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BIOCHEMICAL AND TRANSCRIPTOMIC ANALYSIS OF DISEASE RESISTANCE AND EARLY-MATURITY RELATED GENES IN NMR-191 AND NMR-192 RICE MUTANT LINES

Tuck Mun Yip
BSc (Hons), Applied Biomedical Sciences & Biotechnology, School of Health Sciences, IMU University, Bukit Jalil, Kuala Lumpur, Malaysia,
00000035968@student.imu.edu.my

Sobri Bin Hussein
PhD, Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, Bangi, Kajang, Selangor,Malaysia,
sobri@nm.gov.my

Faiz Bin Ahmad
MSc, Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, Bangi, Kajang, Selangor,Malaysia,
faiz@nm.gov.my.

Yoshihiro Hase
PhD, Takasaki Institute for Advanced Quantum Science, National Institutes for Quantum Science and Technology (QST), Takasaki, Gunma, Japan
hase.yoshuhiro@qst.go.jp

Ying Pei Wong
PhD, Applied Biomedical Sciences & Biotechnology, School of Health Sciences, IMU University, Bukit Jalil, Kuala Lumpur, Malaysia
paye.wong@gmail.com

Rhun Yian Koh
PhD, Applied Biomedical Sciences & Biotechnology, School of Health Sciences, IMU University, Bukit Jalil, Kuala Lumpur, Malaysia
rhunyian_koh@imu.edu.my

Anna Pick Kiong Ling
PhD, Applied Biomedical Sciences & Biotechnology, School of Health Sciences, IMU University, Bukit Jalil, Kuala Lumpur, Malaysia,
anna_ling@imu.edu.my

Abstract

Rice cultivation in Malaysia faces concerns on blast disease, Magnaporthe oryzae attack, and a long maturation period. Generation of new rice mutant lines that possess improved disease resistance and early maturity characteristics are of great importance. Hence, this study aimed to analyze the biochemical characteristics as well as to validate the presence of disease resistance and early maturity-related genes in rice mutant lines (NMR191 and NMR192). After the parent line Pongsu Seribu 2 (PS2) and mutant lines were grown for 14 days, the biochemical tests (total soluble protein content, chlorophyll and proline content) were conducted. The presence of disease resistance and early maturity genes were also analyzed through transcriptomic profiling. The biochemical analyses showed a significant increase in the total soluble protein, chlorophyll and proline content in NMR191 and NMR192 compared to PS2. The transcriptomic profiling analysis revealed that Os07g0129300, Os12g0270300, and Os03g0235000 were the disease-resistance genes whereas Os03g0195300, Os01g0704100, Os11g0143200, Os06g0569900, and Os06g0568600 were the early maturity genes found in NMR191 and NMR192. This study validates that NMR191 and NMR192 could be better varieties than the parent line due to the presence of their early maturity and disease resistance traits.

Keywords:
Chlorophyll Content, Mutation Breeding, *Oryza Sativa*; Proline Content, Total Soluble Protein Content

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1. Introduction

There have been multiple studies conducted to evaluate the causes that led to the decrease in rice cultivation in Malaysia. The major rice disease that hinders rice cultivation in Malaysia is blast disease caused by *Pyricularia oryzae* (NurulNahar et al., 2020). This blast disease is also known as panicle blast disease, which may infect the plant panicle during the reproductive stage. This type of disease may impact the plant by either causing the grain to be partially unfilled or leading to breakage of the panicle (Chuwa et al., 2015). Eventually causes a huge amount of yield loss. Furthermore, the lengthy planting period is another factor that hinders rice cultivation. Generally, rice is cultivated twice in Malaysia annually. The first planting period is during the humid weather in between the month of August to February whereas the second planting period is during the dry weather between March to July (Dorairaj & Govender, 2023). Based on the statement above, the rice cultivated during the humid weather is prone to high yield loss as rice is unable to be harvested before the monsoon season (Vaghefi et al., 2016). There is report showing that floods destroyed 40,828.28 ha of paddy fields in Malaysia from 2017 to 2021 causing huge losses of RM 128.8 million (BERNAMA, 2021). In view of this, drastic action should be taken in the development of new rice varieties that have the characteristics of blast disease resistance and early maturity to minimize yield loss and maximize yield production.

In order to create new rice varieties that pose desirable traits such as pest resistance and early maturity, understanding the biochemical responses of the rice varieties under pest and disease attack as well as early maturity is crucial. This is because the biochemical responses will indicate the mechanism and relation of plants during disease attack and early flowering. Protein, chlorophyll, and proline are among the important biochemicals that play a key role in adapting the plant to combat disease and speeding up the growth stage of plants to achieve early maturity (Kaur et al., 2022). Furthermore, the integration and understanding of the transcriptomic profiles of rice varieties are important as it enables researchers to determine the pattern of gene expression in plants that are responsible for different conditions such as disease resistance and early maturity (Wang et al., 2020). This can be done by comparing the transcriptomes of plants between the parent line and the mutated rice varieties. Transcriptomic studies enable the justification of various genes that are present in the mutant varieties as well as their functions and roles in adapting to different stress conditions (Wang et al., 2020). In short, analyzing the transcriptomic profiles of rice varieties helps us to validate the presence of desired traits in the new plant varieties.

In this study, the mutation breeding method, which is ion beam radiation was applied on the parent line, Pongsu Seribu 2 (PS2) in developing two new rice mutant lines (NMR191 and NMR192) with enhanced characteristics of disease resistance and early maturity. This study aimed to determine and analyze the biochemical characteristics and transcriptomic profiling of disease resistance and early maturity-related genes in NMR191 and NMR192.

2. Materials and Methods

2.1 Plant Materials and Germination of Seeds

The parent line (PS2) as well as NMR191 and NMR192 rice mutant lines were generated using ion beam radiation at QST-Takasaki, Japan and were provided by Malaysia Nuclear Agency. A total of 24 seeds were chosen from each of the NMR191 and NMR192 rice mutant lines and their corresponding parent line. The seeds were soaked in fungicide for 24 hours followed by rinsing with sterile ultrapure water. Then, five seeds from each of the mutant lines were placed on the filter sponge in a transparent glass bottle (6.5 cm X 6.5 cm X 7.5 cm). Sterile ultrapure water was added until the filter sponge was completely soaked in the ultrapure water. Following that, all the bottles were placed at room temperature and the environment with an adequate amount of light and with a photoperiod of 16 hours light and 8 hours dark to facilitate germination. The seedlings were left to grow for 14 days before conducting biochemical and transcriptomic analyses.

2.2 Determination of Biochemical profiles

In the biochemical studies, the content of total soluble protein, chlorophyll and proline in both mutant and parent lines were determined. The total soluble protein and chlorophyll content were determined using Bradford and Lichtenthaler method, respectively (Ling et al., 2013) while the proline content was determined using Bates et al. method (Ghanem et al., 2021).

2.3 Transcriptomic Analysis

The plant samples were sent to the Apical Scientific Sdn. Bhd. for RNA extraction and RNA-Sequencing analysis. The total RNA content of the plant samples was extracted using PrimeWay Total RNA Extraction Kit (1st BASE Kits, KIT-9021-50). The microvolume UV/Vis spectrometer and RNA labchip, RNA 6000 Nano Kit (Agilent, 5067-1511) were utilized to measure the quality and quantity of purified RNA. The transcriptomic analysis was further done by Apical Scientific Sdn. Bhd. STRING database was utilized to analyze the protein-protein interaction of the DEGs. This analysis was used to identify functionally related genes, particularly those associated with disease resistance and early maturity traits.

2.4 Statistical Analysis

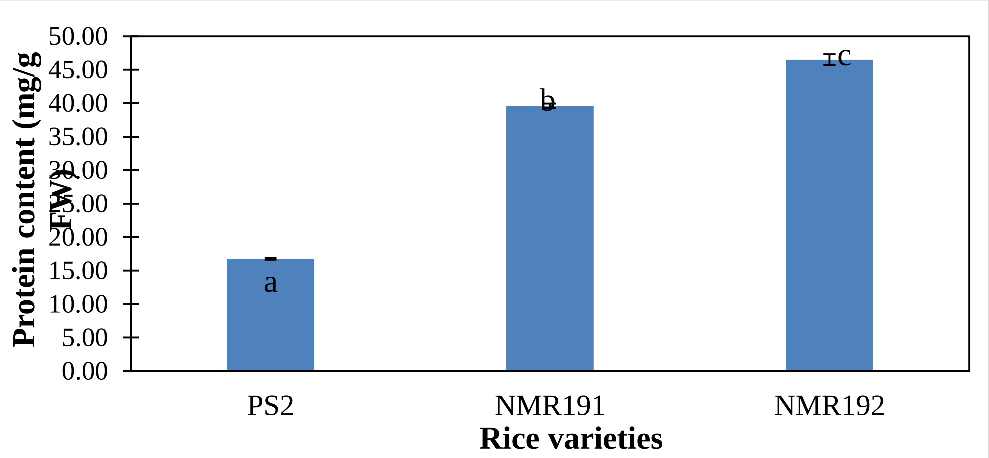
The experimental procedures in this study were conducted with 3 replicates for biochemical analyses. The data obtained was subjected to one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test at a significance level of $P < 0.05$ using SPSS (Version 18.0, SPSS Inc., USA).

3. Results and Discussion

3.1 Total Soluble Protein Content

In general, the study revealed that the total soluble protein content found in the mutant line NMR191 and NMR192 was significantly higher than the parent line PS2. It demonstrated that the protein content found in NMR191 and NMR192 was 236.2 % and 277.34% higher than the parent line PS2. These results indicate that the mutant line could possibly have better disease resistance and early maturity characteristics compared to the parent line.

Figure 1: *Comparison between the Total Soluble Protein Content in the Parent Line, PS2, and the Mutant Line NMR191 and NMR192 after 14 Days of Growing Period*



(Source: Authors' Own Illustration).

Means with different letters significantly differ between rice mutant lines by Tukey's HSD test (*P*<0.05). Error bars indicate the mean ± standard deviation (*n*=3).

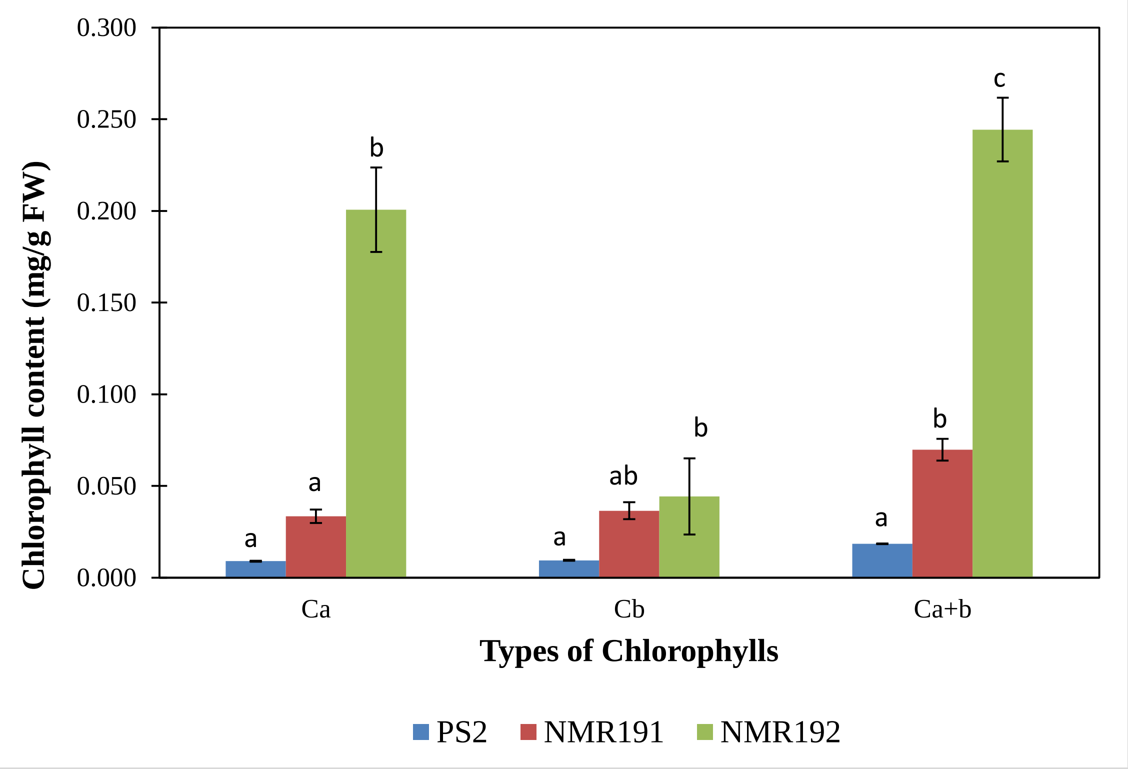
Based on the current findings, NMR191 and NMR192 have been identified as having better disease resistance characteristics than the parent line, possibly due to the presence of significantly higher PR protein as compared to the parent line. Dos Santos and Franco revealed that the PR protein was found to be in the encoded form in the plant and will be expressed during the pathogen invasion in the plant cell (Dos Santos & Franco, 2023, Finkina et al., 2017). The presence of higher protein content in the mutant line enables the plant to express a higher concentration of PR1 protein to inhibit fungal growth and reduces the risk of fungal colonization in the cells (Finkina et al., 2017). The PR1 proteins have antifungal functions enabling the mutant line to have better disease resistance traits than the parent line (Zribi et al., 2021). Besides that, the study conducted by Wu et al. demonstrated that the presence of (R) protein has a crucial role in initiating defense response in plants by recognizing pathogen effectors to combat fungal attack (Wu et al., 2014). In this study, the rice mutant line shows a significantly higher amount of protein content than the parent line, indicating the R protein presence in the mutant line NMR191 and NMR192 could have been upregulated. During fungal pathogen attack, the mutant line was able to activate a higher amount of R protein than the parent line to increase the efficiency of plants to scavenge ROS that will cause cellular damage as well as accelerate the activation of hypersensitive response (HR) at the infection sites leading to apoptosis to prevent the spreading of fungal activity in the plant cells (Wu et al., 2014; Wang et al., 2023).

Additionally, the transport protein that is present NMR191 and NMR192 could also be upregulated and demonstrated early maturity traits due to the fact that the protein content was significantly higher than the parent line PS2. Nitrogen is a type of macronutrient that is required in large numbers to support growth and development in plants (Sandhu et al., 2021). According to the study conducted by Aluko et al. (2023), the transport protein plays a major role in the nitrogen uptake in plants. It was discovered that the nitrogen uptake efficiency (NUE) in rice plants was only between 30% to 50% (Aluko et al., 2023). Therefore, the mutant line that has higher protein content than the parent line was able to increase NUE with the increased expression of transport proteins in the plants, leading to early maturity in rice. Nitrate transporter 1 (NRT1) and nitrate transporter 2 (NRT2) are examples of transport proteins involved in nitrogen uptake in plants (Aluko et al., 2023). Moreover, the studies conducted by Zhou et al. (2022) have discovered that the transmembrane proteins in plants are responsible for reproductive growth and are highly correlated with the plant's early maturity (Zhou et al., 2022). In this study, both the mutant lines have displayed higher protein content than the parent line, which indicates that the expression of transmembrane protein in the plants will be upregulated. The upregulation of transmembrane protein enables the mutant line to reach early maturity by accelerating the growth of pollen tubes in rice through the signaling pathway stimulated by GABA (gamma-aminobutyric acid) and aluminum-activated malate transporter (ALMT) (Suzuki et al., 2015; Zhou et al., 2022).

3.2 Chlorophyll Content

Based on current findings as shown in Figure 2, NMR192 has demonstrated a significantly higher C_a and C_b content compared to PS2. There was also 377.78% and 370% higher of C_a and C_b in NMR191 in respect to PS2. Nevertheless, the increase was not significant. The results in this study indicate that the mutant lines have better disease resistance and early maturity traits compared to the parent line.

Figure 2: *Comparison between the Chlorophyll Content in the Parent Line, PS2, and the Mutant Line NMR191 and NMR192 after 14 Days of Growing Period*



(Source: Authors' Own Illustration).

Means with different letters significantly differ between rice mutant lines by Tukey's HSD test (*P*<0.05). Error bars indicate the mean ± standard deviation (*n*=3).

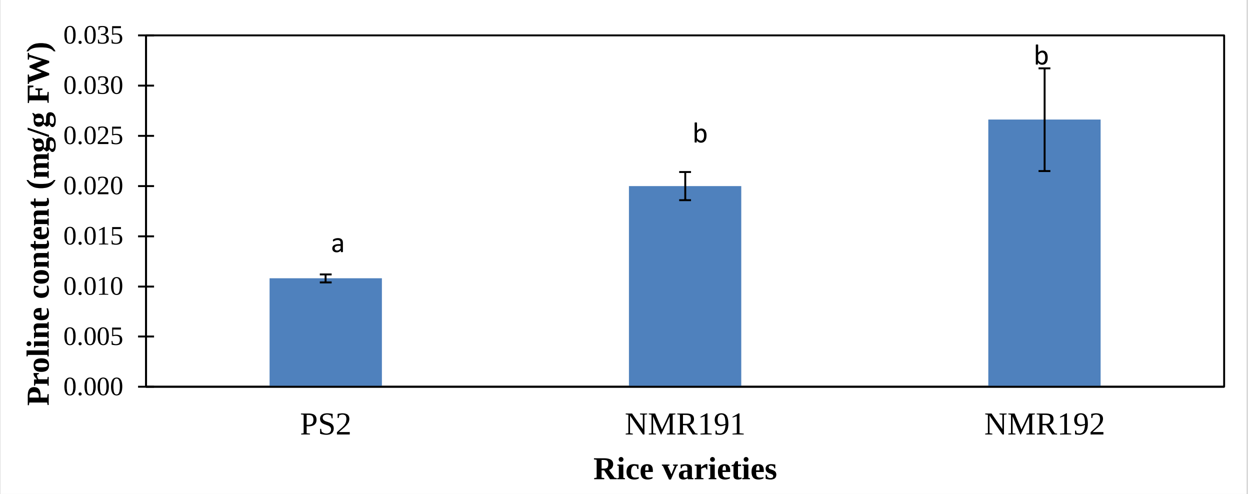
In accordance with the present findings, Kretschmer et al. (2019) reported that C_a plays a major role in synthesizing phytohormones to activate plant defense mechanisms against fungal pathogens. The jasmonic acid (JA) and salicylic acid (SA) are examples of phytohormones that act as defensive hormones in plants (Kretschmer et al., 2019). These defensive hormones in plants play a role in activating the plant defense mechanism to combat fungal pathogens attacks (Wang et al., 2016). Thus, the mutant lines NMR192 in this study that has higher chlorophyll content was able to produce more C_a to upregulate the synthesis of phytohormones allowing the plants to produce more defensive hormones such as SA to trigger fungal pathogen infection in the rice plant. On top of that, Hong et al. and Rossi et al. revealed that C_a is responsible for scavenging peroxyl radicals and has antioxidant properties in plants (Hong et al., 2020). In contrast with the current study, it indicates that NMR192 which has higher C_a content was able to scavenge ROS better, which shows that it has better disease resistance traits than the parent line PS2. This is because there will be a rapid accumulation of ROS in the pathogen infection site to suppress and kill the fungal pathogens (Liu et al., 2013; Hong et al., 2020; Dumanović et al, 2021). However, oxidative stress occurs when the ROS production is overexpressed, and the detoxification rate is much lower leading to imbalance (Hong et al., 2020). During such a scenario, the antioxidant properties of C_a play a role in neutralizing the ROS produced during disease stress conditions (Tripathy & Oelmüller, 2013). As a result, NMR191 that produces higher C_a content than PS2 in the plant was able to upregulate the production of ROS to suppress fungal pathogen growth as well as neutralize the overexpression of ROS produced when the plant is under oxidative stress, leading to NMR191 have better disease resistance trait than PS2.

Besides that, a higher chlorophyll content in plants was able to increase the photosynthesis efficiency leading to early maturity. Studies have revealed that chlorophyll content is crucial to support energy for plants in the stages of early flowering (Ohmiya et al., 2014). In this study, NMR192 has significantly higher chlorophyll content than PS2 indicating that there will be an upregulation of energy produced to support the growth of the plant in the flowering stage. This is because chlorophyll was needed in huge amounts in the early stages of petal development (van der Kooi et al., 2019). As such, the parent line that has a higher content of chlorophyll was able to accelerate the growth and development in rice plants leading to early flowering and shortening the maturation period. Moreover, there have been studies that discovered the upregulation of chlorophyll content in plants was able to increase the production of biomass and carbohydrates in plants enabling robust growth in plants and leading to early maturity. Liu et al. (2019) revealed that there was a high relationship between the chlorophyll index of rice at different growth stages. The study discovered that the chlorophyll content in rice plants was increasing gradually as the plant grew. This is because chlorophyll supports higher energy production, promoting robust vegetative growth, and ensuring that the plant achieves early reproductive development (Liu et al., 2019). Thus, a higher chlorophyll content in NMR192 than PS2 was able to increase energy production in plants thereby supporting the energy required for the robust growth of plants. In addition, Hua et al. (2014) also discovered that the upregulation of chlorophyll concentration in plants may increase the production of carbohydrates. Carbohydrates serve as the energy resources in plants which can be utilized to speed up plant growth and development (Wu et al., 2014).

3.3 Proline content

Based on the current findings, both of the mutant lines demonstrated a significantly higher proline content in the plant samples compared to the parent line as shown in Figure 3. It was shown that the proline content in NMR191 and NMR192 was 181.8% and 245.45% higher than PS2. These results indicate that both mutant lines demonstrated better disease resistance and early maturity traits than the parent line. This conclusion is deduced based on the fact that proline can act as the osmoprotectant and signaling molecules to aid the plant in the defensive system whereas it can accelerate the growth and development of organs and reproductive parts in the plants to shorten maturation period of plants (Hayat et al., 2015; Kavi et al., 2015).

Figure 3: *Comparison between the Proline Content in the Parent Line, PS2, and the Mutant Line NMR191 and NMR192 after 14 Days of Growing Period*



(Source: Authors' Own Illustration).

Means with different letters significantly differ between rice mutant lines by Tukey's HSD test ($P<0.05$). Error bars indicate the mean ± standard deviation ($n=3$).

Based on the study conducted by Liang et al. (2013), proline can act as the osmoprotectant to regulate the osmotic balance in plant cells which are affected by pathogen invasion. Both NMR191 and NMR192 have demonstrated higher proline content than the parent line indicating that they have better disease resistance traits due to their ability to regulate the osmotic balance in the plants better during and after the disease attacks. This is because the fungal pathogens will produce an enzyme known as cell wall degrading enzymes (CWDE) to break the barrier of the plant cell wall to invade the plant cells (Lorrai & Ferrari, 2021). To cope with these conditions, the plant will trigger a signal known as a turgor-dependent signal that functions to strengthen the plant cell wall that was affected by the CWDE enzymes produced by the fungal pathogens. Lorrai et al. (2013) found that the expression of turgor-dependent signals will affect the osmotic pressure in plants to increase cell wall integrity. As such, both of the mutant lines that have higher proline content were able to upregulate the expression of osmotically active substances to manage the plant osmotic balance that was disturbed during the fungal pathogen infections, thereby enhanced the cell wall integrity, allowing the plant to combat against the fungal diseases better.

Besides that, proline has been discovered as a proteogenic amino acid that acts as a beneficial solute in plants. There have been multiple studies that have discovered that proline has been responsible for the growth and development of plants where an increase of the proline content may have the potential to speed up the plant growth leading to plant maturity (Kavi et al., 2015). According to the studies conducted by Hosseinifard et al. (2022) and Vandana et al. (2020), proline metabolism plays an important role in the organ development of rice plants. Their studies discovered that the proline concentrations decreased gradually through the growing stages and decreased rapidly during the flowering stage in plants (Hosseinifard et al., 2022). This suggests that proline was used in the growth and development of plants, particularly during the flowering and maturation stages. As such, NMR191 and NMR192 that have significantly higher proline content was able to accelerate the growth and development of plants, particularly during the flowering stages and maturation stages due to the presence of an adequate amount of proline to support organ development of plants, leading to early maturity.

The research conducted by Kavi and Sreenivasulu (2014) revealed that proline contributes a major role in the development of flowers and reproductive organs of plants including pollen grains and ovules. This signifies that the mutant lines that have higher proline content were able to accelerate the development of reproductive organs in plants leading to early flowering. The biosynthesis of pyrroline-5-carboxylate reductase (*P5CR*) gene was found to express hugely in reproductive parts of the plants, particularly during the pollen growth (Kavi & Sreenivasulu 2014). This is because the *P5CR* gene was expressed to increase efficient nitrogen supply for pollen grain growth and development (Funck et al., 2012).

3.4 Transcriptomic Profiling Analysis

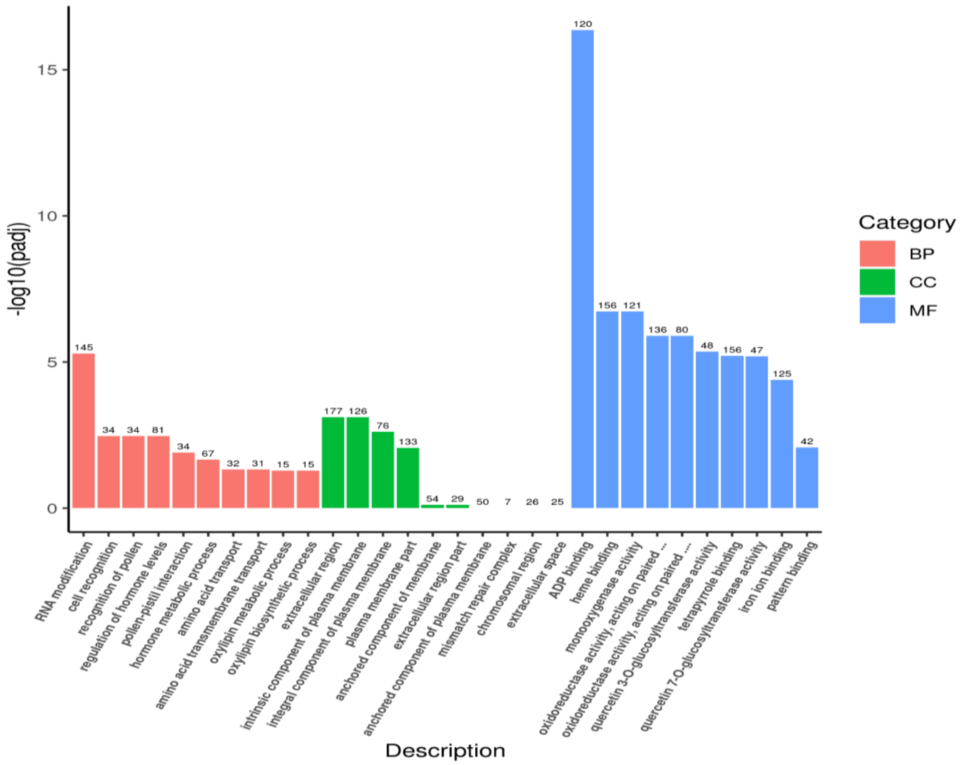
The gene ontology enrichment analysis was used as a bioinformatics tool widely in research analysis due to its ability to interpret high throughput of molecular data and genotype (Tadokoro et al., 2020). In addition, the enrichment analysis can be used to interpret the similarity of genotypes between both of the samples compared (Tadokoro et al., 2020). In this study, the gene ontology (GO) bar was utilized to demonstrate the top 5 representative genes found in both NMR191 and NMR192, when they were compared with their parent line. The top representative genes were further categorized into 3 groups which include biological processes, molecular functions, as well as cellular components as illustrated in Figure 4 and Figure 5. Additionally, the overall top 5 representative genes found in NMR191 and NMR192 were summarized in Table 1 and Table 2. Nevertheless, the majority of the genes were mostly expressed in the groups of cellular components and molecular functions but not in the biological process of the plants. This signifies that the cellular components and molecular functions of the plant cells in NMR191 and NMR192 contribute the major role in disease resistance and early maturity traits, where the biological process of the plant only contribute minor role in these traits.

3.4.1 Disease Resistance Traits Found in the Rice Mutant Line

As shown in Table 1 and Table 2, *Os07g0129300* gene was one of the most representative genes and can be found in NMR191 and NMR192. This gene belongs to the pathogenesis-related 1 (PR-1) proteins family. Multiple studies have revealed that the *PR-1* gene is responsible for plant immunity towards disease and an overexpression of this gene may enhance the plant resistance toward fungus disease attack (Tadokoro et al., 2020; Dos Santos & Franco, 2023; Wang et al., 2023). The upregulation of the *PR-1* gene will enable the mutant rice line NMR191 and NMR192 to equip the rice mutant line with disease-resistance traits. This is because the *Magnaporthe oryzae* disease that causes a huge loss in yield production in Malaysia is a type of rice blast fungus (Wang et al., 2021). The mechanism of *Os07g0129300* gene to fight against the disease was by suppressing fungal pathogen growth by avoiding pathogen colonization in plant cells and acting as an antimicrobial agent. The *PR-1* gene will remain in encoded form in the plant cells and will be expressed during a pathogen attack (Dos Santos & Franco, 2023). Thus, it can be concluded that *Os07g0129300* gene was found to be one of the most highly expressed genes in NMR191 and NMR192 consists of disease resistance traits by the expression of *PR-1* gene to enhanced plant resistance towards diseases. Besides that, *Os12g0270300* gene is one of the most highly expressed genes found in the NMR191 compared to PS2, which is responsible for disease resistance. This gene acts as the disease resistance protein RG-A5 like in rice plants. The RGA5 is a type of immune sensor found in plants. Césari et al. (2014) have revealed that the RGA5 which belongs to the nucleotide-binding and leucine-rich repeat domain proteins (NB-LRRs) plays a role in mediating resistance to the *Magnaporthe oryzae*, which is a type of fungal pathogen. The mechanism of an antifungal is that the RGA5 will recognize the avirulence (AVR) protein produced by the fungal pathogen during a disease attack in plant cells and trigger the plant immune system, leading to cell death on the microbial activity (Césari et al., 2014).

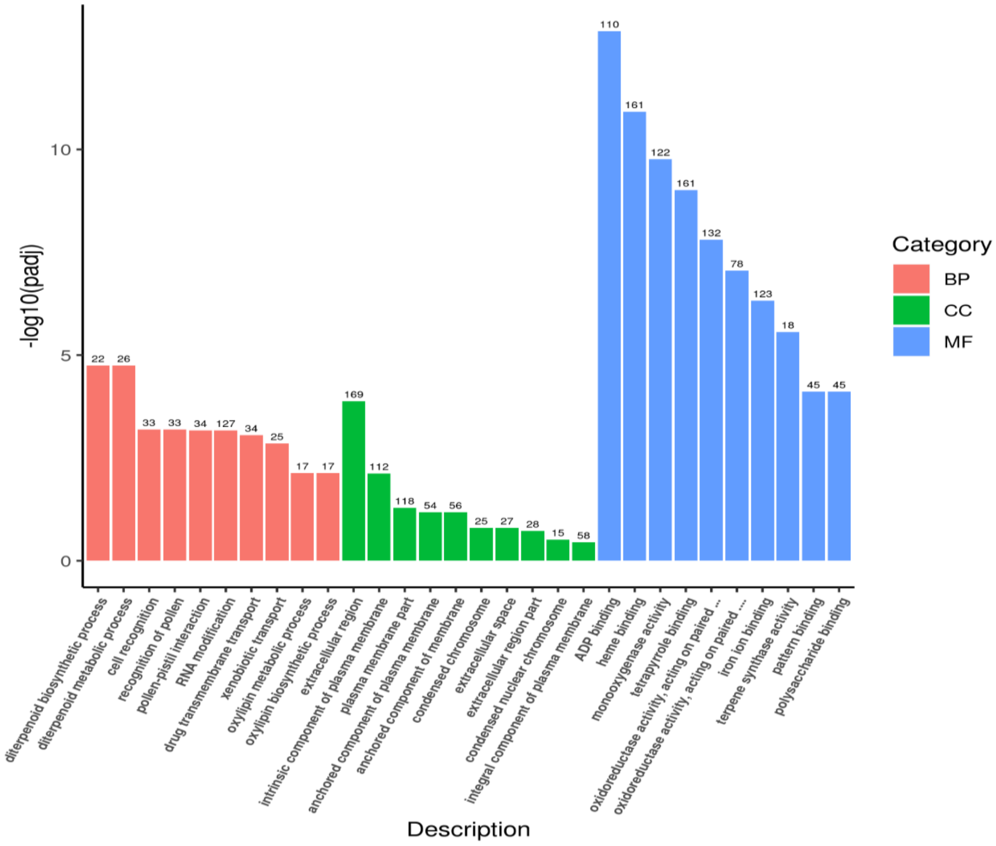
Furthermore, *Os03g0235000* gene is one of the most highly expressed genes and can be found in NMR192. This gene acts as the heme-dependent peroxidase in the plants. Singh et al. discovered that heme-dependent peroxidases are a type of enzyme found in plants that has the purpose to aid in plant defense against fungal pathogen attack (Singh et al., 2013). This is because the enzymes were able to produce ROS in plants during pathogen infections. The ROS that are produced by the heme-dependent peroxidases was hydrogen peroxide (H_2O_2) (Liu et al., 2013). Additionally, Liu et al. (2013) and Huang et al. (2019) revealed that ROS can act as the defensive signal for plants as it activates other defense mechanisms in plants during pathogen attacks. The mechanism of ROS leading to cell death begins with the accumulation of ROS at the pathogen infection site leading to an oxidative burst that is toxic to the pathogen and eventually stimulates the hypersensitive response (HR) in the plants which may cause a zone of cell death preventing the spreading of pathogen in plant cells (Sharma et al., 2013).

Figure 4: *Gene ontology (GO) enrichment bar that illustrates the top 15 most highly expressed genes found in mutant line NMR191 when compared with the parent line PS2*



(Source: Apical Scientific Sdn. Bhd).

Figure 5: *Gene Ontology (GO) Enrichment Bar That Illustrates The Top 15 Most Highly Expressed Genes Found In Mutant Line NMR191 When Compared With The Parent Line PS2*



(Source: Apical Scientific Sdn. Bhd).

Table 1: *Top 5 Most Highly Expressed Genes Found in NMR191 when Compared with the Parent Line PS2*

Type	Gene ID	Description	Traits
CC	*Os07g0129300*	Extracellular region	Disease resistance
CC	*Os03g0195300*	Intrinsic component of plasma membrane	Early maturity
CC	*Os01g0704100*	Plasma membrane part	Early maturity
MF	*Os12g0270300*	ADP binding	Disease resistance
MF	*Os11g0143200*	Oxidoreductase activity	Early maturity

Table 2: *Top 5 Most Highly Expressed Genes Found in NMR192 when Compared with the Parent Line PS2*

Type	Gene ID	Description	Traits
CC	*Os07g0129300*	Extracellular region	Disease resistance
CC	*Os01g0704100*	Intrinsic component of plasma membrane	Early maturity
MF	*Os03g0235000*	Heme binding	Disease resistance
MF	*Os06g0569900*	Tetrapyrrole binding	Early maturity
MF	*Os06g0568600*	Iron ion binding	Early maturity

*MF indicates molecular functions and CC indicates cellular components.

(Source: Authors' Own Illustration).

3.4.2 Early maturity traits found in the rice mutant line

On the other hand, *Os03g0195300* gene was one of the most highly expressed genes found in NMR191, giving the plant early maturity traits, and acting as the high-affinity sulfate transporter 1 (SULTR1) protein in the plants. Sacchi and Nocito discovered that the high-affinity SULTR1 may increase the sulfate uptake at the root of plants (Sacchi & Nocito, 2019). Besides that, *Os01g0704100* gene is one of the most highly expressed genes found in NMR191 and NMR192, which gives the plant early maturity traits and acts as the high-affinity nitrate transporter 2.3 (*OsNRT2.3*) protein in the plants. Luo et al. (2020) found that the expression of the *OsNRT2.3* gene plays a crucial role in plant growth as it increases rice yield and the nutrient uptake efficiency (NUE) in the plants. Sandhu et al. (2021) and Aluko et al. (2023) reviewed that nitrogen and sulfur are the essential macronutrients required for the plant's growth and development. This is because an increase in the nitrogen uptake can increase the expression of transport protein, providing more nutrients for the growth and development of plants such as accelerating the cytokinin production, leading to cell elongation and increased photosynthetic activity (de Vries et al., 2016; Banerjee et al., 2019). Furthermore, there are studies revealed that increasing the sulfur intake in plants will increase the synthesis of the Rubisco enzyme and increase the production of carbohydrates leading to increases in the production of biomass in plants (Erb & Zarzycki, 2018; Narayan et al., 2022).

Besides that, *Os11g0143200* gene is one of the most highly expressed genes found in NMR191, giving the plant early maturity traits. This gene, also known as cytochrome P450 90A3

(*OsCYP90A3*) has the characteristics of photomorphogenic (Tripathi et al., 2019). The photomorphogenic plays a crucial role in plants as it regulates the intake of light towards the plants. During plant growth, light is important to serve as the energy source for the photosynthesis process enabling the plants to produce a sufficient amount of energy for growth and development. The energy was utilized by the plants to continue increasing their branches to capture more light as well as accelerate the growth stage of plants from the vegetative phase to the reproductive phase and eventually to senescence (Tripathi et al., 2019).

In addition, the *Os11g0143200* gene that is found in NMR191 also gives the plant early maturity due to its gene consisting of dwarf traits. Furthermore, *Os06g0569900* and *Os06g0568600* genes, which were among the most highly expressed genes found in NMR192 also gives the plant early maturity due to the fact that both the gene was a type of dwarf gene found in plants. *Os11g0143200* gene is a type of cytochrome P450 90A3 protein in plants whereas *Os06g0569900* gene is a type of cytochrome P450 701A19 protein in plants and *Os06g0568600* gene is a type of cytochrome P450 701A9 proteins. The study conducted by Jun et al. (2015) revealed that the cytochrome P450 family with CYP90 and CYP701 was found to be providing dwarf traits to the plants. Andrew-Peter-Leon et al. (2021) revealed that dwarf plant type can accelerate plant growth leading to early flowering and increased crop yield. Dwarf plant type indicates that the plant will be shorter than 70 cm plant height (Peng et al., 2021). The dwarf plant was able to accelerate the plant growth to early flowering due to the fact that the dwarf plant type consists of gibberellic acid (GA) in the plants (Gupta & Chakrabarty, 2013). The GA in the plants was able to stimulate seed germination, trigger transitions from meristem to shoot growth, juvenile to adult leaf stage, as well as vegetative phase to flowering phase (Gupta & Chakrabarty, 2013). Additionally, the dwarf plant was able to accelerate plant growth since it does not promote the growth of branches and stem of plants to increase their plant height but promote the growth and development of plant including the transition from the vegetative phase to the flowering phase. As such, compared to the plant types that are not dwarf, it conserves the energy and nutrients required by the plants to increase the plant's height.

4. Conclusions

Based on the findings from the current studies, NMR191 and NMR 192 have been validated with disease resistance and early maturity traits with a significant increase in the plant biochemical parameters. This includes a significantly higher content of total soluble protein, chlorophyll content, and proline content. Additionally, it was further supported by the presence of highly expressed disease resistance and early maturity genes. The disease resistance genes that were highly expressed in both the mutant lines NMR191 and NMR192 include *Os07g0129300*, *Os12g0270300* and *Os03g0235000*. Meanwhile, the early maturity genes that were highly expressed in both the mutant lines NMR191 and NMR192 include *Os03g0195300*, *Os01g0704100*, *Os11g0143200*, *Os06g0569900*, and *Os06g0568600*.

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