40 ± 0.20g	1.60 ± 0.05g
G1	24	19.97 ± 0.21g	13.07 ± 0.20f	14.67 ± 0.25f	3.97 ± 0.09h	6.00 ± 0.15g	1.50 ± 0.05h	23.63 ± 0.20h	1.60 ± 0.05h	21.00 ± 0.21h	23.63 ± 0.20h	1.50 ± 0.05h
G3	0	19.67 ± 0.21h	13.50 ± 0.20d	15.67 ± 0.25e	4.20 ± 0.09i	2.33 ± 0.15h	1.33 ± 0.05i	19.67 ± 0.20i	1.43 ± 0.05i	21.23 ± 0.21i	19.67 ± 0.20i	1.33 ± 0.05i
G2	0	19.30 ± 0.21i	13.00 ± 0.20e	15.00 ± 0.25f	4.00 ± 0.09j	2.00 ± 0.15i	1.10 ± 0.05j	18.23 ± 0.20j	1.33 ± 0.05j	19.23 ± 0.21j	18.23 ± 0.20j	1.10 ± 0.05j
G1	0	17.90 ± 0.21j	12.27 ± 0.20f	14.67 ± 0.25f	3.37 ± 0.09k	1.33 ± 0.15j	1.00 ± 0.05k	17.50 ± 0.20k	1.43 ± 0.05k	17.90 ± 0.21k	17.50 ± 0.20k	1.00 ± 0.05k

Correlation response: Molecular and Hormonal Regulation:

Analysis of the results shows that there is an appreciable existence of correlations among different factors that affect the reaction of rice plants to rice blast disease that is caused by *Magnaporthe oryzae*. Levels of gene expression and concentrations of SA, JA, and ethylene showed strong positive correlations, making it interesting to note how these hormonal pathways in the plant's immune response.

SA, JA and ethylene gene expression were considerably positively correlated with the degree of the disease. In particular, the shared gene expression of SA was highly correlate with JA ($r = 0.9971$, $p < 0.0001$) and ethylene ($r = 0.9627$, $p < 0.0001$). Similarly, there was also a strong correlation between JA and ethylene gene expression ($r = 0.9752$, $p < 0.0001$). This implies that regulation of hormonal signaling pathways in the plant could be working in synergy to the pathogen. Deterioration of the disease itself had a significant correlation with expression of SA ($r = 0.9716$, $p < 0.0001$), JA ($r = 0.9617$, $p < 0.0001$), and ethylene ($r = 0.8909$, $p < 0.0001$), which was likely to have a large contribution in the response of the plant to the infection.

The relationship between plant growth traits-such as height, root length, and leaf number-and disease severity was also examined. The lengths of plant and root length were found to have a strong positive correlation to disease severity ($r = 0.7019$, $p < 0.0001$; $r = 0.5174$, $p < 0.0001$ respectively) and the number of leaves was strongly positively correlated ($r = 0.5228$, $p < 0.0001$). These findings indicate that severity of disease has adverse effects on the growth of plants which is additionally supported by the observed reduction in biomass ($r = 0.5864$, $p < 0.0001$). However, biomass showed a strong positive correlation with all the growth parameters measured especially those of plant height ($r = 0.9761$, $p < 0.0001$), root length ($r = 0.9676$, $p < 0.0001$), number of leaves ($r = 0.9649$, $p < 0.0001$), which shows that all the measurements were affecting the overall biomass.

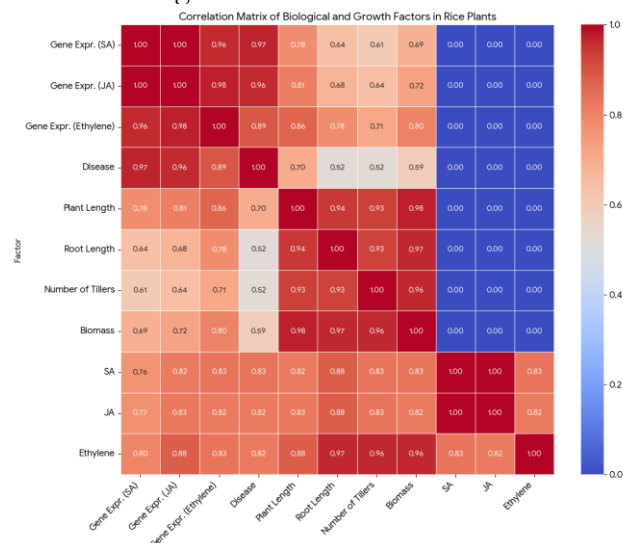


Figure 3. Correlation Matrix of biological and growth factor in rice plant

SA, JA and ethylene concentrations correlated strongly with gene expression of their respective hormones, where SA strongly correlates with JA ($r = 0.9975$, $p < 0.0001$) and ethylene ($r = 0.8342$, $p < 0.0001$). Similarly, JA was strongly correlated with ethylene ($r = 0.8198$, $p < 0.0001$) implying that there was the possibility that the hormonal signaling pathways could synergy in the defense of the rice blast disease. Moreover, disease severity was also highly associated with the presence of the hormones (SA, JA, and ethylene), further supporting the role that such assorted hormones play in the immunity of the plant. Correlation coefficients were significantly high especially with SA ($r = 0.8342$, $p < 0.0001$) and JA ($r = 0.8198$, $p < 0.0001$) which demonstrated their prime role in resistance to diseases.

Table 3. Correlation Matrix of Gene Expression, Disease Severity, Plant Growth Parameters, and Hormonal Concentrations in Rice Plants Infected with *Magnaporthe oryza*

Factor	Gene Expression (SA)	Gene Expression (JA)	Gene Expression (Ethylene)	Disease	Plant	Root	Number	Biomass	SA	JA	Ethylene
Gene Expression (SA)	1.0000	0.9971	0.9627	0.9716	0.7808	0.6430	0.6078	0.6908	0.0000	0.0000	0.0000
Gene Expression (JA)	0.9971	1.0000	0.9752	0.9617	0.8099	0.6789	0.6419	0.7245	0.0000	0.0000	0.0000
Gene Expression (Ethylene)	0.9627	0.9752	1.0000	0.8909	0.8641	0.7808	0.7126	0.8009	0.0000	0.0000	0.0000
Disease	0.9716	0.9617	0.8909	1.0000	0.7019	0.5174	0.5228	0.5864	0.0000	0.0000	0.0000
Plant	0.7808	0.8099	0.8641	0.7019	1.0000	0.9426	0.9319	0.9761	0.0000	0.0000	0.0000
Root	0.6430	0.6789	0.7808	0.5174	0.9426	1.0000	0.9277	0.9676	0.0000	0.0000	0.0000
Number	0.6078	0.6419	0.7126	0.5228	0.9319	0.9277	1.0000	0.9649	0.0001	0.0000	0.0000
Biomass	0.6908	0.7245	0.8009	0.5864	0.9761	0.9676	0.9649	1.0000	0.0000	0.0000	0.0000
SA	0.7625	0.8175	0.8342	0.8342	0.8175	0.8792	0.8342	0.8342	1.0000	0.9975	0.8342
JA	0.7722	0.8257	0.8198	0.8198	0.8257	0.8792	0.8342	0.8198	0.9975	1.0000	0.8198
Ethylene	0.7983	0.8792	0.8342	0.8198	0.8792	0.9676	0.9649	0.9649	0.8342	0.8198	1.0000

Discussion:**Findings Interpretation:**

The findings of this paper elucidated the mechanisms of genetic and hormonal regulation of the rice plants in dealing with rice blast disease launched by *Magnaporthe oryzae*. The significant results showed a substantial effect on genotype, time or genotype-time interaction in many physiological and molecular processes such as the growth of the plant, disease levels, and defense related gene and hormone expression[16][17]. Within three tested rice genotypes, Genotype 3 (G3) had better growth and decreased pathogen-induced visible differences, and it showed greater gene expression of defense mechanisms using salicylic acid (SA), jasmonic acid (JA), and ethylene than Genotypes 1 (G1) and 2 (G2)[18][19]. This indicates that, G3 has stronger resistance mechanism to rice blast probably as a result of the up regulated hormonal and genetic responses that were noted when rice blast pathogen struck it[8].

Even further explanatory and supportive to the idea that these pathways synergize in enhancing defense responses is the fact that the significant correlation amid the gene expression and concentrations of hormones is significant[20]. It is important to note that there was a positive correlation between SA, JA and expression of ethylene genes, hence the suggested interaction between the three pathways in intermediate in the elicitation of immune activities against *M. oryzae*. The present results are consistent with earlier studies on the involvement of plant hormones in disease resistance where it is suggested that hormonal communication is central in regulating the defenses of the plant[21].

Comparison with Past Works:

The findings of the current study are in agreement with a number of earlier researches which have revealed the critical importance of SA, JA, and ethylene as participants in the defense reactions of the plants, based on biotic stress. Indicatively,[22] and [23] identified that SA plays an important role in the defense mechanisms against biotrophs such as *M. oryzae*, whereas, JA is significant in biotic processes relating to necrotrophs. These observations support our findings, in which the expression of SA, JA and ethylene paths is the highest in G3 at the later time-points (72 hours post-inoculation) reflecting the activation of these pathways in the defense response[24]. Also, the findings on its variable reaction among the genotypes are backed up by genetic resistance researches in rice. Similar results were noted by[25] in rice genotypes with differences in blast disease resistance (resistant and susceptible) whereby blast disease-resistant genotypes had increased expression of the genes concerning defense hormones.

Moreover,[26] noted that the genetic loci influence defense responses, and our results support their discovery because we found the multi-locus effect of the genetic resistance loci and hormonal pathways in increasing resistance against the disease. Among the severity of the disease, G1 was the most susceptible, a finding that is consistent with various past literature indicating weak susceptible genotype often experiences poor defense response hence increases severity[27]. This pattern of disease course caused by the G1 makes sense in terms of the idea that those genotypes susceptible to the disease react later to the disease or weaken in their reaction in terms of late or low reaction[28].

Scientific Explanation:

Such physiological and molecular actions that are seen in this study can be explained to be due to the multifaceted interrelationship between genetic resistance and hormonal controls. After infection with *M. oryzae* rice plants launch a defense cascade through the generation of reactive oxygen species (ROS) and the recapitulation of defense genes[29]. The role of SA in interference of plant resistance has been established well and SA comprises an important role of stimulating defense gene like pathogenesis-related proteins (PR1), and NPR1[30]. This hormone also causes a state of systemic acquired resistance (SAR) that may guard the plant against any further attacks of pathogens.

Jasmonic acid (JA) in its turn is implicated in responding to necrotrophic pathogens and insect herbivores[31]. The raised expression of JA in G3 in our study implies that this genotype might be more dependent on jasmonate pathway to fight blast. The increased production of another significant hormone of the plant defense ethylene is likely to reinforce the effect of SA and JA which tend to act defectively as they jointly regulate the defense response. Genetic variation among the rice genotypes can account the identities of the differences recorded in the genotype relating to plant growth, severity of disease and hormonal levels[32]. The genotype 3 that demonstrated the greatest resistance may have good genetic loci that make the plant have innate resistance to identify and react against the pathogen. This could be because these loci may affect the action of genes and pathways involved in the synthesis of defense-related genes, as a result of which the immune response will be stronger[33].

Implications:

The current research has significant significance in view of future research as well as application in rice breeding. Detection of genotypes that have higher resistance to *M. oryzae* introduces new opportunities of producing resistant rice varieties. The identified relationships between genes and the hormonal regulation allow getting a good picture of the molecular complex of blast disease resistance which can be applied to influence the breeding programs for the less stable defense to be selected[34]. In the future, it may be possible to study genetic loci in G3 related to the concept of resistance in more detail and how they may interact with signal transduction involving hormones. Also, further research on the potential of using other plant hormones, e.g. auxins and cytokinins, in enhancing rice blast immunity may help us gain more insight into the immunity system of the plant.

Practically speaking, the results indicate that both genetic resistance and hormonal control may be considered in the breeding processes for the choice of elite rice varieties. A marker assisted selection (MAS) approach of the genes relevant to the SA, JA and ethylene pathways would help in augmenting the benefit of the breeding program that leads to the creation of the blast resistant rice types[35]. Moreover, the research findings are beneficial to the agricultural sector, particularly, on the territories where the risk of instability is high because of the rice blast disease.

Limitations:

Conclusively, although this research provides an important insight on genetic regulation and hormone control of the rice blast resistance, a number of limitations have to be taken into consideration. The research facility is controlled, which makes it possible that some aspects of the field will not be identical to the environment thus manipulating the spread of the disease. Moreover, only three genotypes of rice were used, and an outside application to the study might not be applicable to other varieties of rice growing in various places in diverse environmental conditions.

Moreover, in this study molecular methods employed (qPCR and HPLC) are quite powerful but not free of technical difficulties. An example could be hormone concentration measured by HPLC often needs exact calibration, and error of sample preparation may go wrong by a small amount. These are some points which one should take into consideration when understanding the results more specifically when applying this to the actual breeding activities. The study presents an in-depth discussion on the molecular and hormonal regulation of resistance of rice plant to rice blast disease. Conclusions indicate that genetic resistance, in conjunction with the increased hormonal reactions, is a crucial factor that determines the decreasing disease severity and the plant growth influence. This understanding, besides adding to what we know about how plants interact with their pathogens, also opens a door to the prospect of breeding more resistant varieties of rice, with specific targeting of breeding approaches.

Conclusion:

Finally, this research was able to fulfill its aims namely to examine the molecular pathways involved in resistance of rice plants against rice blast disease and in particular genetic and hormonal regulation. The findings showed that both genetic influences especially through expression of defense related gene and hormonal pathways through both salicylic acid (SA), jasmonic acid (JA), and ethylene systems were important in the immune response of the rice plant. The G3 response was highly resistant, and had higher expression of genes, hormone levels as well as growth and reduced severity of the disease when compared to G1 and G2. The given study is very important as it contributes much to the comprehension of the genetic and hormonal balance of the resistance to rice blast which may be used to breed resistant rice varieties. The results show that genetic loci and hormonal signal pathways must be taken into account to realize the successful development of disease resistant crops. The areas to be explored further by future researchers are to study the molecular dynamics between these pathways and evaluate how it can be applied in various environmental set-ups to widen the distribution of disease-resistant varieties of rice.

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